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PROCEEDINGS

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PAVE THE WAY TO A BETTER LIFE

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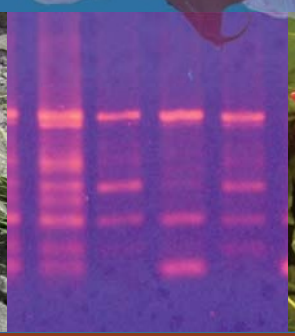

Preface

This proceeding is a compilation of scientific papers presented in the 2nd International Seminar on Biosciences and Biotechnology: "**Pave the Way to A Better Life**" held at the University of Udayana on 23rd – 24th 2010. It includes papers (for oral and poster presentations) presented by Keynote speakers, Invited speakers, and active participants.

This conference was designed in order to gather scientists, engineers, practitioners, and industries in Biological related disciplines, so that they could discuss and share their expertise in the fields of Biosciences and Biotechnology related issues. From this intense discussion, it was expected that some brilliant ideas to be used to improve the quality of human life could be formulated, so that it was in line with the theme of the conference: "**Biosciences and Biotechnology pave the way to a better life**".

This 2nd International conference was held in relation to the Udayana University Anniversary and is expected to be held yearly, so that this event becomes the icon of the Udayana University in the future. The conference consisted of 8 plenary presentations delivered by keynote and invited speakers with International reputations from Japan, Australia, and Indonesia, covering general aspects of Biosciences and Biotechnology. Besides this plenary sessions, we also had four satellite symposia, covering areas of **health**, **agricultural technology and food science**, **agriculture**, and **biodiversity and environment**. Totally, 176 contribution papers (oral and poster presentation) were presented in this conference and they were distributed according to the areas mentioned above. The efforts of the presenters to prepare their contribution papers for this conference are highly appreciated.

This Conference was financially supported by the Rector of Udayana University through the program of Vice Rector I (Vice Rector for Academic Affair) and some sponsors (Monsanto and Kanisius press). Therefore, in this occasion, on behalf of the committee, I would like to acknowledge their financial support.

My thanks should also go to all people who were involved in the committee of the conference. Without their hard working and efforts, I am afraid would not be able to make this event to happen.

Last but not least, I hope you all enjoyed your time in Bali, not only at the venue of the conference, but also enjoyed the beauty of Bali and the friendliness of the people, so that you all brought home some unforgettable memories about the island of Bali. See you again here next year.

Chairman of the Organizing Committee

Drs. Yan Ramona, M.App.Sc., Ph.D.

Forewords-Rector of Udayana University

Dear Distinguished guests, Invited speakers, and all other participants

This second International Conference on Biosciences and Biotechnology with the theme of Bioscience and Biotechnology pave the way to a better life is a continuation of the first International conference successfully held last year, in relation of the Udayana University Anniversary. The main aim of this conference is to gather scientists from all over the world in a venue to share their expertise in Biosciences and Biotechnology and build scientific network, so that they can develop Biosciences and Biotechnology-based methods for improving the quality of human life in the future.

In this opportunity, on behalf of the University, I welcome you all to Bali. Bali is well known as a favorite tourist destination in the world. Recently, it is also a favorite site for holding International events, such as International Conference. When people hear Bali as a site of an International conference, a lot of them will be interested to attend the event. By attending such an event in Bali, they can do two things at once. They can present scientific papers and share their expertise with other scientists known to have International reputation, and at the same time they can also enjoy the beauty of the Bali Island and the culture of Bali which is considered to be unique by foreign tourists.

Here, I would also like to acknowledge the National and International invited speakers for their willingness to come miles away to Bali and present their high standard papers. I understand that you all spend much time for this conference, and therefore I must give high appreciation on all of those effort and dedication.

I hope this International Conference become an annual agenda of Udayana University and become an ideal forum for communication and sharing ideas as well as experience in Biosciences and Biotechnology-related disciplines in the future. I also hope that this forum can serve as a forum for promoting advanced Biosciences and Biotechnology with regard to economic growth and social welfare.

Finally, I wish you most successful conference and hope that it may provide new ideas and strategies for the application of Biosciences and Biotechnology in the industries.

Rector of Udayana University,
Prof. Dr. dr. I Made Bakta, Sp.Pd (K).

KEYNOTE PRESENTATIONS

ORAL PRESENTATIONS: AGRICULTURE

ORAL PRESENTATIONS: AGRITECH AND FOOD

ORAL PRESENTATIONS: BIODIVERSITY AND ENVIRONMENT

ORAL PRESENTATION: HEALTH

POSTER PRESENTATION: AGRICULTURE

POSTER PRESENTATION: AGRITECH AND FOOD

POSTER PRESENTATION: BIODIVERSITY AND ENVIRONMENT

POSTER PRESENTATION: HEALTH

KEYNOTE PRESENTATIONS

USE OF *IN VITRO* BREEDING STRATEGIES IN THE DEVELOPMENT OF NATIVE PLANTS

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ABSTRACT

Plant biotechnology has emerged as a powerful tool of crop improvement and has aroused a great deal of interest in many countries because of its application to agriculture and horticulture. We report here the application of a number of in vitro plant breeding techniques such as micropropagation, in vitro flowering, in vitro pollination and fertilization, anther culture, somatic embryogenesis, and gene technology in the development of Australian native plants. The report covers an overview of our experience working with Australian plants in the past 25 or so years. Many of these plants have the potential to become future floricultural products.

STEM CELL AND ITS MICROENVIRONMENT

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ABSTRACT

Somatic stem cells are maintened and regulated by their surrounding microenvironment (niche). A tissue specific niche is a restricted locale that supports self-renewing division of stem cells. Stem cell niche is a phrase loosely used in the scientific community to describe the microenvironment in which stem cells are found, which interacts with stem cells to regulate stem cell fate. Adult stem cells and their more committed skin, progenitor cells, are prized by medical researchers for their ability to produce different types of specialized cells. The potential of using these cells to repair or replace damaged tissue holds great promise for regenerative medicine. Previous studies on how microenvironments affect the development of adult human stem or progenitor cells have been based on the behavior of these cells in culture (*in vitro*) where they are exposed to a single molecular agent. However, when these cells are in an actual human being (*in vivo*) they are surrounded by a multitude of other cells plus a supporting network of fibrous and globular proteins called the extracellular matrix (ECM), as well as many other nearby molecules, all of which may be simultaneously sending them instructional signals. In our current research, we tried to modify the stem cell microenvironment by adding some of potential agents that resulted in an increasing phenomenon of stem cell growth. Our current results have shown that microenvironment could affect in the proliferation of fibroblast. Hence, in this short communication we could conclude an early suggestion of the importance of stem cell microenvironment.

THE DEVELOPMENT OF REVERSE GENETIC TO DEVELOPE VACCINE TO CONTROL BIRD FLU IN POULTRY IN INDONESIA

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The avian influenza virus (AIV) of H5N1 subtype, popularly known as bird flu virus, is endemic in poultry in Indonesia. The continuous circulation of the virus enhances its possibility to undergo genetic changes which may lead to the generation of fatal and pandemic strain. The most recent data show that the virus has high diversity in Indonesia, in which a monovalent vaccine will not be enough to protect chicken against field isolates. Moreover, we have proofed that the conventional killed influenza vaccine could not reduce virus shedding of the infected chicken. Although the bird remains healthy due to the protection level following vaccination, it is continuously contaminating environment and spreading to other birds and human. To overcome the problem, a polyvalent vaccine that contains some circulating isolates is proposed. To induce protection in the mucosa of the bird, so the virus excretion of infected chicken can be suppressed, a live recombinant Newcastle Disease virus (NDV) carrying the protective immunogenic antigen of AIV, will be important in reducing the economic impact of the disease as well as the risk of the generation of pandemic strain. The recombinant NDV-AIV will be beneficial for poultry industry as it will also reduce the economic impact of NDV disease. Both AIV polyvalent vaccine and recombinant NDV-AIV will be created using reverse genetic technique. In this paper, the academic background of such development is described. The progress of the introduction of reverse genetic in Indonesia is also presented.

Review

GENE ISOLATION BY USING TRANSPOSON AND T-DNA TAGGING METHODS

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ABSTRACT

Transposon used in this study was a Tn5 that harboring a kanamicine resistant gene, and T-DNA used was only a Left Border(LB) and Right Border (RB) of a T-DNA and kanamicine and □-glucuronidase (GUS) were used as reporter genes. Transposon tagging as well as the T-DNA tagging methods is a useful technique and is commonly used in isolating gene of interest. We have used these methods for many of our experiments and it works successfully. This includes isolation of acvB gene of *Agrobacterium tumefaciens* and the isolation of CVPD^r gene. Firstly, the acvB gene of *A. tumefaciens,* which is a chromosomal virulent gene, was found, the latter is known as the first gene found in the world that play an important role in the transfer of ssDNA into host plant cells. The protein that encoded by acvB gene is assDNA binding protein which is located in periplasm of *A. tumefaciens*. Our second experiment discovered CVPD^r gene, that is resistant gene for citrus greening disease that caused by *Liberobacterasiaticum*. This CVPD^r gene can be used to transform citrus plant *in vitro* and *in planta* to generate transgenic plants of citrus. Meanwhile, overexpression of CVPD^r gene resulted in a protein that can be used for producing substances responsible in controlling the citrus greening disease.

Keywords: Transposon, T-DNA, acvB gene, CVPDr Gene, *in vitro, in planta*.

INTRODUCTION

Transposon mutagenesis method has been developed to clone the gene by making use of the features of transposon. Transposon is one of the transposable mobile genetic element which was discovered in 1950 by McClintock in the study of the maize genetic (Watson *et al.*, 1987). Transposon were also found in bacteria. Most of the transposons found in bacteria carry antibiotics resistance gene beside ones essential for the process of transposition. Transposons have the inverted repeats at both end and transpose at high frequency with little target sequence specificity.

The transposition of the transposon inactivates the target loci and causes the mutant phenotype. Such behaviour of the transposons are very useful features for cloning the genes. Transposon used in our study was transposon 5 (Tn5). Tn5 is a composite element in which inverted repeats of a separate mobile insertion element called IS50 bracket a segment containing gene for kanamycine resistance (Sasakawa *et al.*, 1987).

We also used the T-DNA tagging method in the study of Eucaryotic in this case in the isolation of gene resistant to citrus greening disease. The T-DNA used is the left border (LB) and right border (RB) of T-DNA bracket a segment containing genes for kanamycine and b-glucuronidase (GUS). This segment was cloned in a plasmid vector resulted in pBI121 plasmid (Jefferson *et al.*, 1987).

In the study of the isolation of a gene from *Agrobacterium tumefcaciens*, Tn5 cound be inserted into Ti plasmid or into chromosome. In the study of the isolation of a gene from citrus plant the T-DNA could be inserted into chromosomes, mitochondria, and chroloplast. The features of Tn5 and T-DNA tagging are the same which can inactivates the gene in where they are inserted.

Isolation of a choromosomal virulent gene from A. tumefaciens.

A. tumefaciens is a Gram negative bacteria which cause a neoplastic disease called crown gall disease. Crown gall tumor is the undifferentiated growth of plant tissue resulting from the interaction between susceptible plants and virulence strains of *A. tumefaciens*. The tumorous tranformation of crown gall persists in the absence of insiting bacteria (Braun and Mendel, 1948). *A. tumefaciens* contain a large plasmid called Ti-plasmid.

A Ti plasmid is responsible for oncogenicity of *A. tumefaciens*. However, the Ti plasmid alone is not sufficient to cause transformation of plants. The genes on chromosome of *A. tumefaciens* also contributes other function necessary for *in planta* oncogenicity (Powell and Gordon, 1989). Ti plasmid contain two main regions called T-DNA and virulence region (vir-region). Although T-DNA is mobile element, it is not a classical transposable element, since it does not itself encode the products that mediate its movement. Instead, a second component, the virulence (vir) region on Ti plasmid provides most of the *trans*-acting products for T-DNA transit.

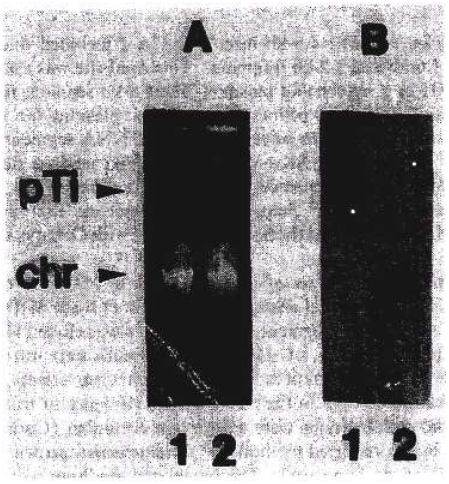

Fig. 1 The Cellular Localization of Tn5 in Mutants of *A. tumefaciens.*The plasmid and chromosomal DNA were prepared from two avirulent mutants (lanes 1 and 2) and electrophoresed on an agarose gel. The electrophoresed gel was stained with ethidium bromide (panel A) and then blotted onto a nylon membrane. The membrane was hybridized with ^{32}P-labeled IS50 probe (probe 1, panel B). pTi, Ti-plasmid DNA; chr, chromosomal DNA.

In the study of the isolation of a chromosomal virulent gene of *A. tumefaciens*, the Tn5 was mobilized into *A. tumefaciens* by triparenal matting method. Tn5 was cloned in the plasmid pJB4J1 which a suicide plasmid (Kang *et al.*, 1992 and Wirawan *et al*., 1993). The Tn5 could be transformed into Ti plasmid or into chromosome. In the isolation of chromosomal virulent gene, we selected the insertion of Tn5 in the chromosome. Five thousand transconyugants were isolated and assayed for virulence on carrot root disks and two avirulent mutants designated as B90 and B119 strains were isolated (Kang *et al.,* 1992 and Wirawan *et al.*, 1993). The B90 and B119 strains have a Tn5 insertion in their chromosomes (Fig. 1). Wild type target DNA segment of B90 and B119 strains were cloned and sequenced. An open reading frame was identified in each of target DNA segment of B90 and B119 strain and designated as *acvI* and *acvB*, respectively. Homology search of both genes found no homologous gene, thus indicating that both genes are new chromosomal virulent genes. The acvB gene is known as the first chromosomal virulent gene of *A. tumefaciens* that play an importance role in the crown gall induction by binding in the single stranded T-DNA (T-complex) and transfer it into

host plant cell. These results suggest that Tn5 was an effective tool for isolation of a gene from Gram negative bacteria, such as *A. tumefaciens*.

Isolation of a Gene Resistant for Citrus Greening Disease

By using the *A. tumefaciens* that contain the pBI121 plasmid which harbouring the LB and RB of the T-DNA and bracket a segment containing genes for kanamycine resistance and □-glucuronidase (GUS) we transformed the *Triphacia trifolia* L., a citrus that is known to be resistant to citrus greening disease or in Indonesia is well known as Citrus Vein Phloem Degeneration (CVPD). The transformation were conducted through *in vitro* and *in planta*. The plantlets of *T. trifolia* were inoculated by *A. tumefaciens* LBA4404 harbouring both pAL4404 and pBI121 plasmids and culture on MTOK and LS media which contain kanamycine for the selection of the transformants. On the other hand, the *in planta* transformation was conducted by inoculation of a *T. trifolia* plants or seedling resulted in a chimera phenotype. Three thousand of the transformants were isolated and then subjected to the assayed for susceptible to citrus greening disease. One succeptible tranformants was isolated and the DNA fragment of T-DNA insertion was identified and isolated (Wirawan *et al*., 2004). Wild type target DNA was then isolated and sequenced. One ORF was identified and homology search found no homologous to other genes, indicating that this ORF was a new gene that responsible for resistance to citrus greening disease, and designated a CVPDr gene (Fig. 2).

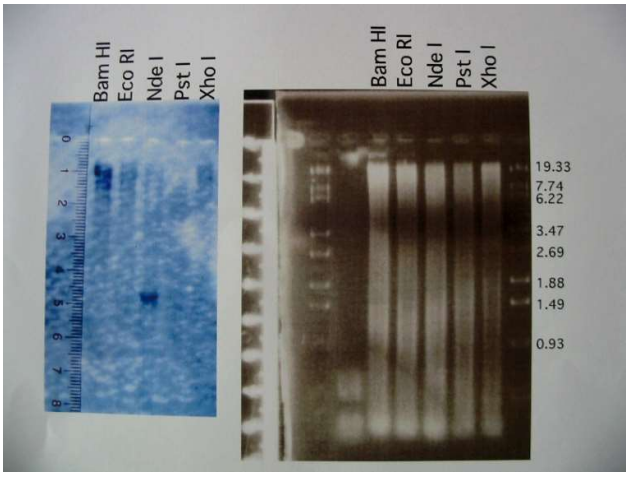

Fig. 2. Identification and isolation of a wild type target DNA from *T. trifolia* by using various restriction enzymes and the subjected agarose electrophoresis and Southern Blotted using a flanking DNA as a probe.

The overexpression of CVPDr gene was also done (Fig. 3). The overexpression protein of CVPDr gene can be used for formulating substances for growth hormon and control the citrus greening disease as well. The results suggest that the pBI121 plasmid containing the fragment of RB and LB of the T-DNA and bracket a segment of kanamycine resistant gene and the GUS gene was an effective plasmid for isolation of a gene from citrus plant.

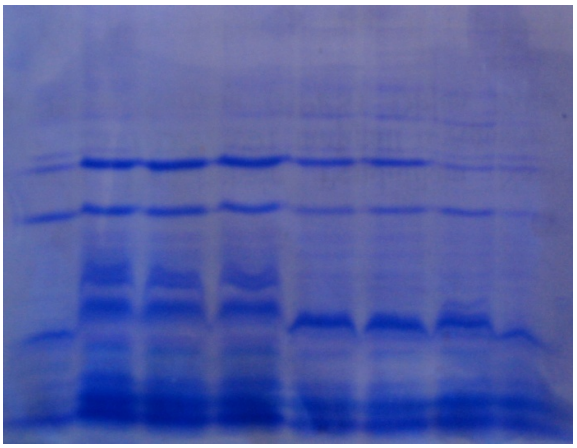

Fig. 3. An Overexpression of cloned CVPDr gene in *Escherichia coli*

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WHY ARE THE NETWORKS FOREST ECOSYSTEM? FROM THE BIOLOGY OF *ARMILLARIA* AND *TERMITOMYCES*

Jooyoung Cha[1] and IGP Wirawan[2]
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ABSTRACT

In 1992, the prestigious journal Nature reported that the largest and oldest organism that is alive on earth is *Armillaria*. It is neither a plant nor an animal. Then, what is the secretive life style that makes it possible to have 1,500 years of longevity? How big is it? Also, I reported the relationship that *Armillaria* and other fungi have with other living creatures like plants and animals in the forest. *Armillaria* has an important role of the network formation in the forest ecosystems. And then, I will introduce termites that cultivate mushrooms, *Termitomyces.* It had mentioned from the previous study that mushrooms grow on dead and wasted comb. But that is obviously misleading. Based on the findings from our study, we can conclude that the comb that grows mushrooms are used and actively controlled by termites.

ONLINE SIMULATION OF BIOPROCESSES

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SUMMARY

Modeling and simulation is a widely accepted tool for bioprocess optimization. The mathematical description of the reactor and the biology assisted the engineering process in a wide range of applications, for example in plant development and control (Heinzle et al. 2007). Thus the theoretical analysis offers a lot of insight into the biological behavior and helps to drive the bioprocess in a more efficient state (Sarwari et al., 2009). Normally the numerical calculation will be done in an offline fashion. That means, the output is a complete time-trajectory of the simulated bioprocess without direct coupling to the process.
Running the simulation in parallel to the bioprocess (in real-time) enables the wide field of online process diagnosis, state prediction and optimization (Fig. 1). A common example is a Kalman-Filter based approach for the estimation of biomass and product concentration in a fed-batch fermentation (Zhang, 2009).

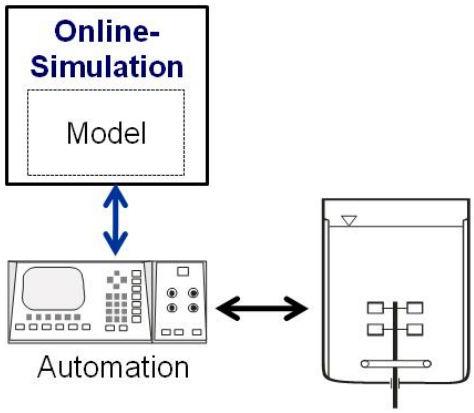

Fig. 1 Software-sensors and state estimation: Online-simulation is running in parallel to the real process.

The substitution of the real process by a dynamic model leads to another common application of online-simulation.

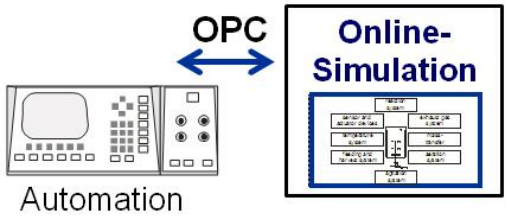

Fig. 2 Hardware in the loop approach: Real process is replaces by a simulation.

This hardware in the loop technique is a well suited tool for the development of automation systems. One application is the tuning of low level control loops, such as temperature control, were the optimal PID parameter could be obtained by simulation.

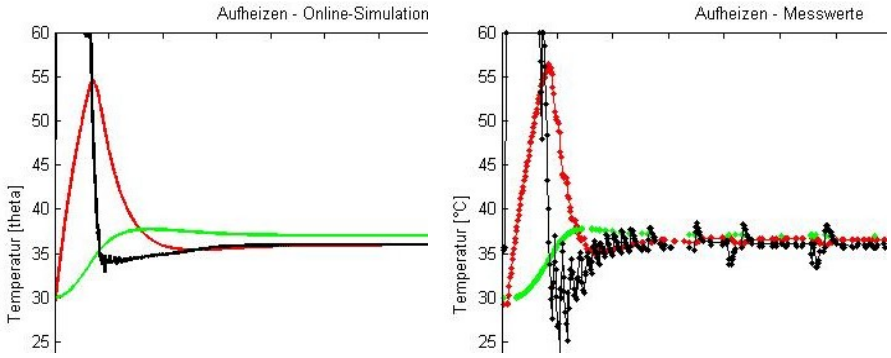

Fig.3. Hardware in the loop approach. Simulation results (top) and real process (bottom).

Replacing the complete system by a virtual bioreactor, which is a full-scope model of the bioprocess including the reactor periphery, allows for a new type of application (Fig. 4).

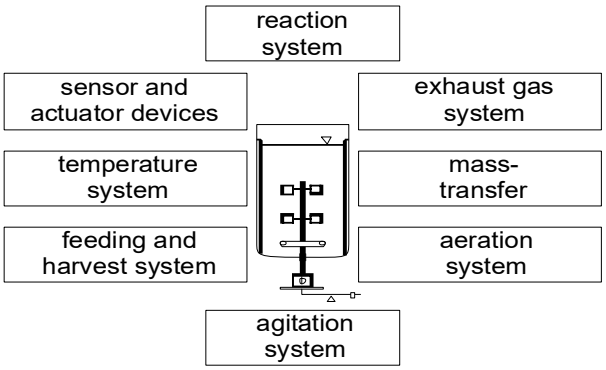

Fig. 4. Subsystems of a full-scope model.

Such a tool could be very helpful for student education (Hass and Pörtner, 2009), operator-training and during the development of process strategies (Luttman and Gollmer, 2000). The virtual bioreactor acts like a "flight-simulator" and enables the user to grasp the complex impacts of the applied control actions (Fig. 5).

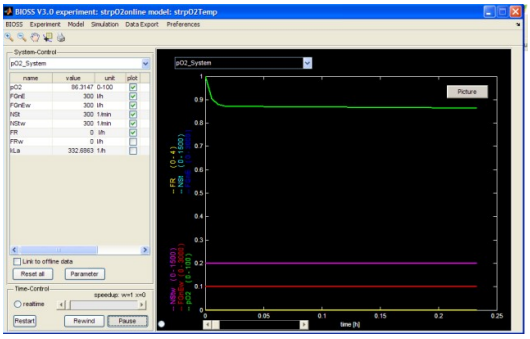

Fig. 5. Simulation of a virtual bioreactor.

Due to rewind and fast-mode operation the trained scenarios could be repeated as long as the suggested process output is reached. Thus the virtual bioreactor prevents the real hardware from damage, saves a lot of time and natural resources (media, energy, human power).

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ELICITORS INDUCING PLANT DEFENSE RESPONSES

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ABSTRACT

In plants, reactive oxygen species (ROS) and nitric oxide (NO) play crucial roles in the regulation of various physiological processes. ROS and NO have been also shown to be an important messenger in plant defense signaling against microbial pathogens. They participate in the induction of the hypersensitive response (HR), defense gene expression and production of antimicrobial compounds (phytoalexins). Elicitors are defined as compounds produced by pathogens which induce defense responses in host plants. They include a wide variety of molecules, such as peptides, glycoproteins, carbohydrates, fatty acids, lipids, and extracellular microbial enzymes. However, current knowledge on elicitors triggering ROS and/or NO production in host plants is still limited. First, we isolated a ceramide elicitor from *Phytophthora infestans* which activates O_2^- production of potato suspension-cultured cells. Secondly, we employed nine ceramide-related chemicals to test their elicitor activity. Although, none of the tested chemicals induced O_2^- production, *N*,*N*-dimethylsphingosine (DMS) induced accumulation of phytoalexin in potato tubers. In potato, tobacco and *Nicotiana benthamiana*, DMS also induced rapid cell death. Thirdly, we screened synthetic chemicals with NO producing activity. Two compounds induced NO generation in potato suspension cultured cells, potato leaves and *N. benthamiana* leaves. O_2^- generation by these compounds was also observed in potato suspension cultured cells, while phytoalexin production was not detected in potato tubers. They induced hypersensitive cell death in potato suspension cultured cells and the expression of NO production-related genes in *N. benthamiana* leaves.

Keywords: Defense response, Elicitor, Nitric oxide, *Nicotiana benthamiana, Phytophthora infestans*, Potato, Reactive oxygen species

I. Reactive oxygen species and reactive nitrogen species

Plants defend themselves against pathogen attack through various reactions. Reactive oxygen species (ROS), such as superoxide (O_2^-) trigger plant defense responses. The rapid production of O_2^- (oxidative burst) can inhibit pathogen infection, by inducing various other defense responses including direct microbicidal action (Peng and Kuc 1992), restriction of pathogen penetration via cross-linking of cell wall glycoproteins (Bradley et al. 1992), induction of phytoalexin accumulation or defense-related genes (Apostol et al. 1989, Desikan et al. 2001), or activating salicylic acid biosynthesis (Leon et al. 1995). However, several reports state that although O_2^- is essential, it is not the only component required for defense responses. O_2^- alone cannot induce hypersensitive responses (HR) and hypersensitive cell death (HCD) in plants. We focused on nitric oxide (NO) as a collaborator of O_2^- in defense responses.

NO is a free radical gas that is involved in the intra- and intercellular signal transduction pathway (Stamler 1994) and it plays a prominent role in the activation of disease resistance and various defense-associated responses in plants. NO-donor treatment triggered the accumulation of phytoalexin (Noritake et al. 1996), the expression of several genes responsible for synthesis of defense-related metabolites (Durner et al. 1998, Yamamoto et al. 2003) and jasmonate accumulation (Wang and Wu 2005) in plants.

We pharmacologically characterized NO production of tobacco Bright Yellow-2 cells that followed treatments with INF1 preparations from *Escherichia coli*, the major secreted elicitin of the late blight pathogen *Phytophthora infestans* (Yamamoto et al. 2004). NO production rapidly occurred within 1 h and reached maximum level 3 h after

the addition of INF1. Using carboxy-PTIO, a NO specific scavenger, we investigated the relationships between NO generation and several defense responses induced by INF1. Carboxy-PTIO suppressed the activation of a 41-kD protein kinase and cell death by INF1 completely. Moreover, carboxy-PTIO also suppressed the induction of hypersensitive-related (hsr) genes, *HSR515* and *HSR203J*, of which expressions are strongly correlated with hypersensitive response in plants. Our results suggest that NO plays a crucial role in induction of hypersensitive cell death.

O_2^- and NO are generated simultaneously in defense responses (Kozak et al. 2005) and react with each other very rapidly to form peroxynitrite ($ONOO^-$) without requiring enzymatic catalysis and hypothetically resulting in the mobility of NO and redox activity of O_2^- (Padmaja and Huie 1993). $ONOO^-$ has a strong nitration activity for tyrosine residues. Tyrosine nitration is one of the $ONOO^-$ specific and direct reactions.

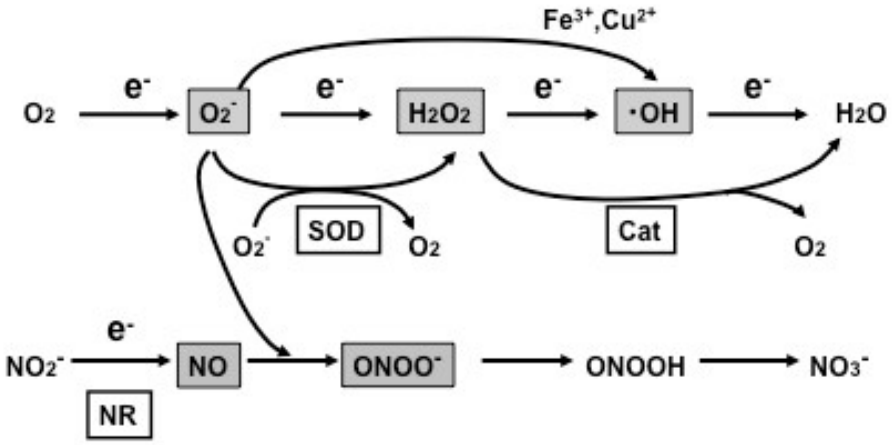

Fig. 1 Reactive oxygen species and reactive nitrogen species. SOD, superoxide dismutase; Cat, catalase; NR, nitrate reductase.

II. Elicitors inducing disease resistance reactions

Plants have the ability to recognize molecules derived from potential pathogens to induce various disease resistances. Surface-derived structural molecules from plant pathogens, such as fungal cell wall constituents (chitin, glucan, protein and glycoprotein) and bacterial lipopolysaccharide (LPS) and flagellin, elicit defense responses in a wide range of plant species. Such nonspecific elicitors are generally conserved structural components of microorganisms termed microorganism-associated molecular patterns (MAMPs) or pathogen-associated molecular patterns (PAMPs), which are recognized as a result of the innate immunity of plants (Jones and Takemoto 2004). Another group of elicitors are recognized by specific range of plant species or cultivars. Originally, specific elicitors were named avirulence (Avr) proteins because they were identified as determinants of avirulence of plant pathogens, which now are commonly called "effectors" because they are very often also virulence factors for pathogens during the interactions with susceptible plants (Greenshields and Jones 2008).

Both non-specific and specific elicitors generally induce a similar set of disease resistance reactions (Mysore and Ryu 2004). Production of ROS, a hallmark of plant resistance reactions, is involved in the induction of resistance reactions such as the expression of defense genes, accumulation of phytoalexins and induction of

hypersensitive cell death. Because the ROS produced by animal phagocytes is microbiocidal to attacking pathogens (Klebanoff 2005), the ROS produced by plant cells after challenge by pathogens was initially considered to be a toxic agent that inhibits the growth of microorganisms. However, recently emerging evidence indicates that ROS is a universal second messenger of plant signaling for various physiological activities including the induction of disease resistance (Torres et al. 2006).

One such disease resistance mechanism is hypersensitive cell death, a form of plant programmed cell death that limits the attacking pathogen to the infection site. The processes that induce programmed plant cell death are largely unknown, in contrast to well-studied programmed animal cell death, but animal and plants share many of the common morphological and biochemical changes, such as chromatin condensation, transition of mitochondrial permeability and DNA fragmentation (Lam et al. 2001). Recent reports also suggest that programmed cell death in plants and animals in regulated by similar mechanisms. Caspases are a family of cysteine proteases essential for the induction of programmed cell death in animal cells (Cohen 1997). Although caspase activity was detected in plant cells, no orthologous sequence of animal caspases has been found in plant genomes (Bonneau et al. 2008). Hatsugai et al. (2004) identified vacuolar processing enzyme (VPE) as a caspase of plant that is essential for TMV-induced hypersensitive cell death. Bax is a mammalian pro-apoptotic protein that has been shown to induce programmed cell death of plant cells when overexpressed in plant cells (Kawai-Yamada et al. 2001). While there is no obvious homologue of the *Bax* gene in the plant genome, there is a homologous gene for Bax inhibitor (*BI-1*), which can inhibit Bax activity, H_2O_2 generation and salicylic acid-induced cell death (Kawai-Yamada et al. 2004). These reports strongly suggest that machineries for inducing programmed cell death are evolutionally conserved between animal and plant systems even though sequences of genes for the factors involved are not necessarily conserved.

III. Elicitor inducing hypersensitive cell death and phytoalexin accumulation

Hyphal wall components (HWC) elicitor is a crude fraction extracted from mycelia of *Phytophthora infestans*, which induces various defense responses of potato, such as ROS production, expression of defense genes, phytoalexin accumulation and hypersensitive cell death. We aimed to purify elicitors of *P. infestans*, and identified a ceramide-related compound as an elicitor, which induced ROS production of suspension-cultured potato cells (unpublished data). We tested commercially available ceramide-related chemicals, including C2-ceramide, C2-dihydroceramide, C6-ceramide, C8-ceramide, C18-ceramide, *N*,*N*-dimethylsphingosine (DMS) and sphingosine-1-phosphate (Fig. 2) for their activity as elicitor of resistance reactions (Uruma et al. 2009).

ROS production

Five-day-old suspension-cultured potato cells were treated with water, HWC or ceramide-related chemicals, and ROS production of potato cells was analyzed 3 h after the treatment of elicitors. Only HWC elicitor had strong activity in inducing ROS production whereas none of the ceramide-related chemicals were active for ROS production of potato cells.

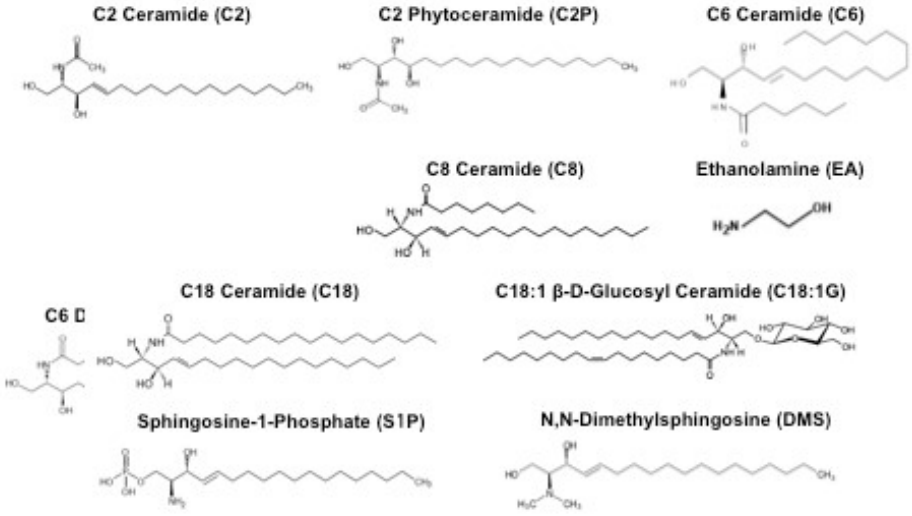

Fig. 2 Structure of ceramide-related chemicals used in this study

Phytoalexin accumulation

Potato tubers were treated with water, HWC or ceramide-related chemicals and accumulation of the potato phytoalexin, rishitin, was analyzed by TLC. Twenty-four hours after the treatment, HWC induced production of rishitin. Of the tested ceramide-related chemicals, only DMS was active in eliciting phytoalexin production.

Induction of HR-like programmed cell death

Cell viability of suspension-cultured potato cells was investigated after treatment with either HWC elicitor or DMS using Evans blue staining. Treatment with 50 to 100 μM DMS induced death of potato cells within 1 h, and at 6 h after the DMS treatment at 100 μM, cell death was six-fold higher than that of HWC-treated cells. Within 1 day after the treatment with HWC, treated area of potato tubers developed browning, inductive of hypersensitive cell death, and DMS-treated potato tubers also developed dead cells. Chromatin condensation paralleled by DNA fragmentation is one of the most important criteria to identify apoptotic cells. To investigate the nature of cell death induced by DMS, chromatin condensation and DNA fragmentation of DMS-treated potato cells was investigated. Nuclear DNA of potato cells was visualized by DAPI staining. Treatment with 100 μM DMS induced chromatin condensation. Genomic DNA was isolated from potato cells 3 h after treatment with the elicitors, and DNA degradation was monitored. Six hours after treatment with 100 μM DMS, smearing of genomic DNA was obvious and laddering of DNA was detected in DMS-treated cells. Altogether, treatment with HWC and DMS induced chromatin condensation of potato cells paralleled by fragmentation of genomic DNA, indicating that DMS can induce HR-like programmed cell death. DMS also showed activity to induce HR-like cell death of *Nicotiana* species without ROS production.

DMS is perhaps not recognized as an exogenous elicitor but acts later in the signaling pathway, inside the plant cell, leading to the induction of defense reactions.

IV. Elicitor with NO producing activity

Present information on the role of NO in plants is still limited by the lack of experiments based on manipulation of endogenous NO levels, and experimental strategies still rely almost entirely on the use of NO-donors and NO-scavengers. In this study, we screened synthetic chemicals with NO producing activity.

Among them, two compounds, namely Compounds 2 and 3, potentially have NO producing activity as elicitor. NO and ROS producing activities of these chemicals were measured by using potato and tobacco (*Nicotiana benthamiana*) plants. Induction of cell death, expression of defense-related genes and resistance inducing activity against pathogen infection were also investigated.

NO generation

NO producing activities of Compounds 2 and 3 were investigated in potato suspension cultured cells (cv. Sayaka), potato leaves and *N. benthamiana* leaves. NO sensitive fluorophore DAF-2DA in conjugation with fluorescence spectrophotometer or fluorescence stereomicroscope was used to detect NO generation. Compounds 2 and 3 induced NO in potato suspension cultured cells and carboxy-PTIO decreased the amounts of NO production.

O_2^- production in potato suspension cultured cells

O_2^- producing activity of potato suspension cultured cells was measured by treating with Compounds 2 and 3 using O_2^- unique luminous reagent L-012 in a plate reader. Compounds 2 and 3 generated O_2^- at 3 h after treatment.

Cell death induction in potato suspension cultured cells

Hypersensitive cell death inducing ability of Compounds 2 and 3 in potato suspension cultured cells was investigated using Evans blue staining. After 3 h treatment of the cells by Compounds, cell death was increased.

Phytoalexin producing activity on potato tuber

Phytoalexin producing activity of potato tuber was measured by treating with Compounds 2 and 3, using potato cultivar Sayaka. The compounds did not produce phytoalexin in potato tuber 48 h after treatment.

Resistance induction in potato and *N. benthamiana* leaves

To examine whether the NO generating compounds induce resistance in potato and *N. benthamiana* against *P. infestans*, leaves of potato and *N. benthamiana* were infiltrated by Compounds 2 and 3. Compound 2 and 3 induced moderate resistance against pathogen in compare to 0.02 % DMSO infiltrated leaves.

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THE CONTROL OF GONADOTROPIN-RELEASING HORMONE (GNRH) IN MAMMALS: A WAY TO IMPROVE THE FERTILITY IN DOMESTIC ANIMALS

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INTRODUCTION

Anovulation or anestrus is one of the major constrains in the animal production, especially in the milking cow. Ovarian activities are inhibited during lactation in most mammalian species, because the secretion of gonadotropins, especially luteinizing hormone (LH), are profoundly suppressed during lactation (McNeilly, 2001). The cause of the gonadal suppression during lactation has been a target for the animal scientists and intensively investigated. Epidemiological studies suggest that high milk yield is related to the lactational anestrus in milking cows (Stevenson and Britt, 1979) and thus the negative energy balance of a milking cow could be a cause for the gonadotropin secretion.

From the lactating rat model, we found that there might be two mechanisms mediating the suppression of LH secretion during lactation (Tsukamura and Maeda, 2001). In the first half of the lactation, the suckling stimulus suppresses LH secretion through a direct neural pathway, hypothalamic deafferentation, especially a roof deafferentation, quickly restores the suppressed pulsatile LH release in lactating rats (Tsukamura et al., 1990). On the other hand, the suckling stimulus could suppress LH secretion through another pathway in the second half of lactation.

In the present article, we describe the mechanism mediating the energy deficiency-induced suppression of gonadal activity in rats to serve as a model for the anestrus during lactation in milking cows. The mechanism is divided into 3 parts, such as signals, sensors and pathways to simplify the structure of the issue. For instance, the signals involved in the suppression should be identified to develop an animal feed or drug for the therapy of the reproductive disorders during malnutrition. We, thus, believe that the mechanism obtained from the experimental animal models would be quite useful for the solution of the problems emerging in farm animals, such as milking cows.

Signals, sensors and pathways for the energetic control of reproduction

Pathways

Experimentally, energy deficiency, such as fasting, suppresses gonadal activity through inhibiting gonadotropin release in hamsters, rats and sheep. Fasting is one of the good models to look for the neuroendocrine pathway mediating gonadal suppression during malnutrition, because 48-h fasting profoundly suppresses estrous cyclicity through the inhibition of gonadotropin secretion in female rats (Cagampang et al., 1991). It should be noted that fasting disrupts estrous cyclicity by inhibiting tonic (pulsatile) GnRH/LH release and then follicular development, but not by inhibiting GnRH/LH surges or ovulation.

The 48-h fasting suppresses gonadotropin secretion through a specific neuroendocrine pathway, suggesting that the fasting-induced suppression of gonadotropin secretion is a physiologic response to the environmental pressure endangering the existence of an individual (Maeda and Tsukamura, 1996). The neuroendocrine pathway involves the afferent vagus nerve, emanating from the upper digestive tract and projecting to the solitary tract nucleus (NTS), especially the noradrenergic neuron in the A2 region. The pathway also involves the noradrenergic input to the hypothalamic paraventricular

nucleus (PVN), which finally suppresses GnRH release through corticotropin-releasing hormone (CRH).

Interestingly, the fasting-induced inhibition of gonadotropin secretion is estrogen-dependent, because ovariectomized rats does not show any suppression of gonadotropin after 48-h fasting but estrogen replacement restores the suppression (Nagatani et al., 1994). The action site of estrogen has been identified to be the PVN and A2 region of the NTS, which correspond to the nuclei of origin and projection of the noradrenergic neuron (Maeda et al., 1996).

Glucose and fatty acid as a positive energy signal controlling reproduction

There have been a numerous number of studies identify peripheral signals relaying the nutritional information to the brain to control the food intake as well as reproduction (Wade et al., 1996). Sensors for the signals are also important to be identified for the application of the mechanism.

Glucose is the first and most prominent candidate for the signal regulating food intake and reproduction, because the inhibition of glycolysis (glucoprivation) profoundly stimulates feeding (Smith and Epstein, 1969) and suppresses gonadotropin secretion (Bucholtz et al., 1996; Funston et al., 1995). The localization of the sensors has been under debate because there are several inconsistent findings. Oomura et al. found neurons responding to the change in extracellular glucose concentrations in the ventromedial hypothalamic nucleus (called satiety center) and lateral hypothalamus (called feeding center) and suggested that the neurons are the part of the glucose sensor controlling feeding behavior (Oomura et al., 1992). On the other hand, Ritter et al. found that 5-thioglucose, a glucose antagonist, stimulates the feeding behavior by acting on the hindbrain but not forebrain (Ritter et al., 1981).

We have found that a small amount of 2-deoxyglucose (2DG) injected into the hindbrain causes an increase in food intake and suppression of luteinizing hormone (LH) release in male rats (Murahashi et al., 1996), suggesting that glucose is positive energy signal to induce satiety and maintain the normal gonadal functions. In addition, immunohistochemical study demonstrated that glucokinase (hyxokinase type IX), an enzyme for glucose sensing in the pancreatic B cell, is found in ependymocytes lining the wall of the fourth cerebroventricle in rats (Maekawa et al., 2000). The ependymocyte taken from the hindbrain can respond to the change in extracellular glucose concentration *in vitro* (Moriyama et al., 2004). These finding suggest that the ependymocyte is a glucose sensor for the regulation of feeding and reproductive functions.

Free fatty acid would also be a positive signal to regulate reproductive functions. Peripheral administration of mercaptoacetate (MA), a inhibitor of the b-oxidation of the palmitate, a long-chain fatty acid, caused a marked inhibition of pulsatile LH secretion in rats (Sajapitak et al., 2008). The action site of the MA would be the hindbrain, because the administration of MA into the fourth cerebroventricle suppresses pulsatile LH secretion in female rats. The target cell of the MA in the hindbrain would most probably the ependymocyte, because palmitate causes an in vitro increase in intracellular calcium concentration in the ependymocyte taken from the fourth cerebroventricle (unpublished observation).

Ketone body as a negative energy signal controlling feeding and reproduction

Ketone bodies are the byproducts of the b-oxidation of free-fatty acids. Ketosis is found to be one of the major symptom during energy deficiency, such as fasting, diabetes and lactation, which accompany the increase in food intake and suppression of reproductive functions. Ketone bodies, therefore, could be a negative energy signal for feeding and reproduction.

The injection of ketone body into the fourth cerebroventricle profoundly suppresses pulsatile LH secretion (unpublished observation). The suppression is mediated

by a specific neuroendocrine pathway involving noradrenergic inputs to the PVN, because the blockade of catecholamine synthesis in the PVN negates the effect of ketone body on LH secretion (unpublished data). We explored the possibility that ketone body sensing at the ependymocyte is involved in diabetic ketosis-induced increase in food intake. For this end, monocarboxylate transporter 1 (MCT1), a ketone body transporter, was inhibited by a specific inhibitor. Food intake was dramatically increased by streptozotocyn-induced type 1 diabetes in rats. In this model, plasma ketone body concentrations are kept at a high level and positively correlated with the level of cerebrospinal fluid. When the MCT1 inhibitor is injected into the fourth cerebroventricle, food intake is restored to the normal level. The result strongly suggests that ketosis is a primary cause for the diabetic hyperphagia. Thus, the MCT1 blockade would be a therapy for various disprders under ketosis.

CONCLUSION

We have found that the noradrenergic input to the PVN from the NTS is the common pathway mediating LH suppression during malnutrition. In addition, glucose, fatty acids and ketone bodies would be signals, which sensed by the hindbrain ependymocyte to control feeding and reproduction. Of these signals, ketone bodies are most likely to serve as a negative energy signal to induce hunger responses, such as suppression of reproduction and stimulation of feeding behavior.

We, therefore, propose that anti-ketogenic drugs or feeds would be a possibility for the therapy of lactatinal anestrus in milking cows.

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CONSERVATION ACTIVITIES OF AN ENDANGERED ANIMAL

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ABSTRACT

The Bali starling (*Leucopsar rothschildi*) is endemic bird of Bali islands, assigned as critical endangered species. Several conservation activities for the bird had been carried out. However, any wild birds could not be observed at 2006. Newly reintroduction program started at 2007. More then one hundred captive birds were released in Bari barat national park where is known as the last natural habitat of the bird. As a result, the wild bird increased, and the numbers of wild bird were about fifty in 2009. And surprisingly, breeding among released birds also succeeded. Preservation and research center, city of Yokohama, where breeds over one hundred captive Bali starling, cooperated this reintroduction program thorough supplying captive Bali starling, development of educational program for local conservation activity, and cooperating DNA analysis of the bird. These supports will be continued until the wild population recovers perfectly.

BACKGROUND

The Bali starling (*Leucopsar rothschildi*) is one of the most endangered animals in the world (Fig.1). The bird is endemic species of the island of Bali. In past, its wild population size was thought to be about 300 to 900. However, any wild bird could not be observed in 2006. Therefore, this bird was considered critical endangered animal by International Union for Conservation Nature (IUCN). Habitat destruction and illegal hunting is thought to be the cause of its decline (van Balen et al., 2000). On the other hand, there were more than 1,000 captive birds in the world. Therefore, several reintroduction programs were carried out, although the number of wild bird did not increase. Then, the number of captive Bali starling in Japan was over one hundred in 2003. Based on the situation, we started to support reintroduction activities in Indonesia.

Fig.1 *Leucopsar rothschildi*

SUPPORTING RE-INTRODUCTION ACTIVITIES

1. Supplement of captive birds

We entered into a MOU with Ministry of forestry, republic of Indonesia about supplement of one hundred captive birds from Yokohama to Indonesia. We had already finished it. These birds were bred in several Indonesian captivities, and some of them were released into the wild. In addition, we tried to support reintroduction activities in Indonesia as follows. Following works were supported by Japan International Cooperation Agency (JICA).

2. Stopping illegal hunting

In order to stop illegal hunting of the wild Bali starling, we asked Ministry of forestry to organize a union for conservation of Bali starling. Asosiasi Pelestari Curik Bali (APCB) was organized at 2005 for conservation of the bird. We supplied microchip to APCB for supporting registration of the Indonesian captive Bali starling. Most of the captive Bali starlings were attached microchip.

3. Monitoring technique

Figure 2. The use the radio transmitter

In 2007 and 2009, about one hundred birds were released into Bali barat national park, where is the natural habitat of the bird. According to the guide line of reintroduction, reintroduction programs should be modified based on the result of monitoring after releasing (IUCN 1995). Therefore, monitoring of the releasing birds is essential study to succeed the reintroduction program. We tried to advise the technique of monitoring to the staff of national park.

Firstly, we told the way of daily observation using the binoculars, which is the most basic and the simplest technique. This observation will be able to appear the habitat range of wild birds, which is essential information for reintroduction program. Next, we tried to use the radio transmitter, which is one of most popular technique of monitoring (Fig.2). This method is usually used for large size birds such as eagle. So, this was the first trial for small bird. But unfortunately, we could not get a good result. Therefore, we have to continue supporting radio tracking technique.

4. Recovery of natural habitat

The main cause of wild Bali starling decline is thought to be habitat destruction. Natural habitat of the bird was changed to coconut and kapok plantation and human settlements (van Balen et al., 2000) (Fig.3). These developments resulted in habitat fragmentation of the bird. Such fragmentation accelerated population decline, which is well known as Allee effect (Allee, 1949). Therefore, in order to succeed the reintroduction program, recovery of natural habitat is very important. We support the recovery program conducted by local organizations through supplement of some educational book, and developing educational program based on Japanese local culture (Fig.4).

Figure 3

Figure 4

5. Genetic analysis of Bali starling

According to the IUCN guideline, all reintroduction programs of endangered species should be conducted to maintain their genetic diversity. Maintaining genetic diversity is same as preventing inbreeding. The bad effects of inbreeding are well known. In order to maintain genetic diversity of Bali starling, we tried to clarify the genetic diversity using some DNA markers. Firstly, we attempted to the RAPD analysis which is easy and simple technique of population genetics, using the Japanese captive population. However, we could not clarify the genetic variation of them. Next, we tried to analyze DNA sequences from mitochondrial D-loop region, where is thought to be one of the most variable region in many vertebrate species. We used six birds having different captive

maternal origins, respectively. We, however, could not appear any DNA variations from them. Since the above results indicated there is maybe no genetic diversity existed in Japanese population, we tried to analyze DNA variation of the bird using the Indonesian captive individuals. For this study, we cooperated with Prof. I G P Wirawan, Prof. I G Ngurah K Mahardika and Dr. Sari nindhia. This work is in progress. Additionally, we tried to develop new primers for microsatellite DNA of Bali starling. Analysis of microsatellite DNA polymorphism is a popular technique for population genetics although there was no information about microsatellite DNA sequences of Bali starling. We could get 13 primer sets of microsatellite DNA of the bird, and conducted its polymorphic analysis. This work also is in progress.

CONCLUSION

Fortunately, the number of wild Bali starling in Bari barat national park increased, and the breeding among releasing bird succeeded. In order to recover of the wild birds in the future, we should continue reintroduction activities cooperating with many counterparts not only in Indonesia, also in other countries having a lot of captive Bali starling.

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CELL WALL DEGRADATION AND MODIFICATION ENZYMES OF GRAM-POSITIVE BACTERIA: HISTORY, IMPORTANCE AND FUTURE ASPECTS

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ABSTRACT

Gram-positive bacteria contains more than 10 cell wall hydrolase genes, and the spore-forming bacteria like *Bacillus subtilis* generally contains more than 30 genes. The cell wall hydrolases are associated with various cellular functions such as cell growth, cell separation, cell wall turnover, motility, cell lysis, infection, and differentiation (sporulation, and germination). These enzymes contain various substrate specificities. On the other hand, cell wall modification enzymes are also important for antibiotic sensitivity, and also sporulation and germination. In this presentation, I introduce various cellular functions of cell wall degradation and modification enzymes from *B. subtilis* and propose future aspects of these enzymes.

INTRODUCTION

Spore-forming bacteria were widely distributed in various environments and Bacillus subtilis is a model organism for spore-forming bacteria as well as Gram-positive strain. Until early '90, researches did not pay attention to autolytic enzymes and in 1990 we first reported the autolytic enzyme gene in B. subtilis [1]. Since 1995, whole genome sequencing of bacteria are developed and the whole genome sequence of B. subtilis was completed in 1997 [2]. The approach for investigation of autolytic enzymes was drastically changed, and Foster's group in University of Sheffield reported that 35 potential cell wall hydrolase genes are found in B. subtilis [3]. Recently autolytic enzymes are associated with various cellular functions. Since the name of autolytic enzyme is used for very limited function such as autolysis, here I would like to use cell wall degradation enzymes instead of autolysins. Moreover, cell wall degradation enzymes are also important for cellular functions, I will explain some new results found recently in our laboratory. Especially, novel cell wall hydrolase CwlQ from B. subtilis has one catalytic domain which exhibits two enzymatic activities (muramidase and lytic transglycosylase). PdaC is a polysaccharide deacetylase which could deacetylate chitin oligomer consisting of N-acetylglucosamine, but did not deacetylate N-acetylglucosamine but N-acetylmuramic acid in peptidoglycan. Two other peptidoglycan hydrolases associated with cell growth are also presented and discussed.

MATERIALS AND METHODS

Bacterial strains and culture conditions *B.subtilis* 168 and its derivatives were used in this study. Cells were cultured in LB medium or LB agar at 37°C. If necessary, antibiotics were added to the cultures. Escherichia coli was used as the host of enzyme production. Proteins were purified as histidine-tagged proteins by nickel-affinity column chromatography.

RESULTS AND DISCUSSION

Life cycle of *B. subtilis* associated with some cell wall degradation enzymes and the structure of peptidoglycan are shown in Figs. 1 and 2. Cell wall contains peptidoglycan (approximately 50 %) and the rests are anion polymers such as teichoic acid and teichuronic acid.

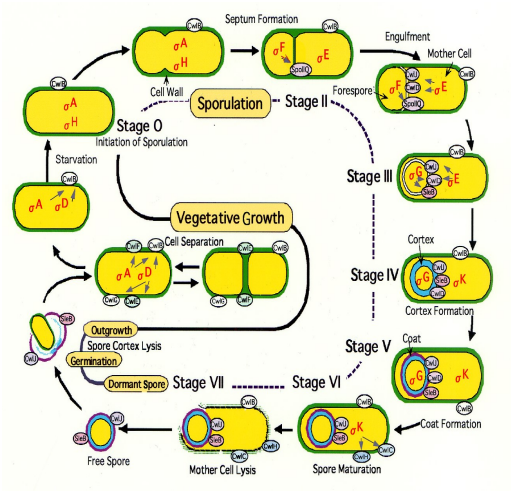

Fig. 1 Illustration of the life cycle of B. subtilis and association between cellular functions and some cell wall degradation enzymes. σ^A is a house-keeping sigma factor of RNA polymerase, and σ^F, σ^E, σ^G and σ^K are sporulation sigma factors.

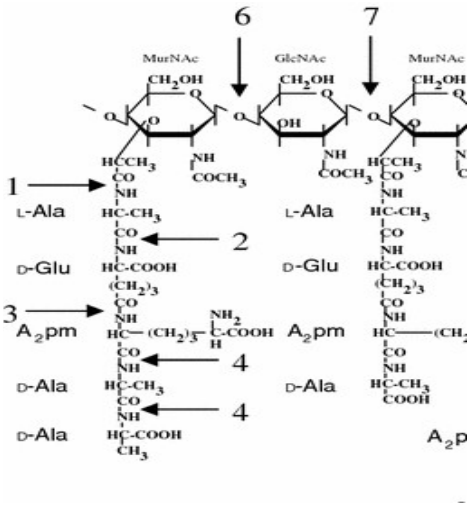

Fig. 2 Peptidoglycan structure of B. subtilis and the cleavage sites of cell wall lytic enzymes. Numbers 1 to 7 correspond to N-acetylmuramoyl-l-alanine amidase, ld-endopeptidase, dl-endopeptidase, carboxypeptidase, dd-endopeptidase, N-acetylmuramidase/lytic transglysosylase, and N-acetylglucosaminidase, respectively

Phage and tranposon-related cell wall degradation enzymes

This group contains XlyA and XlyB of PBSX phage, BlyA and CwlP of SPβ prophage and gp13 of phi29 phage. CwlA in the skin region may belong to this group.

Sporulation and germination -associated cell wall degradation enzymes

CwlC, CwlD, SpoIID, SpoIIP, SpoIIQ, CwlH, YaaH, LytH, SleB, CwlJ are associated with sporulation and/or germination. LytC (CwlA) is associated with both turnover of peptidoglycan during vegetative growth phase and mother cell lysis at the end of sporulation. CwlD is required for making mature spores [delta lactam formation of cortex (spore peptidoglycan)] [4].

Cell separation-associated cell wall degradation enzymes

LytE, LytF, CwlS, CwlO, and PgdS (YwtD) are in the dl-endopeptidase family by the amino acid sequence similarity. LytE and CwlS are cell separation enzymes, and LytE has two functions (cell separation and cell growth). IseA is a proteinaceous inhibitor of B. subtilis, and overproduction of IseA leads to filamentation from rod cells [5].

Cell growth-associated cell wall degradation enzymes

LytE and CwlO belong to this group. They exhibit synthetic lethal phenotype.

Other functions of cell wall degradation and modification enzymes

PgdS is a polyglutamic acid (PGA) hydolase, but did not digest peptidoglycans. In contrast, dl-endopeptidases (LytF, LytE, CwlS, and CwlO) had PGA degradation activity. CwlT is located in the Integrative and Conjugational Element (ICEBs1) and it may be associated with conjugation [6]. PdaA and PdaC are polysaccharide deacetylases. PdaA is related to biosynthesis of delta-lactam formation of cortex as well as CwlD, and PdaC is associated with lysozyme sensitivity.

Novel enzymatic properties of cell wall degradation and modification enzymes

The N-terminal catalytic domains of CwlT (CwlT-N) and CwlP (CwlP-N), and CwlQ exhibit high sequence similarities with E. coli soluble lytic transglycosylase (SLT) and goose lysozyme. CwlT-N and CwlP-N are not SLT but muramidase. Surprisingly CwlQ has one catalytic domain which exhibits bifunctional enzyme activities (muramidase and SLT activities) [7]. Among the polysaccharide deacetylases in B. subtilis, PdaA strictly recognizes the substrate $[\text{-GlcNAc-MurNAc-}]_n$, releasing the acetic acid from MurNAc, and it is a member of biosynthetic genes of spore cortex. PdaC deacetylates peptidoglycan and the target site is the deacetylation of MurNAc. MurNAc with a peptide stem (-l-Ala-d-Glu-) is a more preferable substrate.

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STRUCTURAL ANALYSIS OF THE PHOTOREACTIONS OF FLAVIN-BINDING PROTEINS BY FTIR SPECTROSCOPY

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ABSTRACT

Of photoreceptors in organisms, phototropin (Phot),cryptochrome (Cry) and BLUF-domain-containing proteins have flavin (FMN or FAD) as a chromopore. Interestingly, light-signal transductions in LOV domain of Phot,Cry and BLUF domain are initiated by different photoreactions, flavin-cysteine adduct formation, reduction and rearrangement of hydrogen-bonding network, respectively (Figure 1). My purpose of the research is to elucidate the control mechanisms of the photoreaciton of flavin. We have used light-induced difference FTIRspectroscopy in order to reveal molecular mechanism of photoreactions of flavin-binding photoreceptors. FTIR spectroscopy is a powerful method to obtain information as to structural changes of peptide backbone (from C=O stretching vibration), protonation states and hydrogen-bonding alteration of X−H groups (X = N, O, S), which are difficult to be obtainedfrom the X-ray crystallographic analysis. For example, we determined theprotonation states of the reactive cysteine in the unphotolyzed [1] and triplet-excited [2] states of LOV domain from the analysis of S-H stretching vibration. In this symposium, I will present our recent FTIR studies of flavin-binding photoreceptors andphotolyase (Phr). Phris a DNA repair protein by use of near UV light.Though physiological functions of Phrare entirely differentfrom those of Cry, Phrand Cry have the same protein architecture. Unique protein structural changes obtained by the FTIR analysis will be discussed in relation to their functions.

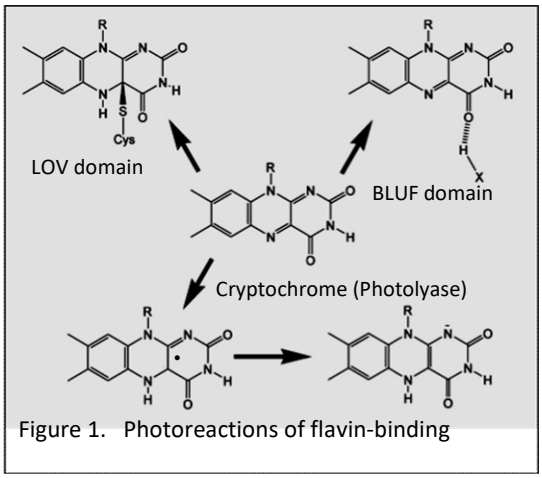

Figure 1. Photoreactions of flavin-binding

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ORAL PRESENTATIONS: AGRICULTURE

GROUND WATER USE EFFICIENCY BY MAIZE CROP UNDER DIFFERENT IRRIGATION TECHNIQUES[1]

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ABSTRACT

Ground water use efficiency for growing crops, especially in a dry climate, needs to be improved. The objective of this study was to evaluate the efficiency of four irrigation techniques in using ground water for irrigating maize crop. The four irrigation techniques were: big gun sprinkler, impact sprinkler, drip and gravitation irrigation. An unreplicated experiment was conducted in Amor-amor, North Lombok (8°14'29"S and 116°17'01E) in a dry season of 2009. The results show that the highest maize yield (11.06 ton Ha^{-1}) was achieved under drip irrigation and the highest water use efficiency (1.57 ton ML^{-1}) was achieved in big gun sprinkler plot. Drip irrigation produced the highest economics efficiency with 385.23 g of grain yield per rupiah spent for irrigation as compared to 19.46 g per rupiah in impact sprinkler.

INTRODUCTION

Water is the main limiting factor in plant growth and its availability is limited. Qadir *et al.* (2003) stated that 98% of available fresh water in the world is ground water and only 2% is surface water, such as rivers and lakes. From the available fresh water, agricultural sectors use the water the most, at around 69%, followed industrial sectors, 23% and the rest is for household (Prathapar, 2000 *in* Turner, 2001). Based on that data, the surface fresh water alone is not suffucient to meet water demands for agriculture. That is why, good management of ground water to optimize agricultural production is needed.

The main source of ground water is precipitation. How much precipitation is needed to refill the ground water is depend on climate. Qadir *et al.* (2003) explained that 30% to 50% of precipitation can turn into ground water at high humidity condition. However, in a hot and dry climate conditions, the ability of precipitation to refill the ground water is much less than those numbers. Therefore, there is an urgent need to use efficient irrigation techniques in using ground water in areas with dry climate, such as in North Lombok, West Nusa Tenggara. For North Lombok conditions, in addition to improve ground water productivity, water use efficiency needs also to be considered.

In North Lombok, most of tube wells built by government are not well utilized. The reason is mainly due to the high cost of operation. Most of farmers irrigate their crops using an open irrigation system (gravitation), and this system is too costly to operate in a sandy soil, the predominat soil type in that area. The use of high pressure irrigation technique using a big gun sprinkler irrigation was reported to improve maize production and to decrease the irrigation cost in that area (Anon. 2008). On the other part of the world, there was a report saying that drip irrigation is much more efficient in using water as compared to a big gun sprinkler irrigation and to an open irrigation systems, and hence, drip irrigation system is considered the most appropriate technique to be used in a limited water condition (O'Neill *et al*. 2008).This study examined irrigation techniques that have already been used by farmers, such as gravitation, drip irrigation and high pressure irrigation using big gun and impact sprinkler.

MATERIALS AND METHODS

The experiment was conducted on an Entisol soil in Amor-amor, North Lombok (8°14'29"S dan 116°17'01"E) at elevation of 6 m above sea level. Soil texture was sandy loam with 0.09% N, 820 ppm of P, 0.76 me/100 g of K, pH 6.6 with field capacity of 27% (w/v). The experiment was unreplicated but the plot size of each treatment was large (12 m x 100 m), except for big gun treatment (100 m x 100 m) and yield prediction was taken from 100 m^2 from each treatment plot. Each plot was separated by 5 m empty space to avoid mix effect among the treatments.

There were four irrigation techniques tested, namely big gun sprinkler (Komet Twin 22), impact sprinkler (Naan 5022), surface drip (emitters distance 30 cm with 1.5 L h^{-1} of capacity and an open/gravitation irrigation. Plot size for big gun sprinkler irrigation was 1 Ha and for this irrigation, the pump machine was rotated at 1400 rpm and the pressure at the nozzle was 3.5 bars. With this rotation speed and pressure, a 30 mm nozzle of the big gun able to spray 16 L s^{-1} of water and the diesel consumed was 6 L h^{-1}. For impact sprinkler treatment, to run four sprinkler points with 1.5 bars of pressure, the pump was operated at 1100 rpm and diesel consumption was 1.5 L h^{-1}. Drip water source come from a 350 L tank and water used calculation was based on the ammount of the water used for irrigating the treatment plot. Water used in the gravitation irrigation treatment was calculated manually based on the ammount of water sent out from the main pipe per second. Water was applied when the soil moisture in the plots at about 40% of field capacity (monitored by Takemura DM5 Soil Moisture Tester). The ammount of water given at a time to reach field capacity in each plot then was recorded.

Variables measured were yield components, volume of water used in each irrigating time of each irrigation technique, number of water application and water use efficiency for biomass production, yield production and economic efficiency.

RESULTS AND DISCUSSION

The lowest yield was obtained from gravitation irrigation technique and the rest of the techniques resulted in relatively the same yield (Table 1). Yield componets of the maize, such as cob length, cob weight and grain yield was the highest in drip irrigation technique. Maize yield from drip irrigation was 156% higher than yield from gravitation technique. The high yield in drip irrigation was possibly due to an even distribution of water around maize root zone (O'Neill *et al*. 2008). Those plants irrigated with sprinkler techniques yielded better than those irrigated with gravitation technique because the plants received sufficient water and better aeration under sprinkler irrigation (Humphreys *et al*. 2008).

Table 1. Effect of irrigation techniques on some maize yield parameters

Treatments	Cob length (cm)	Cob weight (g)	Cob dry weight (g)	Grain weight (kg/100 m^2)
Big Gun	17.1±0.60*)	260.0±19.08	202.8±14.88	108.4
Impact Sprinkler	17.2±0.39	256.5±8.53	200.1±6.66	93.5
Drip	17.4±0.43	272.0±21.23	220.7±17.20	110.6
Gravitation	13.9±0.53	137.5±20.50	106.6±16.19	46.2

*) mean ± standard error

Amount of water used in surface drip irrigation technique was around 9 ML Ha^{-1}, much higher than that water used in other irrigation techniques (Table 2). This was possibly due to higher water loss through evaporation during the early stage of plant growth. With sub-surface irrigation, water requirement for maize crops was reported at

5.1 ML Ha^{-1} (O'Neill *et al*. 2008). The advantages of sub-surface irrigation from surface drip irrigation have many been discussed, such as by Ayars *et al*. (1999).

Table 2. Ammount of water used, number of irrigating times and ammount of diesel consumed in each irrigation technique

Treatments	Ammount of water used (L Ha^{-1})	Number of irrigating times	Diesel consumption (L Ha^{-1})
Big Gun	6,917,293	40	107.4
Impact Sprinkler	6,457,500	52	106.8
Drip	8,968,750	64	6.4
Gravitation	6,204,082	13	9.5

O'Neill *et al*. (2008) reported that maize crops require around 6.2 ML Ha^{-1} water under big gun sprinkler irrigation in New South Wales, Australian. In the current study, both big gun and impact sprinkler irrigation techniques used more water than the one in Australia. The difference possibly was caused by high wind speed in the current study area (average of 2 m s^{-1}, measured with Anemometer, EXTECH Instrument Type 45118) that resulted in high water loss (Dechmi *et al*. 2003; Lorenzini & Wrachien, 2005). In contrast, water used in gravitation technique in this study was slightly lower than those reported elsewhere earlier of around 6.6 to 7.5 ML Ha^{-1} (Pang & Latey, 1998 *in* Zand-Parsa *et al*. 2006).

Ammount of diesel consumed for irrigation by using the two sprinkler techniques was higher than that in other irrigation techniques (Table 2). The high pressure (around 3.5 bars) needed by sprinkler irrigation techniques to rotate the nozzle and to spray the water is the main reason for the high energy consumption. Drip and gravitation techniques, on the other hand, required less energy because of the low pressure requirement and hence less energy consumption. The pressure for drip irrigation in this study was gained from the water tank that was placed in a tower and for the gravitation technique, the pressure came from the main pipe.

Big gun sprinkler technique was the most efficient in using water followed by impact sprinkler, drip and gravitation, consecutively (Table 3). The low yield efficiency in gravitation technique was mainly caused by the low yield under this irrigation. In term of biomass formation efficiency, that based on plant biomass taken from sample plants on week seven after sowing, gravitation technique produced the highest efficiency. Drip irrigation was found to be the most efficient in term of irrigation cost (economic efficiency), resulted in 385.23 g of dry grain for every rupiah spent in buying diesel.The lowest economic efficiency of the four irrigation techniques tested was in impact sprinkler (Table 3).

Table 3. Yield, biomass efficiency (BE), yield efficiency (YE) and economic efficiency (EE) of the four irrigation techniques tested

Treatments	Yield (ton Ha^{-1})	BE (g L^{-1})	YE (g L^{-1})	EE (g Rp^{-1})
Big Gun	10.84	0.51	1.57	22.42
Impact Sprinkler	9.35	0.51	1.45	19.46
Drip	11.06	0.38	1.12	385.23
Gravitation	4.62	0.88	0.74	108.07

The most efficient irrigation technique in using ground water in this study is big gun sprinkler. This is shown from yield efficiency of the technique at 1.57 g L^{-1} (1.57 ton ML^{-1}). This water use efficiency result is higher than that reported by O'Neil *et al*. (2008) in Australia that only 1.4 ton ML^{-1}. However, yield efficiency of sub-surface drip

irrigation reported by O'Neill *et al*. (2008) was 1.7 ton ML^{-1}, while yield efficiency of the drip irrigation in this study was only 1.12 ton ML^{-1}. From all those the above facts, it is clear that drip irrigation is actually has a higher water use efficiency than sprinkler irrigation as long as the technology used is appropriate, such as sub-surface drip irrigation instead of surface drip irrigation. To use ground water efficiently and wisely to grow maize in a low wind speed condition, it is suggested to use big gun sprinkler irrigation or to use sub-surface drip irrigation whenever the technology is feasible.

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DISTINCT CHARACTERISTICS OF CHRYSANTHEMUM VIRUS B (CVB) ISOLATED FROM CHRYSANTHEMUM IN INDONESIA

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ABSTRACT

Infection of Chrysanthemum B Carlavirus (CVB) has been reported from many countries where chrysanthemum plants are cultivated. An isolate of CVB obtained from a chrysanthemum (*Chrysanthemum morifolium*) plantation in Indonesia was characterized and found to be unique. The virus isolate, designated as CVB-Ina, shared similar characters with other CVB isolates based on electron microscopy analysis and serological reactions. However, the results of bioassay showed that CVB-Ina was very distinct from most CVB isolates reported earlier. CVB-Ina induced vein banding and mottling on the leaves of chrysanthemum plants, could infect *Nicotiana benthamiana*, and was transmitted by the chrysanthemum aphid, *Macrosiphoniella sanborni*. In contrast, most CVB isolates were not able to induce any symptoms on chrysanthemum leaves, were not able to infect *N. benthamiana*, and were transmitted at low efficiency by the aphids. Furthermore, molecular characterization of the coat protein gene exhibited some differences in amino acid sequences. This might explain the distinct biological characteristics of CVB-Ina.

Keywords: *Chrysanthemum B Carlavirus* (CVB), chrysanthemum (*Chrysanthemum morifolium*), *Macrosiphoniella sanborni* (chrysanthemum aphid).

INTRODUCTION

Chrysantemum (*Chrysanthemum morifolium*) is a very popular flower world wide due to its variety in colour, shape, and size. Vegetative propagation of chrysanthemum is common practice because it can be done relatively simple. This type of propagation may facilitate the spread of diseases, which is especially true for virus infection. Chrysanthemum B Carlavirus (CVB) is a carlavirus that has been reported as one of the major viruses infecting chrysanthemum world-wide. Leaf mottling or mild vein-clearing is associated with CVB infection, although most of the cultivars are symptomless. The infection may also result in reduced flower quality (Hollings & Stones, 1972; Verma *et al.* 2003). The host range of CVB is narrow, it infects about 10 plant species in 5 dicotyledonous families (Hollings, 1957). CVB is transmitted in a non-persistent manner by several aphid species such as *Myzus persicae*, *Macrosiphum euphorbiae*, *Aulacorthum solani*, *Coloradoa rufomaculata*, and *Macrosiphoniella sanborni*. It could also be transmitted by grafting, and mechanical inoculation. Transmission by contact between plants and seed transmission has not been demonstrated (Hollings & Stone, 1972). Particles of CVB are filamentous, slightly flexuous, 680 – 685 nm long and 12 – 13 nm wide.

Infection of CVB in chrysanthemum cultivation in Indonesia was reported previously (Temaja *et al.* 2007). Symptoms diversity was recorded, including mild motlle, mild mosaic, vein-clearing and vein-banding in leaves, and color breaking in flowers. The Indonesian isolate of CVB was able to infect 7 out of 26 plant species tested in host range study, *i.e*. *Nicotiana benthamiana, N. tabacum* var. Havana, *N. clevelandii, N. tabacum* var. Burley, *Chenopodium amaranticolor, C. quinoa,* and *Petunia x hybrida*.

In this paper we report the further characterization of an Indonesian isolate of CVB to reveal its genetic diversity based on insect transmission study, electron-microscopy observation, analysis of the coat protein and DNA sequencing.

MATERIALS AND METHODS

Aphid transmission

Aphids were collected from a flower cultivation site in Cianjur, West Java. Species identification was done based on morphological characters, *i.e.* head, siphunculi, and cauda, of apterous aphids using methods described in Blackman & Eastop (2000).

Three-day-old non-viruliferous nymphs were given a starvation period of 2-3 h in a plastic box before they were placed on infected CVB plants for a 5-min acquisition feeding period. The effect of vector number on the relative efficiency of virus transmission was determined by allowing 1, 7, 14, or 21 aphids a 5-min inoculation access period on healthy chrysanthemum plants following the previous acquisition feeding period. Incubation period and symptom development was observed for up to 4 weeks and virus infection was confirmed using ELISA.

Virus Purification

Purification was done following a method described by Foster (1998) using CVB-infected tobacco (*N. benthamiana*). Virus concentration was calculated based on the A_{260} value of the purified sample and an *extinction coefficient* ($E^{0.1\%}_{1\,cm.260\,nm}$), of 2.3 for a typical carlavirus (Koenig, 1982). The purified virus was then subjected to electron microscopic observation (JEOL 1010, Japan) following staining using 2% phosphotungstate acid (pH 7.0) (Dijkstra & de Jager, 1998).

SDS-PAGE dan *Western Blot*

Protein analysis was conducted using sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) following the Laemmli procedure (Gall *et al.* 1980) prior to Western blotting using CVB specific antisera (Towbin *et al.* 1979).

Nucleic acid analysis

Total RNA was extracted from leaf tissue using *RNeasy Plant Mini Kits* (Qiagen, Germany). The total RNA was then served as a template in Reverse Transcription-Polymerase Chain Reaction (RT-PCR) which was performed using specific primers for CVB coat protein: CVB5 (5' CAAAGAGGTGATCATCCGTCTAG 3') and CVB3 (5' CTCGGTTACTTTATCGCACCTAG 3'). The amplified products of each CVB isolates were then subjected to nucleic acid sequencing.

RESULTS AND DISCUSSION

Aphid transmission efficiency

Aphid colonies collected from a chrysanthemum cultivation site in Cianjur, West Java were identified as *Macrosiphonoiella sanborni*. Aphid colonies usually developed on the stem or on the underside of young leaves.

Neither single nor seven aphids were able to transmit CVB when given a 5-min acquisition access period and a 5-min inoculation access period. Transmission was observed when 14 or 21 aphids were used with a disease incidence of 12.5%, and 62.5%, respectively for the CVB isolate from Indonesia (Cianjur) (CVB-Ina) (Table 1). Different results were obtained when the same transmission experiment was conducted using a CVB isolate from Japan (CVB-S), where the minimum number of aphids required to cause transmission was 21 aphids per plant. Previously Ohkawa *et al.* (2007) also reported that transmission efficiency of CVB-S using aphids was relatively low.

Table 1. Aphid transmission of CVB by *Macrosiphonoiella sanborni*

No. aphids/plants	Disease incidence (%)	
	CVB - Indonesia	CVB - Japan
1	0	0
7	0	0
14	12.5	0
21	62.5	12.5

Virus purification and electron microscopy observation

A clear viral band was detected after cesium sulfate gradient centrifugation. Absorbance value of the purified preparation indicated that the quality of purified virus was good, with an A_{260} of 1.449 and A_{280} of 1.183 (A_{260}/A_{280} ration 1.22). Using an extinction coefficient ($E^{0.1\%}_{1\,cm.260\,nm}$) value for carlaviruses of 2.3, we estimated that the concentration of our purified virus was 0.630 mg/ml. Therefore total virus recovered from 200g of fresh leaves of *N. benthamiana* was 6.250 mg. Similar attempts for virus purification had been reported earlier: Hollings & Stone (1972) reported that they were able to obtain a higher A_{260}/A_{280} value (1.55); and Suastika *et al.* (1997) were able to obtain 12 mg of purified virus from 100 g fresh leaves of *N. clevelandii*.

Electron microscopic observation revealed the characteristic shape of CVB, *i.e* flexuous and slightly filamentous rods 685 nm long and 12 nm wide (Fig.1). This observation was confirmed by previous reports by Hollings & Stone (1972), Suastika *et al.* (1997), and Verma *et al*. (2003). The CVB particles observed by Suastika *et al*. (1997) had a long, rather flexuous shape 650-700 nm long and 13 nm wide; whereas Verma *et al*. (2003) reported flexuous particle, 680 x 12 nm in size.

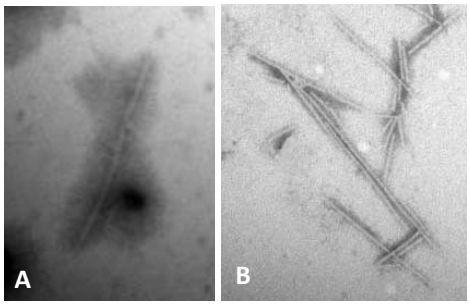

Fig 1. Electrone microscopic observation of CVB-Ina (40.000 x magnification) : leaf dip of CVB-infected tissue (A); purified virus (B)

Analysis of SDS – PAGE and *Western Blot*

The protein bands of about 34 kDa were observed in SDS-PAGE when the samples were prepared from CVB-infected *N. benthamiana* plants and from purified virus, but the band was not seen when healthy *N. benthamiana* was used. Reaction with CVB antisera in the Western blot confirmed that the band was indeed coat protein of the virus (Fig. 2).

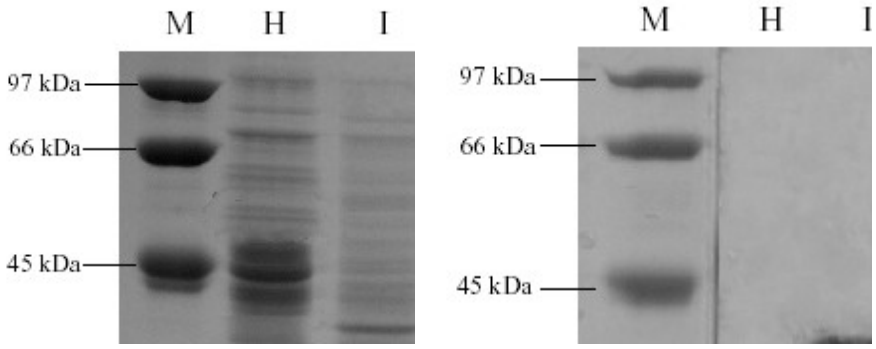

Fig 2. SDS-PAGE analysis (A) and western blot (B) of CVB coat protein. The samples are: (M) Protein marker , (H) Healthy leaf of *N. benthamiana*, (I) CVB-infected leaf of *N. benthamiana*, and (C) Purified virus. Sizes of protein markers are shown on the left margin of each panel and arrows on the right margin show the position of the CVB coat protein band.

Analysis of nucleic acid

Infection of CVB was confirmed in leaf samples collected from different chrysanthemum cultivation areas in Indonesia by RT-PCR using primer pair CVB 5/CVB 3. Amplicons of 739 bp were successfully amplified from all samples from Medan, Cianjur, Malang and Bali (Fig.3).

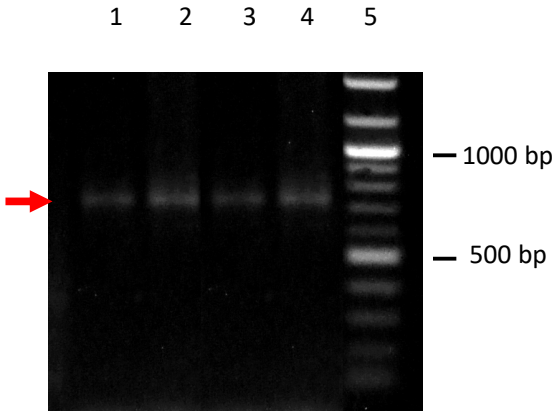

Fig. 3. Amplification of CVB by RT-PCR using primer pair CVB 5/CVB 3. The samples in lanes 1 to 4 are leaf tissue collected from Medan, Cianjur, Malang and Bali, respectively. Lane 5 is a DNA marker with the 500 and 1,000 bp bands marked on the right margin. An arrow on the left margin marks the position of the CVB amplicon.

The PCR product was then directly submitted for DNA sequencing and phylogenetic analysis, conducted using the PAUP 4.10 program based on the UPGMA approach. It was shown that all Indonesian isolates of CVB are clustered together with the CVB isolate from Japan and two CVB isolates from India, *i.e*. Chattisgarh and Jammu isolates, whereas the other Indian isolates were clustered together with CVB isolates from Italy (Fig.4). Although all CVB isolates from Indonesia clustered with CVB from Japan, they formed 2 sub groups with relatively close genetic distances, 0.01–0.05. Isolates from Cianjur and Medan belonged to one sub group, whereas isolates from Bali and Malang belong to another group. The results of further analysis of derived amino acids sequences

showed that CVB isolates from Indonesia have a high amino acid homology with CVB from Japan, 86 – 90% (Table 2), and differences in some regions of the sequences were observed.

Based on the preliminary characterization we have done, it can be concluded that the CVB from Indonesia showed a slightly different characteristics from other CVB isolates reported earlier. CVB from Indonesia induced vein-clearing and mottling on the leaves of chrysanthemum, could infect *N. benthamiana*, and was transmitted by the chrysanthemum aphid *M. sanborni* efficiently.

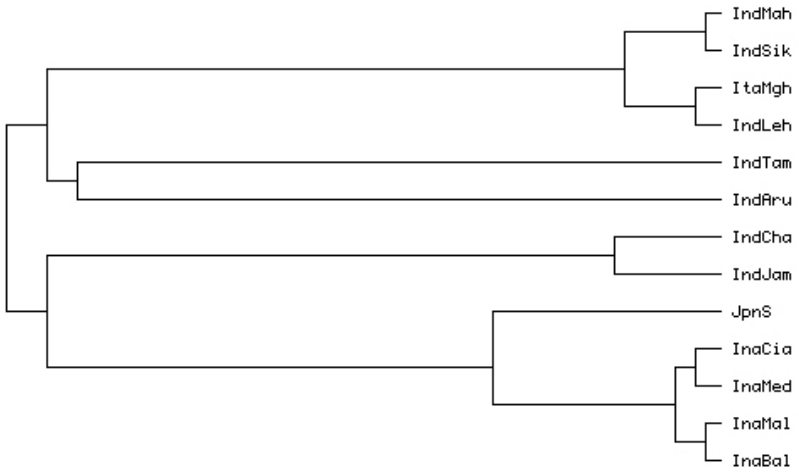

Fig 4. Phylogenetic relationship between 4 isolates of CVB from Indonesia with other CVB from different geographic location available from GenBank. *). Ind: India, Ita: Italia, Ina: Indonesia, Jpn: Japan; Mah: Maharashtra, Cha: Chattisgarh, Jam: Jammu, Sik: Sikkkim, Tam: Tamilnadu, Mgh: Mgh57, Aru: Arunachal P, Cia: Cianjur, Med: Medan, Mal: Malang, Bal: Bali

Table 2. Amino acid sequence homologies between four isolates of CVB from Indonesia (Ina) and CVB isolate from Japan CVB S (Jpn) based on their coat protein gene.

No	Isolate	Percent homology				
		1	2	3	4	5
1	Jpn:S	-				
2	Ina:Cianjur	90	-			
3	Ina:Medan	89	98	-		
4	Ina:Malang	86	96	95	-	
5	Ina:Bali	87	97	95	99	-

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ALLELOPATHIC EFFECT OF *Wedelia trilobata, Ageratum conyzoides, Chromolaena odorata* AND *Mikania micrantha* ON GREEN MUSTARD GROWTH

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ABSTRACT

Many weeds are allelopathic, that is, they can produce and release allelochemicals that can either inhibit or benefit other plants. Greenhouse study was conducted to determine the allelopathic potential of some weeds namely creeping daisy (*Wedelia trilobata*), goat weed (*Ageratum conyzoides*), Siam weed (*Chromolaena odorata*) and bittervine (*Mikania micrantha*) on the growth and yield of green mustard (*Brassica rapa*). Weeds leaves were cut into 2 cm length, and for each 100, 200, 300, 400, and 500 g of leaves were soaked in 1 L of distilled water for a period of 36 hours to get 100 through 500 g/L allelophat concentrations treatment. The extract were filtered with Whatman No.1 paper. One week after transplanting, green mustard were treated with 0.2 L extract every 2 days for 20 days. The results shows that shoot length, shoot and root dry weight of green mustard decreassed progressively when exposed to increasing concentrations of aqueous leaf extract of creeping daisy and increased progressively when exposed to increasing concentrations of leaf extract of siam weed. Goat weed and bittervine leaf extract at different concentrations resulted in inconsistent growth and yield of green mustard. Creeping daisy had more inhibitory effect on green mustard growth as compared to the other extracts. Percent reduction of creeping daisy extract on shoot length, shoot dry wight and root dry weight of green mustard was 8.4%; 16.0% and 43.0% respectively as compared to the untreated control. The extract of creeping daisy also resulted in the lowest leaf area, followed by bittervine, siam weed and goat weed extract. The greenest leaves were resulted from the 500 g/L allelophat concentration

Keywords: Allelopathy*, Wedelia trilobata, Ageratum conyzoides, Chromolaena odorata, Mikania micrantha*, mustard

INTRODUCTION

In their communities plants may interact in a positive, negative, or neutral manner. It is more common that neighboring plants will interact in negative manner, where the emergence or growth of one or both is inhibited. The adverse effect of a neighboring plant in an association is termed interference. The potential causes of interference include allelospoly, allelopathy and allelomediation. Allelopathy refers to the beneficial or harmful effects of one plant on another plant, both crop and weed species, by the release of chemicals from plant parts by leaching, root exudation, volatililization, residue decomposition and other processes in both natural and agricultural system (Ferguson and Rathinasabapathi, 2003; Dailey, 2008).

Several studies showed that several weeds species such as *Cyperus rotundus, Imperata cylindrica, Shorgum halapense, Cyrcium arvense* and *Agropyron repens* (Sastroutomo, 1990; Setyowati, et al, 1999), *Wedelia trilobata* (Togatorop, et al., 2010; ChengRong et al, 2006; Jinrong et al, 2008; YuHu et al.), *Chromolaena odorata* (Ambika and Poornima, 2006; Adetayo et al, 2005; Onwugbuta and Enyi, 2001), *Ageratum conyzoides* (Kong et al, 2002; ChuiHua et al, 2006), and *Mikania micrantha* (Bao-Ming et al, 2009; Ismail and Chong, 2002) produce substances which are toxic to other plants.

W. trilobata (creeping daisy) occurs in agricultural areas, coastland, natural forests, planted forests, range/grasslands, ruderal/disturbed, and urban areas. It has a very wide ecological tolerance range, and seems to be equally suited to dry and moist sites. It grows well on almost all soil types, including bare limestone and nutrient poor sandy beaches and swampy or waterlogged soils (ISSG, 2007). Study in Indonesia indicated

that creeping daisy and Siam weed were to be the most promising natural fallow species to enhance soil quality improvement (Handayani et al, 2006).

C. odorata also known as Siam weed, it forms dense stands that prevent the establishment of other plant species. It is an aggressive competitor and has been nominated as among 100 of the "World's Worst" invaders. Siam weed is a fast-growing perennial shrub, an aggressive competitor and may have allelopathic effects. It is also a nuisance weed in agricultural land and commercial plantations. Siam weed grows on a wide range of soils and grows in a range of vegetation types (Wilson, 2006; Ismail and Sugau, 1993)

A. conyzoides (goat weed) is a weed distributed in many tropical and subtropical countries and is often difficult to control. Goat weed is a herb present in many tropical and subtropical environments. Goat weed grows as a monoculture in grasslands, forests, agricultural, plantations and horticultural fields in India. Goat weed is a weed in many annual and perennial crops (ISSG, 2009). Some studies have demonstrated allelopathy in the weed and crops (Kong *et al*. 2002).

Mikania micrantha (bittervive) is a widespread weed in the tropics, grows very quickly and covers other plants. This species has been nominated as among 100 of the "World's Worst" invaders,occurs in agricultural areas, coastland, natural forests, planted forests, ruderal/disturbed, urban areas and wetlands. Bittervine is one of the three worst weeds of tea in India and Indonesia and of rubber in Sri Lanka and Malaysia. It also causes serious problems in oil palm, banana, cacao and forestry crops, and in pastures (ISSG, 2005). It contains allelochemicals (Maoxin et al, 2003).

An experiments were conducted in greenhouse to determine the allelophatic potential of creeping daisy, goat weed, siam weed and bittervine in the growth and yield of green mustard.

MATERIALS AND METHODS

Extract preparation. Creeping daisy, goat weed, siam weed and bittervine leaves were collected from area around Agriculture Faculty, University of Bengkulu as source of allelopathy extract for these studies. The leaves were washed with water, cut into 2 cm length, and for each 100, 200, 300, 400, and 500 g of leaves were soaked in 1 L of distilled water for a period of 36 hours to get 100 through 500 g/L allelophat concentrations treatment. The extract were filtered with Whatman No.1 paper.

General experiments procedures. These studies were conducted in November 2008 through January 2009 in greenhouse of Agriculture Faculty, Bengkulu University. Green mustard seeds were germinated in germination trays for 2 weeks. Seedlings were transplanted into plastic pots (20 cm diameter x 15 cm depth) filled with a mixture of one part topsoil, one part manure fertilizer and one part sand. These plants were then treated with 0.2 L extract every 2 days for 20 days. All treatments were fertilized with inorganic fertilizers of urea (50 kg/ha), KCl (75 kg/ha) and SP-36 (100 kg/ha) at planting date except for urea applied twice at planting date and 3 weeks after planting. Green mustards were harvested 5 weeks after planting. The experimental design was a Completely Randomized Design (CRD) with 3 replications. Orthogonal coefficients were used to separate the treatment means in the ANOV.

RESULTS AND DISSCUSSION

Green mustard shoot length in all treatments increased following the time (Figure 1-4)

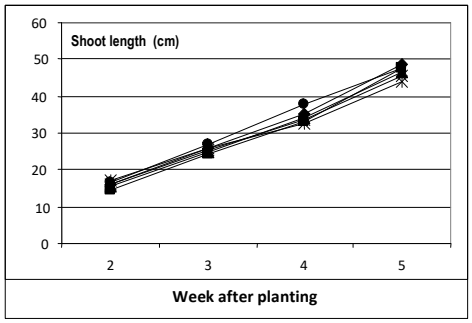


Figure 1.Effect of creeping daisy extract on green mustard shoot length growth

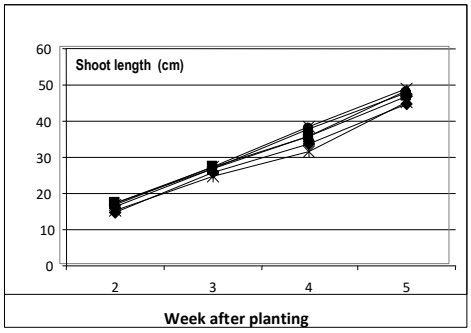


Figure 2. Effect of goat weed axtract on green mustard shoot length growth

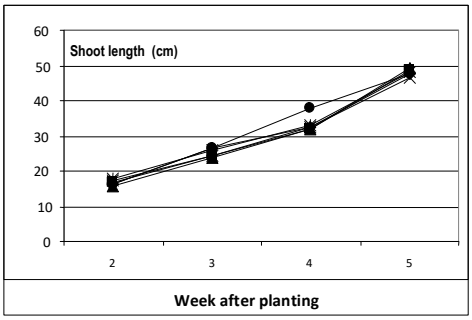


Figure 3. Effect of Siam weed extract on green mustard shoot length growth

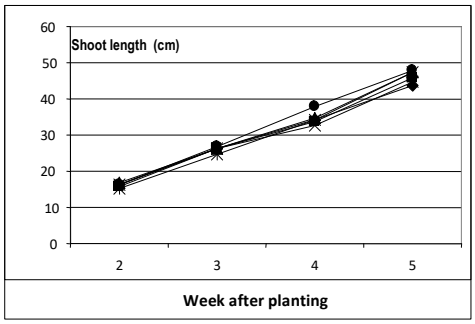
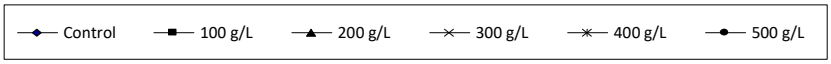


Figure 4. Effect of bittervine extract on green mustard shoot length growth



In general, green mustard have similar growth pattern up to 3 weeks after transplanting. The growth pattern varies at 3 to 5 weeks after transplanting. Extract of weeds resulted in lower shoot length compare to that of control (no extract addition). Haig (2008) reported that plants may contain secondary metabolite such as glucosinolates, phenolic compounds, terpenoids, alkaloids, hydroxamic acids, flavonoids and quinines. Glucosinolates are considers the precursors of the allelochemicals possessing phytotoxicity.

The addition of creeping daisy and Siam weed extract reduced green mustard shoot length at all concentrations tested (Figure 5).

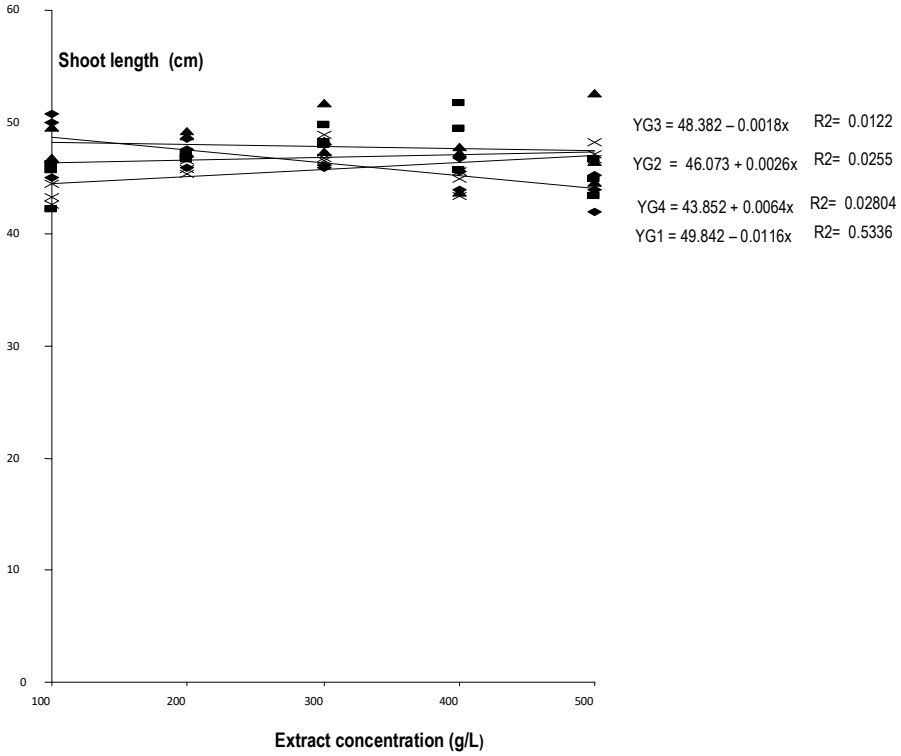


Figure 5. Interaction of extract concentration and weeds type with green mustard shoot length

Increasing 10 g/L extract of creeping daisy and Siam weed reduced shoot length as much 0.0116 cm and 0.0018 cm respectively. On the other hand, at the same concentration , extract of goat weed and bittervine stimulated shoot growth of green mustard.

Extract creeping daisy was also decreased shoot dry weight at all concentrations testes while extract goat weed and Siam weed increased shoot dry weight (Figure 6).

On the other side, extract bittervine at concentration 100 g/L up to 300 g/L increased shoot dry weight while at higher concentrations the extract suppressed shoot dry weight. Creeping daisy, goat weed, Siam weed and bittervine extracts suppressed shoot dry weight as much as 16.96%; 3.58%; 10.58% and 11.95% respectively. Therefore, extract of creeping daisy was the most toxic to green mustard leaves growth.

There were also interactions between extract concentration and weeds type with green mustard root dry weight (Figure 7).

Creeping daisy extract treatment, significantly reduced the root dry weight at all concentrations tested. On the other sides, goat weed stimulate the root dry weight (Y = 2.8013 + 0.0005 x). Compare to control treatment, creeping daisy, goat weed, Siam weed and bittervine extract decreased root dry weight as much as 42.97%; 33.07%; 25.77% and 25.77% respectively.

As extract concentration of all weeds increased, the leaves greenest also increased (Figure 8). The highest leaves greenest were found at extract concentration 500 g/L. Component of the extract may contain N that play an important role on increasing chlorophyll content.

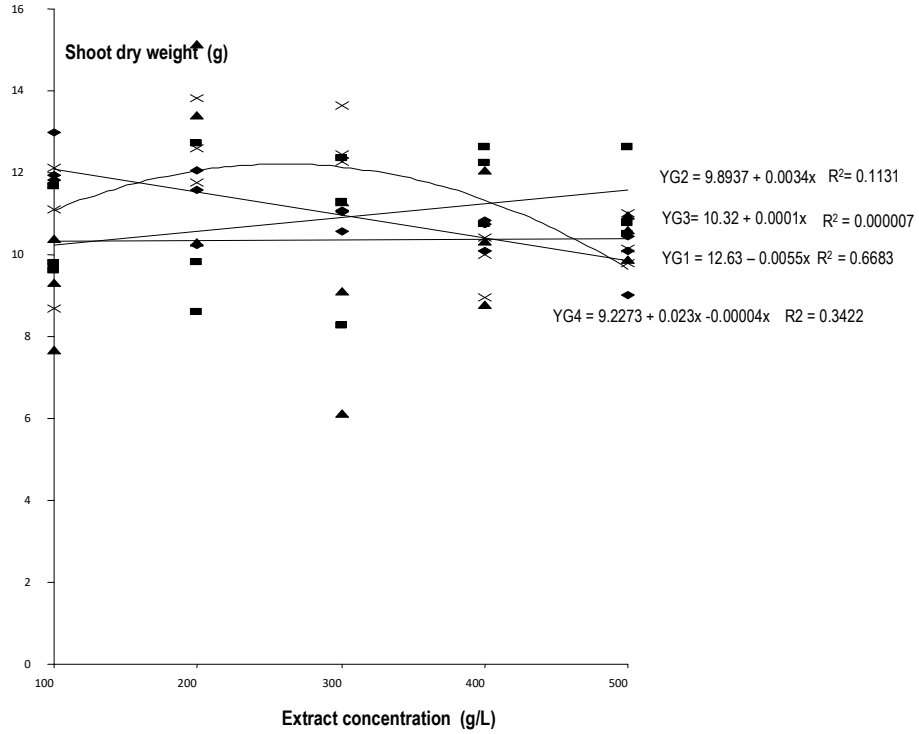


Figure 6. Interaction of extract concentration and weeds type with green mustard shoot dry weight

Result of this experiment shows, in general, creeping daisy was more toxic to green mustard than the other three weeds species. Similar result were reported by Jinrong et al. (2008), that the residue leachates of creeping daisy inhibited the germination of lettuce and the inhibition was also increased with the increment of residue content in the soil. Moreover, the chlorophyll content of lettuce leaves increased in the non-sterilized soil but decreased in sterilized soil. ChengRong et al (2006) also reported that creeping daisy extract reduced root and aerial parts of *B.parachinensis* as well as the plant height, chlorophyll content and net photosynthetic rate. The aqueous extract inhibit the activity of catalase, peroxidase, nitrate reductase as well as nitrogen metabolism in the plant.

The inhibitory effects of goat weed volatiles on peanut, redroot amaranth, cucumber and ryegrass were reported by Kong et al (2002). Monoterpenes and sesquiterpenes were the major volatile component of goat weed. Similar to our results, ChuiHua et al (2006) reported that allelopathic potential of goat weed was also vary with growth stage and environmental conditions. In our experiment, effect of goat weed allelochemical on green mustard was vary with extract concentrations. Goat weed also suppress the germination and the growth of roots and shoots of *Amaranthus caudatus, Digitaria sanguinalis* and *Lactuca sativa* (Kato-Noguchi, 2001).

Shoot dry weight of green mustard increased as siam weed extract increased. Similar results were reported by Ambika and Poormina (2006), Siam weed allelochemicals increased the vegetative growth, metabolite content and yield of grain crops and vegetables. Siam weed extract inhibit soybean growth at later stage of crop growth however the growth of maize, cowpea,and siam weed itself was stimulated by siam weed extract (Adetayo et al, 2005; Onwugbuta and Enyi, 2001).

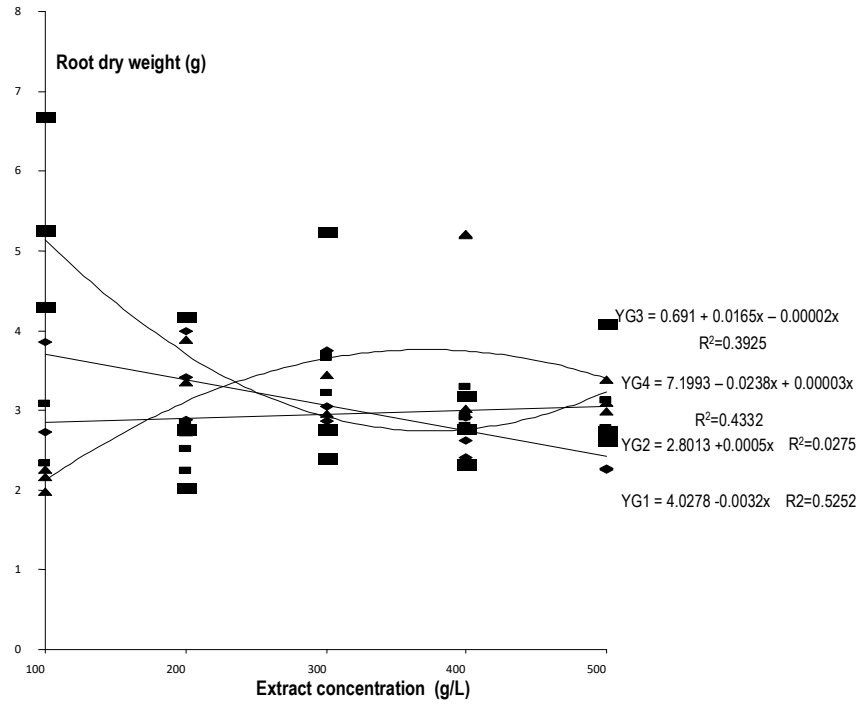


Figure 7. Interaction of extract concentration and weeds type with green mustard root dry weight

Aqueous extract of bittervine leaves and roots had inhibitory effects on plants. Allelopathic activity depended on the concentration of the extracts, target species and the extract sources (Ai-Ping et al, 2009; Ismail and Suat, 1994). In this experiment, the bittervine allelopathy activity were also depended to the extract concentrations. Bittervine also had inhibitory effects on seed germination, radical length and fresh weight of Chinese cabbage and tomato but did not affect those of corn and long bean seedling (Ismail and Chong, 2002).

As extract concentration of all weeds type increased, the leaves greenest were also increased (Figure 8)

The highest leaves greenest were resulted from the extract at concentration 500 g/L. The extract component may contain N that play an important role on increasing leaves greenest. Therefore, as leaves extract concentration increased, the leaves greenest was also increased. The degree of leaves greenest mostly depend on N-content in the soil (Gardner et al, 1991)

CONCLUSIONS

Shoot length, shoot and root dry weight of green mustard decreassed progressively when exposed to increasing concentrations of aqueous leaf extract of creeping daisy and increased progressively when exposed to increasing concentrations of leaf extract of Siam weed. Goat weed and bittervine leaf extract at different concentrations resulted in inconsistent growth and yield of green mustard. Creeping daisy had more inhibitory effect on green mustard growth as compared to the other extracts. Percent reduction of creeping daisy extract on shoot length, shoot dry wight and root dry weight of green mustard was 8.4%; 16.0% and 43.0% respectively as compared to the

untreated control. The extract of creeping daisy also resulted in the lowest leaf area, followed by bittervine, Siam weed and goat weed extract. The greenest leaves were resulted from the 500 g/L allelophat concentration.

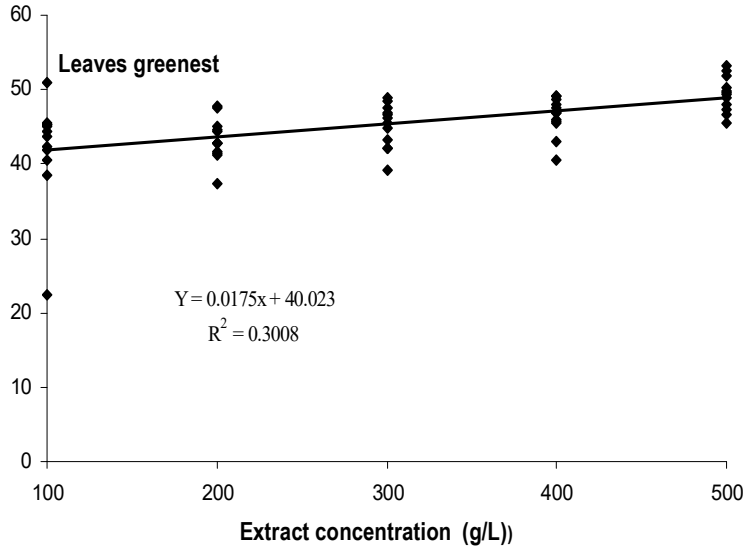


Figure 8. Effect of extract concentrations on leaves greenest

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PHYSIOLOGICAL RESPONSE OF TOMATO (*LYCOPERSICUM ESCULENTUM* MILL.CV. KALIURANG) TREATED WITH NPK FERTILIZER AND PACLOBUTRAZOL

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ABSTRACT

The purposes of this research were to evaluate the physiological responses of tomato plant treated with NPK fertilizer and paclobutrazol. Tomato seeds were obtained from a horticulture seed center in Ngipiksari, Kaliurang, Yogyakarta. The experiment design was factorial 3 x 4 that was arranged in Complete Randomized Design. The first factor was application of NPK fertilizer (15:15:15), which consisted of 3 levels, 0 g / plant (control), 1 g / plant, and 2 g / plant. The second factor was a variation of paclobutrazol concentration, which consisted of 4 levels 0 µM (control), 20 µM, 40 µM, and 60 µM. For each treatment the combination 5 replicates were used. The growth parameters that were observed were days to flowering, plant height, chlorophyll content, the thickness of stem vascular tissue, the total of sugar content levels and vitamin C contents in the fruit. Data were analyzed using ANOVA, if there were significant difference among treatments, and then continued by DMRT with P = 5%. The results of this research show that application of paclobutrazol tends to suppress plant height, if paclobutrazol is combined with NPK fertilizer, the effectiveness of paclobutrazol in reducing growth become less. Application of paclobutrazol 20 µM or 40 µM combined with NPK fertilizer of 1 g / polybag or 2 g / polybag increased the total of chlorophyll content, promoted flowering time, increased fruit production as well as total sugar content levels and vitamin C content in the fruits. In addition, a paclobutrazol application of 20 µM increased the thickness of vascular tissues in the stem.

Keywords: *Lycopersicum esculentum* L, NPK fertilizer, paclobutrazol, flowering.

INTRODUCTION

Lycopersicum esculentum, Mill.is a major vegetable being developed by the Indonesia Research Centre of Horticulture due to its economical value and good market prospect (Rukmana,1994). One of the tomato cultivars, called "Kaliurang", becomes a priority cultivar that has been developed by the Horticulture Development and Promotion Unit in Sleman Regency (Pitojo, 2005). Compared to other tomato cultivars, the "Kaliurang" cultivar has better characteristics, such as high fruit yield, resistant of bacterial wilt disease and able to survive on lowland up to highland areas. However, there are still limited literatures about factors which influence the "Kaliurang" tomato growth and fruit yield.

Both internal and external factors influence the growth and productivity of crop plants. Amongst the external factors, synthetic compound, such as packlobutrazol and inorganic fertilizers are commonly applied to crop plants to alter the growth and to obtain better yield quality. Packlobutrazol is a growth retardant that inhibits gibberellin biosynthesis. The application of packlobutrazol can cause dwarfism. However, at an appropriate concentration, the plant productivity can still be maintained whereas the chlorophyll content and assimilate allocation to the sink often can be improved (Seda *et al*., 1995). It has been reported that in *Brassica carinata* L., packlobutrazol application of 5, 10, or 20 $\mu g.mL^{-1}$ increases chlorophyll content and assimilates translocation to the seeds (Bhathal *et al*., 1995). According to Berova and Slatev (2004), paclobutrazol application on tomato inhibits the stem growth but accelerate roots growth and fruit development. NPK fertilizer is commonly applied to increase vegetative and / or reproductive growth. In tomato, nitrogen ion is required for flower development and it determines fruit productivity (Morgan, 2006). The availability of NH4 – N (*ammonium*–

nitrogen) with concentration proportion of 0:100, 10:90, 20:80, 30:70, or 40:60 in tomato plants grown under light intensity of 2,667 or 5,030 $Wh^{-1}m^{-2}$ increases vegetative growth, flowering and fruit formation (Villa *et al.,* 2007). Hochmuth and Cordasco (1998), reports that an application of NPK fertilizer in tomato increases the vegetative growth, flower development, as well as fruit formation. This experiment aims to evaluate the effect of packlobutrazol and NPK fertilizer on the growth, fruit development and quality of tomato cv. "Kaliurang".

MATERIALS AND METHODS

The CV "Kaliurang" tomato seeds were obtained from the Horticulture Development and Promotion Unit in Ngipiksari, in the Sleman district of Yogyakarta. Tomato seeds were germinated in a plate containing soil: organic fertilizer (2:1 v/v) and seedling were grown for 3 weeks. Each seedling was then grown in a polybag containing a mixture of soil (9 kg/polybag) and organic fertilizer (0.3 Kg/polybag). Each polybag was placed within the distance of 70 cm x 50 cm in the field experiment which has an elevation of $\pm$600 m above sea level. The design of the experiment was 3 x 4 factorial. The first factor was NPK application which consisted of 3 levels, namely control (without NPK), 1 g NPK/polybag, and 2 g NPK/polybag. The second factor was paclobutrazol application which consisted of 4 levels, namely control (without packlobutrazol), 20 µM, 40 µM, and 60 µM paclobutrazol. Each treatment combination has 5 replicates. NPK fertilizer was applied three times (at planting date, 4 and 8 weeks after planting). Foliar application of packlobutrazol was done 25 and 50 days after planting. Watering was done twice a week. The growth and development parameters monitored were plant height, flower initiation, and fruit yield which were determined by weighing the first cluster of fruits. Chlorophyll content was determined according to Harborne (2006), total sugar was determined by Nelson Somogyi method, and vitamin C of fruits was determined by AOAC method (Sudarmadji *et al*., 1997). Anatomical structure of stem was observed through semi permanent preparate. Data were analyzed using ANOVA and the significant difference was determined using Duncan's Multiple Range Test with P = 5%.

RESULTS AND DISCUSSION

The application of packlobutrazol reduced plant height mainly on plants without NPK fertilizer. The higher concentration of paclobutrazol applied the greater the reduction in plant height was observed, but additional of NPK fertilizer counteract the effect of packlobutrazol so that there was no significant difference in the height of plants that received NPK fertilizer (Fig. 1). This might be due to the availability of nitrate from NPK increased the synthesis of chlorophyll (Fig.2), and as a result it increased photosynthesis rate and the availability of assimilate for plant growth also greater compared to those plants without NPK fertilizer (Chaturvedi, 2005). Haughan *et al*. (1989) in Hammes and Tsegaw (2006) reported that growth repression caused by packlobutrazol is due to reduction in the number of cells that carried out the proliferation. However, in other plant species the retardation in vegetative growth was due to inhibition in cell elongation (Nazarudin *et al.,* 2007). Iremiren *et al.* (1997), reported that packlobutrazol application of 12 and 24 ml. L^{-1} on corn plants (*Zea mays* L.) increased chlorophyll-a and chlorophyll-b contents. Similar finding is also reported in tomato, in which packlobutrazol of 30 $g.mL^{-1}$ and 60 $g.mL^{-1}$ increased chlorophyll and carotenoid content (Jaafari *et al*., 2006). The mechanism by which chlorophyll content of those plants applied with increased packlobutrazol can be through the conversion of geranylgeranyl pyrophosphate to phytol and rather than to *ent*-kaurenoic acid (Chaney, 2005).

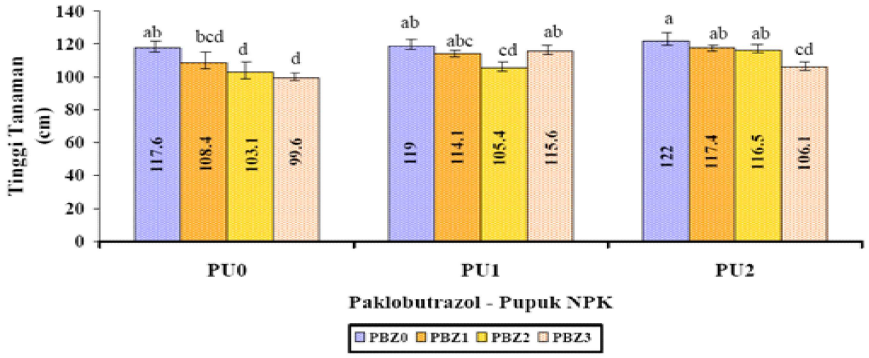

Fig. 1. Effects of paclobutrazol and NPK on the plant height at 8 weeks after planting
Note : The same alphabeth on each bar showed no significant difference at P = 5%, n = 5
PBZ_0 : without paclobutrazol ; PBZ1: paclobutrazol 20 µM; :PBZ2 : paclobutrazol 40 µM ; PBZ_3 ; paclobutrazol 60 µM
PU_0 : without NPK; PU_1 : NPK 1g / polybag; PU_2 : NPK 2g / polybag

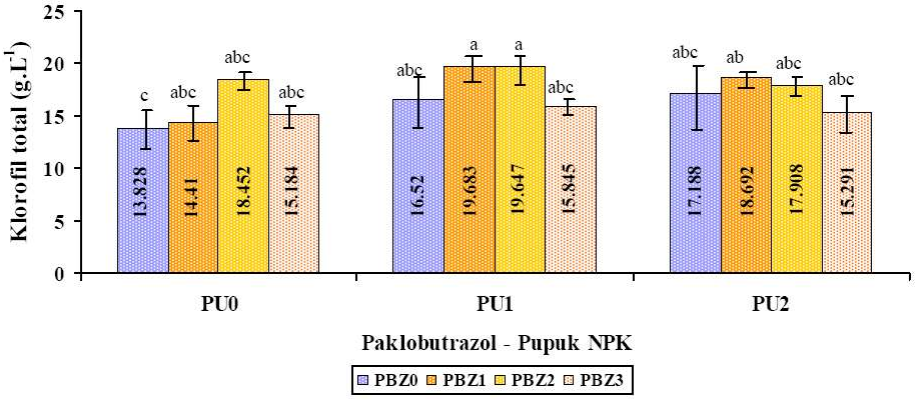

Fig. 2. Effects of paclobutrazol and NPK on chlorophyll content of leaves.
Note : The same alphabeth on each bar showed no significant difference at P = 5%, n = 5
PBZ0 : without paclobutrazol ; PBZ1: paclobutrazol 20 µM; :PBZ2 : paclobutrazol 40 µM ; PBZ3 ; paclobutrazol 60 µM
PU0 : without NPK; PU1 : NPK 1g / polybag; PU2 : NPK 2g / polybag

Flower initiation of plants treated with packlobutrazol of 20 µM or 40 µM and NPK fertilizer of 1 or 2 g/polybag was 3 to 4 days earlier compared to control plants. However, higher concentration of packlobutrazol (60 µM) applied to the plants that received no NPK fertilizer did not speed up flower initiation (Fig 3.). It is probably that the endogenous abscisic acid increased by application of high concentration of packlobutrazol, and as a consequence the plant growth and development becomes slow.

The vegetative growth plants treated with packlobutrazol were inhibited, but chlorophyll content increased. These conditions probably lead to the change in the assimilating allocation to reproductive stage rather than to the vegetative stage (Shuju *et al*., 2004). The availability of NPK in plants treated with packlobutrazol 20 µM or 40 µM increased fruit production significantly. The fruit weight of the first fruit cluster was about 750 g whereas fruit weight of controlled plants is only about 350 g. In addition, the total sugar content and vitamin C content increased significantly in the fruits of plants that were treated with 40 µM paclobutrazol and NPK fertilizer of 1 g/polybag (Fig. 4).

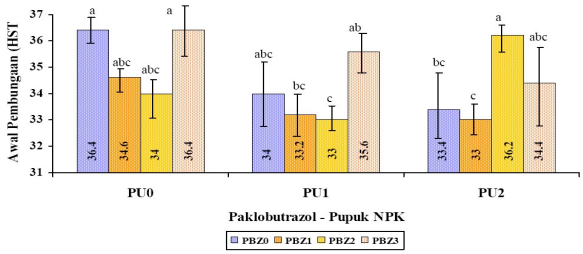

Fig.3 Effect of packlobutrazol and NPK fertilizer on flower initiation
Note : The same alphabeth on each bar showed no significant difference (P = 5%, n = 5)
PBZ0 : without packlobutrazol; PBZ1 : packlobutrazol 20 µM
PBZ2 : packlobutrazol 40 µM ; PBZ3 : packlobutrazol 60 µM
PU0 : without NPK ;PU1 : NPK 1g / polybag; PU2 : NPK 2g / polybag

The value of total sugar and vitamin C contents were 4.7% and 41.5 mg.100^{-g} respectively, whereas those of controlled plants were 3.3% and 25.8 mg.100^{-g} respectively. Plants subjected to stress conditions normally formed antioxidant such as karotenoid, ascorbic acid (vitamin C), glutathione, α-tokoferol, and flavonoids (Sopher *et al*., 1999; Vijayarengan *et al*., 2007). Thus, the increase of vitamin C in the fruit of plants treated with packlobutrazol was probably due to a stress response of those plants. Similar finding was reported on ocra (*Albomoschus esculentus* (L.) Moench) and two species of citrus: *Citrus reticulata* Blanco and *Citrus aurantium* L. in which vitamin C content increased in the fruits of plants treated with packlobutrazol (Benjawan *et al.,*2007).

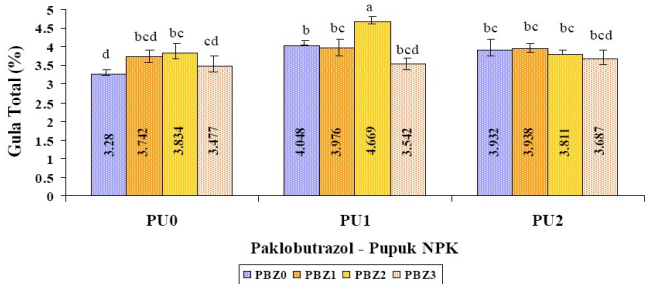

Fig.4. Effect of paclobutrazol and NPK on total sugar content of first cluster tomato
Note : Similar alphabeth on each bar showed no significant difference at P = 5%, n = 3
PBZ0 : without paclobutrazol ; PBZ1 : paclobutrazol 20 µM; PBZ2 : paclobutrazol 40 µM; PBZ3 : paclobutrazol 60 µM; PU0 : without NPK; PU1 : NPK 1g /polybag; PU2 : NPK 2g / polybag

The observation on the anatomical structure of stems showed that the width of vascular bundle and cortex in plants treated with packlobutrazol (40 or 60 µM) and NPK of 2 g / polybag were 879.7 µM and 377.7 µM respectively, whereas those of controlled plants were 674,4 µM and 243,4 µM respectively. These increases in the width of vascular bundle and cortex were probably the consequence of growth retardation, so that the plant height was reduced but the stem diameter was increased. It has been reported that packlobutrazol application on tomato and potato plants through soil surface or through leaves increased the stem diameter (Berova and Zlatev, 2004; Hammes and Tsegaw, 2004).

Based on the results and discussion it can be inferred that paclobutrazol represses the vegetative growth of tomato plants. However, additional NPK fertilizer lightens the growth retardation. The application of paclobutrazol 20 µM or 40 µM combined with

NPK fertilizer of 1 or 2 g / polybag increased the total chlorophyll content, hasten flowering time, increased fruit yield as well as total sugar and vitamin C content in the fruits. Additionally, the thickness of vascular tissues and cortex layer in the stem is increased through packlobutrazol treatment.

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PROMOTING GROWTH AND TUBER FORMATION OF POTATO PRODUCTION AT LOWLAND BENGKULU BY APPLYING ANTI-GA (GIBERELLIC ACID) AND LOWERING SOIL TEMPERATURES

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ABSTRACT

The overall goal of this experiment was to establish a technology for growing potato at the low elevation. There were three groups of experiment with its own separate objective. The first experiment was to determine the best time (1, 2, 3, and 4 week after emergence) for applying retardant at their effective concentration (4 ppm ANZ, 1200 ppm CCC, 50 ppm COU, and 4000 ppm PBZ) for promoting potato tuber formation. The second experiment was to determine the best combination of time for watering potato crops and time for applying retardant (Coumarin) to promote tuber formation. The third experiment was to find out the best mulch type in reducing soil temperatures. Results showed that each retardant had its own best timing for application, 12:00 WIB was the best time for watering potato crops, irrigation significantly reduce maximum soil temperatures, and silver mulch along with organic mulches were the best mulches for reducing maximum soil temperature.

INTRODUCTION

The centre production for potato (*Solanum tuberosum* L.) in Indonesian generally take places in highland altitude, such as Pengalengan, Lembang, and Cipanas (West Java), Dieng Highland (Cebtral Java), Batu (East Java), Brastagi (North Sumatera), and South Sulawesi highland (International Potato Center, 2001). This is agronomically well-understood since potato plants will optimally produce its tuber at the temperature of 17-20 ºC (Haynes *et al*., 1988; Stark and Love, 2003; Suharjo, 2006). Several Indonesian researchers, however, have tried to develop technology production of potato in lower altitude in order to increase national production of potato (Sutater *et al*., 1987; Syarif, 2004; Wicaksana, 2001). Unfortunately, the results were unable to answer whether potato grown in lowland produces tubers as good as highland potato production.

According to Suharjo (2006) the main constraint for potato production in lowland altitudes is related to high temperatures, particularly night temperatures and soil temperatures. At high temperatures, the tuber formation (change from stolon to tuber) is significantly (Stark and Love, 2003), and increases the biosynthesis of gibberellic acid (GA) in leaf buds (Menzel, 1983). It has been well reported that GA inhibited the tuber formation (Vreugdenhil *et al.,* 1998). Many researchers, however, reported that the negative effects of GA can be removed by anti GA applications, such as CCC (Menzel, 1980; Mardalena, 2006), Ancymidol (Escalante and Langille, 1998), Paclobutrazol (Wang and Langille, 2005), or Coumarine (Andrianie, 2006). High temperatures also has been well-known to increase crop respiration, decrease rate of photosynthesis, decrease assimilate partitioning to roots and tubers, decrease sucrose conversion to starch (Reynold *et al.*, 1990; Sarquis *et al.,* 1996). These all significantly inhibit growth and tuber formation of potato plants.

High temperature decreased potato production by inhibiting starch synthesis in the tuber (Krauss and Marcheshner, 1984) or decrease the assimilate partitioning to tuber (Basu and Minhas, 1991), and increase assimilate partitioning the above-ground parts (Gawronska *et al.,* 1992). In addition, high air temperatures (30-35 ºC) have bigger negative effects to the tuber formation than high soil temperatures do (Ewing and Struik, 1992). High soil temperatures will not inhibit the signaling for tuber formation, it will

inhibit the development of stolon to become tuber (Jackson, 1999). It is therefore crucially important to decrease temperatures in potato's rizosphere for lowland potato production.

Research conducted by Ewing and Struik (1992); Fernie and Willmitzer (2001); Jackson (1999) indicated that mode of action of tuber formation inhibition by high temperature takes place as a result of increased GA in stolon. This result was confirmed by the applications biosynthesis inhibitor anti-GAs, such as chloroethyltrimethylamonium chloride (CCC) (Jackson, 1999), ancymidol (Jackson and Prat, 1996; Escalante and Langille, 1998). Paclobutrazol and CCC (Kusmawati, 2006), as well as Coumarine (Andriani, 2006) to minimize the negative effects high temperatures.

It is also important to mention, however, that anti-GA may reduced potato growth when it is applied inappropriately. *In vitr*o research conducted by Kusmawati, 2006) reported that when CCC is applied at the early stage of potato growth, potato grew well at 0 ppm CCC, decreased at 250 ppm, and completely stopped at 750 ppm, either at 18 ºC or 25 ºC air temperatures. However, when it is applied on six-week old explants, CCC increased the total tuber number. This increased was getting better at higher concentrations, particularly at high temperatures. Similar results were also reported when coumarine (Andriani, 2006) and the combinations of CCC and cytokinin (Mardalena, 2006) were applied. Those results indicated that the time of anti-GA application is very important to be determined in order to get good tuber formations at high temperature condition. Since GA biosynthesis takes place in leaf buds (Menzel, 1983), the application of anti-GA should be more effective through foliar application would be more efficient than through soil or irrigation. However, which technique is more efficient has not been well documented and need to be further investigated.

The objectives of this experiment are (a) to determine the proper time of application of growth retardants with effective concentration b) to determine the type mulch that effective to decrease the rizosphere temperatures, and c) to determine the effective time for growth retardant application and time of watering to promote tuber formation and development.

METHODS

Serial experiments were conducted in the screen-house and experimental site of Agronomy laboratory, faculty of Agriculture, Universitas Bengkulu from February to October 2008. The experiments were arranged in bi-factorial Randomized Completely Design. This experiment used recommended concentrations of growth retardant from the previous *in vitro* experiments at 30/25 ºC, i.e., 4 ppm Ancymidol, 4000 ppm Paclobutrazol, 1200 ppm CCC, and 50 ppm Coumarin. Data were analyzed with Analysis of Variance and DMRT at 5%.

(1) Evaluation of time of growth retardant's application. Four types of growth retardant with its respective effective concentration were applied to potato plants grown in polybag at 1, 2, 3, and 4 weeks after emergence.

(2). Evaluation of growth retardant application and time of watering. Four types of growth retardant with its respective effective concentration were applied to potato plants grown in polybag at 1, 2, 3, and 4 weeks after emergence in combinations with time of plant watering.

(3). Evaluation of mulch application. Plastic mulches (red plastic mulch, transparent plastic mulch, black plastic mulch, and black-silvered plastic mulch), and organic mulches (rice straw and hulk) were used to reduce rizosphere temperatures of potato plants grown in polybags at 10 m altitude.

RESULTS AND DISCUSSION

Time of Growth Retardant Application

Results indicated that four weeks after plant emergence could be considered as the ideal time for application the applications of growth retardant to promote tuber growth and formation (Table 1), except for Ancymidol (4 ppm) can be applied at anytime. Four-weeks old of potato plants generally have 6-8 leaves where foliar application of retardant could effectively absorbed by plants.

Table 1. Effects of growth retardant and time of retardant applications on tuber formation (tuber number)

Type of Growth Retardant	Time of application (weeks after emergence)			
	1	2	3	**4**
Ancymidol	7.1 c	8.3 d	8.4 d	8.7 d
Paclobutrazol	3.7 a	4.1 a	3.9 a	5.7 b
CCC	5.3 b	7.6 c	7.9 cd	7.2 c
Coumarin	5.9 bc	5.0 ab	5.6 b	5.7 b
Average	5.4	6.3	6.5	6.8

Mean treatments followed by the same letter in the same column are not significantly different according to DMRT at 5 %

Table 2. Effects of growth retardant and time of retardant applications on total tuber weight (g)

Type of Growth Retardant	Time of application (weeks after emergence)			
	1	2	3	**4**
Ancymidol	4.64 a	4.22 a	2.21 b	**2.41 b**
Paclobutrazol	3.72 a	4.13 a	0.00 b	**0.00 b**
CCC	5.12 a	3.81 ab	2.61 b	**3.13 ab**
Coumarin	4.34 a	3.12 b	0.00 b	**0.00 c**
Average	4.43	5.3	2.41	2.77

Mean treatments followed by the same letter in the same column are not significantly different according to DMRT at 5 %

Table 3. Effects of growth retardant and time of applications on tuber diameter (cm)

Type of Growth Retardant	Time of application (weeks after emergence)			
	1	2	3	**4**
Ancymidol	1.21 a	1.16 a	0.53 b	**0.65 b**
Paclobutrazol	1.00 a	0.88 a	0.00 c	**0.00 b**
CCC	0.34 b	0.29 b	0.67 a	**0.23 b**
Coumarin	0.72 a	1.15 b	0.00 b	**0.00 c**
Average	0.82		0.23	0.22

*Mean treatments followed by the same letter in the same column are not significantly dif**ferent according to DMRT at 5 %***

Combination between Time of Growth Retardant Application and Watering

Watering significantly decreased the maximum rizosphere temperatures, but not the minimum temperatures (Table 4). Although the minimum temperatures did not decreased, time of watering significantly affected the number and the quality of tubers (Table 5 and Figure 1).

Table 4. Effects of watering on soil / rizosphere temperatures

Treatments	Temperatures (°C)	
	Minimum	**Maximum**
Watering	24.5	**37.5**
Not watering	25.5	31.0
Watering at 06.00 hour	24.9	31.5
Watering at 12.00 hour	24.9	31.9
Watering at 18.00 hour	24.9	32

Table 5. Effects of time of watering on stolon number (SN), number of tuber (NT), percentage of stolon to become tuber (PSBT), total weight of tuber (TWT), average tuber weight (ATW), weight of the biggest tuber (WBT), weight of the smallest tuber (WST), average tuber diameter (ATD), diameter of the biggest tuber (DBT), and diameter smallest tuber (DST).

Time of watering (hours)	Variables									
	SN	NT	PSBT (%)	TWT (g)	ATW (g)	WBT (g)	WST (g)	ATD (mm)	DBT (mm)	DST (mm)
06:00	9.9b	3.69ab	37.2b	50.9b	17.2b	26.1a	6.3ab	20.3a	26.5a	11.7a
12:00	8.3b	6.91a	83.3a	98.2a	25.4a	30.4a	9.2a	19.7a	28.8a	11.6a
18:00	12.9a	2.25b	17.5c	60.1b	15.4b	30.9a	3.9b	19.3a	28.2a	10.8a

Watering and Mulch Application

The application of mulches significantly decreased maximum soil temperatures, though it was not significantly reduced the minimum soil temperatures.

Table 6. Effects of mulch type on soil temperatures

Jenis Mulsa	Suhu minimum (oC)	Suhu maksimum (oC)
Tanpa Mulsa	24,8	37,5
Jerami Padi	25,2	35,0
Sekam Padi	25,0	35,0
Plastik Hitam	25,0	35,0
Plastik Bening	25,2	37,0
Sekam Merah	24,8	34,0
Jerami Perak	25,5	34,0

CONCLUSIONS

1. Each growth retardant has different effective time of application to promote the tuber growth and formation of potato plants.
2. Although watering reduced rizosphere's temperatures, time of watering did not decrease the minimum temperature.
3. Potato plants that irrigated during the day-time produced the highest number of tuber compared to other watering treatments
4. Silvered-black plastic mulch decreased maximum soil temperatures as good as organic mulch does. While transparent plastic mulch increased the rizosphere's temperatures during the day time

We recommend that retardants be applied at four weeks after plant emergence, e combined with day time irrigation, as well as by using the organic mulch or black-silvered plastic mulch.

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ANTI SURFACE UNIT (SU) ANTIBODY RESPONSE OF BALB/C MICE IMMUNIZED WITH SPLEEN AND TISSUE CULTURE VACCINE OF JEMBRANA DISEASE VIRUS

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ABSTRACT

Bali cattle are one of the Indonesia's national assets which need to be conserved as they have many advantages. They are however susceptible to many infection disease such as jembrana disease. Currently, the disease is prevented by immunized using vaccine derived from jembrana disease virus (jdv)-infected bali cattle. An alternative vaccine using jdv-infected lymphocyte culture is expected to increase the virus yield and is therefore likely to increase the antibody response in the vaccinated animals. A study was therefore conducted to compare the anti-surface unit antibody response of balb/c mice immunized with vaccine derived from the spleen of infected bali cattle (spleen vaccine) and those immunized with vaccine with vaccine derived from infected lymphocyte culture (tissue culture vaccine). As many as 32 female balb/c mice were divided into two groups, each group was immunized 4 times every 2 week's respectively with spleen and culture vaccines. The antibody response against the surface unit protein of jdv was determined using enzyme-linked immunosorbent assay (elisa) and the absorbance reading of mice sera from each group was compared. t-student and univariate analysis showed that the average absorbance reading sera of sera derived from mice immunized with spleen vaccine (0.36) was significantly different from those immunized with tissue culture vaccine (0.75). It appears that culture vaccine is able to induce anti-surface unit antibody response higher than spleen vaccine.

Keywords: anti-surface unit antibody, spleen vaccine, tissue culture vaccine, jembrana disease virus.

INTRODUCTION

Bali cattle are one of the Indonesia's national assets which have to be conserved properly because they have advantages, such as contributes to meat production, acts as a source of income, generates employment, proves manure for improving soil fertility and can provides drought power. However, the Bali cattle are very sensitive to some diseases specially jembrana disease caused jembrana disease virus (jdv). Prevention to jdv has been done by vaccination using whole inactivated virus isolated from infected spleen, namely spleen vaccine. This kind of vaccine is able to induce immune response with 70% of protection. Non-maximal protection produced by this vaccine would lead to new infection of jdv in the farm. This protection is believed can be increased. Culturing the virus in vitro is one of methods to increase the number of virus (jdv-infected lymphocytes cultures) to develop an alternative vaccine to increase antibody response namely tissue culture vaccine.

MATERIALS AND METHODS

In this study, the jembrana virus (jdv) was cultured in lymphocyte cells in the presence of concanavalin-a (con-a) and interlekin-2 (il-2). Normal Bali cattle lymphocytes cells were cultured for 3 days before coinfected with infected peripheral mononucleus cell (pbmc) collected from jdv infected cattle. This cells mixture was then incubated for another 2-3 weeks before it was used as a seed virus for tissue culture vaccine. The virus was inactivated with triton x-100, emulsified with mineral oil adjuvant and called as a tissue culture vaccine. Efficacy (humoral immune response) of this tissue culture vaccine then compared to the available conventional (whole

inactivated spleen) vaccine in balb/c mice using post test only control group design. before the tissue culture vaccine inoculated into mice, the quantity and quality of both kind of vaccines tested by immunohistochemistry (ihc) using monoclonal antibodies to know the number of cells infected by jdv and with sds page using monoclonal anti-surface unit antibodies (abmo anti su) and polyclonal antibodies (abpo) to show the presence of jdv's specific proteins. As many as 32 female balb/c mice were divided into two groups, each group was immunized 4 times every 2 weeks respectively with spleen and tissue culture vaccines. The antibody response against the surface unit protein of jdv was determined by using enzyme-linked immunosorbent assay (elisa) and by comparing the absorbance reading of mice sera from each group.

RESULTS AND DISCUSSIONS

The result of the immunohistochemistry test indicated that number of cells infected in lymphocyte cell culture was approximately six times higher (57.4%) than in infected lymphocytes spleen cells (9.5%). The western immunoblotting showed that major jd viral protein of p 45 kda was detected in both lymphocyte culture and spleen cells with monoclonal antibody. Other important jd viral proteins of 16 kda, 21.5 kda, 26 kda, 29.7 kda, 40 kda and 50 kda were also detected if polyclonal antibodies were used.

While in enzyme linked immunosorbance assay (elisa), based on the absorbance value, both spleen and tissue culture vaccine induced antibodies against su antigen. Analysis of univariat showed that the absorbance value of antibodies against su protein of the culture vaccine was significantly higher ($p<0.01$) than response of antibodies against su protein of the spleen vaccine. t-student and univariat analysis showed that the average absorbance reading sera of sera derived from mice immunized with spleen vaccine (0.36) was significantly different from those immunized with tissue culture vaccine (0.75). Despite the fact, that the tissue culture vaccine against su antigen, it can be considered as an alternative vaccine for jd.

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SUCCESSIVE SPAWNING STUDY ON AUSTRALIAN RED CLAW CRAYFISH (*Cherax quadricarinatus*): I. EFFECT OF PROTEIN AND ENERGY CONTENT OF FEED ON DURATION INTER SPAWNING

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ABSTRACT

Observation on successive spawning on Australian Red Claw Crayfish (Cherax quadricarinatus) has been conducted in Laboratory of Animal Physiology, School of Life Science and Technology, Bandung Institute of Technology, from October 2009 until July 2010. Crayfish (8 adult female: 3 adult male per aquaria) were kept in 3 glass of aquaria (100 x 50 x 25 cm) until the female completed two successive spawning. Three kinds of experimental feeds were tested, varied in protein and energy content, i.e. 33%:3.8 kcal/g feed; 36%:3.8 kcal/g feed and 36%:4.2 kcal/g feed, respectively. Variables observed were duration inter spawning, defined as the length (in days) between two consecutive "manually released embryo" from bearing female, and some nutritional status of female measured after two consecutive spawning. The results of the study showed that the female has been kept from 90 to 279 d with mean 175, to complete two consecutive spawning. Duration inter spawning ranged from 49 to175d and mean ± standard deviation and minimum-maximum for the three experimental feeds were: 82.25 ± 38.477 (49-147) d ; 115.50 ± 45.211 (56-175) d and 110.25 ± 15.313 (84-126) d, respectively. Analysis of variance showed that those data were not significantly different for all experimental feeds. Molting between two consecutive spawning that occurs on some female, presumably extend the length of duration inter spawning content of hepatopancreas showed the lowest in 33% : 3.389 kcal/g feed and significantly different with other treatments. Biochemical analysis on hepatopancreas showed protein content was not significantly different, while lipid and carbohydrate content are.

Keywords *: Red Claw Crayfish, Successive spawning, Nutritional State, Feed Effect.*

INTRODUCTION

Australian Red Claw Crayfish (*Cherax quadricarinatus*) were introduced to Indonesia as ornamental crustacean from Queensland-Australia in 1990. However, in the last few years, its culture paradigm has changed to consumption crustacean, and nowadays it has spread to the whole of Indonesia (Lukito dan Prayugo, 2007). In Indonesia, red claw crayfish hatchery unit, commonly operate as backyard scale using a relative small glass aquaria. Usually, female crayfish were fed by commercial shrimp feed that is not specifically made for crayfish. That kind of feed presumably does not give optimal result, as Fotedar (1998) reported that the same nutrition applied for different culture system will give a different response. It also is known that commercial feed is more suitable for relatively wide natural pond, whereas for the smaller media like aquaria, semi purified diet is more suitable Fotedar (1998). In addition, protein and energy content of the commercial feed is not certainly optimal for supporting reproduction process of crayfish.

In some observations on feed effect on reproduction, effect of feed were measured directly on "the following spawning", and the period of feeding of experimental feed was relatively short about 70 days (Rodrìguez-Gonzáles *et al*., 2006). This method was assumed can not reflect the effect of experimental feed, since effect of feed on reproduction is presumably "long term effect". Food intake firstly is used to develop the nutritional status (i.e. hepatosomatic indices), then this nutritional status determines

reproduction. Based on this hypothesis, this research was conducted with extended observation, through two consecutive spawning using reproductive parameter called "duration inter spawning" (DIS). This parameter was on the basis of individual observation, different from previous population based study. The research was conducted to verify whether nutrition manipulation (rising protein and energy content, and combine both commercial feed and purified raw material to get semi purified diet) can make duration inter spawning shorter.

MATERIALS AND METHODS

This study was conducted in Laboratory of Animal Physiology, School of Life Science and Technology, Bandung Institute of Technology, from October 2009 until July 2010. Three aquaria (100 x 50 x 25 cm) were prepared to evaluate the effect of three experimental feed (Feed J: Protein 33% : Energy 3.8 kcal/g feed; Feed K: Protein 36% : Energy 3.8 kcal/g feed; Feed L: Protein 36%: Energy 4.2 kcal/g feed) on 24 adult female crayfish (8 replications for each feed treatment). Aquarium equipped with aerator that supplied oxygen continuously and plastic chicken egg racks as previously described (Idris *et al*., 2009), as shelter. Water temperature was set to 28 ± 1ºC with 200 watt water heater. Animal test (average weight 45.9482 ± 9.0650 g; range 31.0563 – 69.0732 g) were tagged individually (Idris *et al*., 2009) and were stocked with sex ratio 3 male: 8 female. They were kept until the female completed the successive spawning. Crayfish were fed twice a day, 1% of biomass per each, but it was reduced on the next feeding if the previous feeding was uneaten. Molting crayfish was recorded, and the animal retagged when exoskeleton harden. Every week, shelters were remove from the aquaria, female crayfish were checked and egg bearing females were recorded as the first day of spawning. Then, the 50% of water was replaced. Egg bearing female was kept in the same aquaria. When embryo attached the pleopod at reaching the 5th stage of embryonic developmental characteristics (eyes visible, Jones, 1995, usually 28 days after the first day of egg of spawning indicated), embryos was manually released. Female was then retransferred to aquarium and were kept until the next spawning. The day when embryos manually was released, it was determined as the first day of duration inter spawning (DIS). On the second spawning, the same criterion was applied, and that day of manually released embryos was determined as the last day of DIS. After manually releasing embryos, the female was euthanized in freezer about 3 hours. Isolated organ was also weighed and all measured data were used in the following formula:

1. Gonadosomatic Index base on Body Weight [GSIB = 100*(ovary weight)/(body weight – ovary weight)] (Mc Rae, 1998)
2. Hepatosomatic Index base on Body Weight [HSIB = 100*(Hepatosomatic weight)/(body weight-hepatopancreas weight)] (Mc Rae, 1998)

Protein (Kjeldhal method), lipid (Soxhlet) and carbohydrate (by different) content of hepatopancreas and experimental feed were analyzed in Laboratory of Ruminant & Chemical Nutrition, Fac.of Animal Husbandry, Univ.of Padjadjaran, Bandung. Energy content of hepatopancreas and tail muscle (bomb calorimeter) also measured in that laboratory.

Experimental design method applied in this study was completely randomized design (CRD). Differences in all measured parameters were analyzed by one-way Anova. Statistical analysis was performed by using computer software SPSS 16. If the result of one-way Anova was a significantly different between groups, then Post Hoc Multiple Comparison "Least Significant Difference" or "Tamhane" (if Equal Variances Not Assumed) was applied.

RESULTS AND DISCUSSIONS

Female has been kept from 90 to 279 days to complete two consecutive spawning (Feed J: 140.75 ± 41.286; Feed K: 191.12 ± 70.023 and Feed L: 194.62 ± 49.460 days). Female can interrupt two consecutive spawning with molting─ physiological event that clearly extend the length of DIS. Although energy reserve needed for spawning was greater than in this experiment, both physiological events were assumed equal. So, duration from the first spawning to the next molting could be used to exchange DIS. Figure 1 present in comparing DIS and combination of D to MIS +28 and DIS (for the female without molting), it shows that no significant difference was found for DIS, but it was on DIS or D to MIS +28

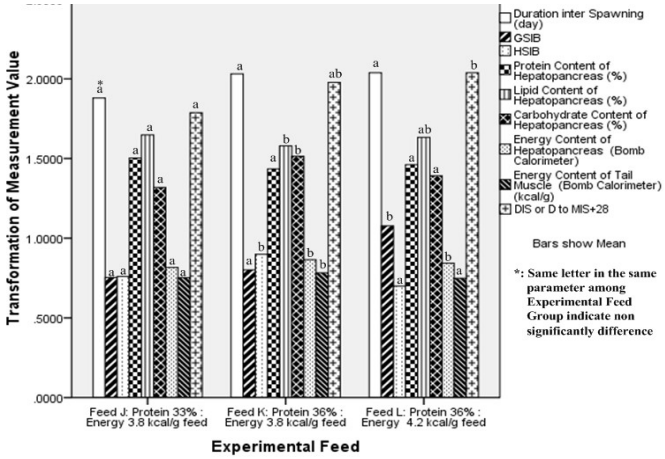

Figure 1. Response of some parameters of Red Claw Crayfish (*Cherax canquadricarinatus*) female Fed 3 Kinds of Experimental Feed

Parameter of DIS or D to MIS +28 showed that 33% protein and 3.8 kcal/g energy ~~/g~~ (Feed J) was a better for the female, but the highest level of protein and energy content (Feed L) showed the best for GSIB. HSIB, a nutritional status that reflect energy content of hepatopancreas, showed the best response on Feed K, parallel with that on energy content of hepatopancreas. This result implies that protein and energy level of Feed K can support female to deposit energy reserve in hepatopancreas, and increase energy level to 4.5 kcal/g feed (Feed L) enable female to maximize vitellogenesis, the process of forming vitellin─ substance which form yolk on mature oocyte─ from vitellogenin, precursor substance in the hemolymph and hepatopancreas (Mc Rae, 1998). This process resulted in the highest level of GSIB on Feed L but the rest of reserved energy in hepatopancreas depleted under that in Feed K. Rodrìguez-Gonzáles *et al*. (2006) stated that GSI and maturation stage of *Cherax quadricarinatus* closely related to the level of protein and lipid in gonad. In this study, it was presumably concurrent with the level on feed. It also shows that the experimental feed didn't affect protein content of hepatopancreas. Increasing protein level of feed from 33% (Feed J) to 36% (Feed K) increased carbohydrate level in hepatopancreas, on the contrary, decreased protein and lipid level of that organ. It is quite different from Rodrìguez-Gonzáles *et al*. (2009) report, that the increasing of crude protein in diet also increased both protein and carbohydrate of hepatopancreas. In Rodrìguez-Gonzáles *et al*. (2009) study, biochemical composition of hepatopancreas could be explained by composition of the diet, but in this study, it couldn't be explained. It might be different result of them was caused by the difference in method. In Rodrìguez-Gonzáles *et al*. (2009) study, biochemical analysis was conducted after feeding for 70 days, but in this study biochemical analysis was conducted after two

consecutive spawning (feeding for 90 to 279 days (mean 175 days) and the hepatopancreas organ isolated from egg bearing female.

It can be concluded, protein and energy level of 33% and 3.8 kcal/g feed is recommended (Feed J) to support spawning or molting of the female. This protein level was similar to Rodrìguez-Gonzáles *et al*. (2009) recommendation, that was 33%, but differ slightly in energy content (15.6 mg kJ^{-1}, converted to kcal/g feed = 4.66, for Rodrìguez-Gonzáles *et al*. 2009). The highest GSIB was longer in DIS for Feed L, revealed that energy content of feed for shortening DIS still need for further study.

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THE 5'-END NON-CODING REGION AND CODING REGION OF POLYMERASE GENE COMPLEX OF BIRD FLU VIRUS FROM POULTRY AND SWINE IN INDONESIA

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ABSTRACT

Studies on the viral genetic factors that influence host specificity and pathogenecity of avian influenza virus of H5N1 subtype (AIV H5N1) or bird fllu virus are invaluable to predict the pathogenicity of the isolates. This study was done to elucidate the possibility of nucleotide (nt) sequence variation of non-coding region (NCR) of 5'-ends of positive strand cRNA as well as nt and deduced amino acid (aa) sequences of 5- end coding region (CR) of PB2, PB1, and PA genes of isolates from chicken, duck, quail and swine. The object of the study was AIV H5N1 isolates that were isolated from chicken, duck, quail, and swine from Java and Bali in 2004-2007. The isolates belong to Faculty of Veterinary Medicine of Udayana University Bali, Gadjah Mada University Yogyakarta, and Bogor Agricultural University. The isolates were propagated in specific pathogenic free (SPF) chicken eggs of 9 days-old embryo. The targeted gene fragments were produced with reverse transcriptase-polymerase chain reaction (RT-PCR) using published universal forward primers and specifically designed backward primers. Reverse Transcriptase Polymerase Chain Reaction products were sequenced using automated DNA sequencer in Eijkman Institute for Molecular Biology, Jakarta. All nt sequences and the corresponding aa sequences were aligned and deduced using Clustal W method of Mega 4. It was concluded that sequence variation of NCR of PB2 and PA genes between AIV isolates of poultry (chicken, duck, and quail) and mammal (pig) was not found. Sequence variation of NCR of PB1 gene between AIV isolated from those species was also not found, except that one variant sequence of 5'-AGCGAAAGCAGGCAAAC*T*ATTTGA-3' (the substituton C-7T is written in italic and underlined) was observed in one isolate from duck (Duck/Badung/2006). Host diversity and geographical distribution did not influence the pattern of the 5-end CR sequences of PB2, PB1, PA genes. Furthermore, the 5-end CR of PB2, PB1, and PA genes of all isolates under study do not show any specific geographical and species pattern.

Keywords: 5'-End non-coding region, coding region, bird flu virus, polymerase genes complex, sequence analysis.

INTRODUCTION

H5N1 influenza viruses are transmitted from poultry to humans in Asia cause high mortality and pose a pandemic threat. viral genes important for cell tropism and replication efficiency must be identified to elucidate and target virulence factors (Salomon *et. al*., 2006). Studies on the viral genetic factors that influence host specificity and pathogenecity of avian influenza virus of H5N1 subtype (AIV H5N1) are invaluable to predict the biology of the isolates (Wasilenko *et. al*., 2008). As widely accepted, those determining factors are located in various gene segments (Wright and Webster., 2001). However, recent studies show that species adaptation determinants have been located in the polymerase gene complex (Salomon *et. al*., 2006). The amino acid at 627 position is adaptation factor of avian influenza virus on mamalia spesies (Gabriel et.al., 2005). Both the coding and the segment-specific non-coding region of the influenza A virus NS segment for its efficient incorporation into virions (Fujii *et. al*, 2005).

This study was done to elucidate the possibility of nucleotide (nt) sequence variation of non-coding region (NCR) of 5'-end of positive strand cRNA as well as nt and deduced amino acid (aa) sequences of coding region (CR) of PB2, PB1, and PA, of various isolates from chicken, duck, quail and swine. Those gene fragments have never

been studied previously. The study was based on the knowledge that those gene fragments influence various phases of viral replication and interact with viral and cellular proteins, so it probably play a significant role in host specificity.

MATERIALS AND METHODS

Twenty six isolates avian influenza (AI) virus H5N1 from chicken, duck, quail, and swine from Java and Bali in 2004-2007 were isolated. The total number of isolates under study was 26, which included nine chicken, seven duck, five quail and five swine isolates. The isolates were collected during AI outbreak in troughout Indonesia between 2004 to 2007. The isolates were the collection of the Faculty of Veterinary Medicine of Udayana University Bali, Gajah Mada University Yogyakarta, and Bogor Agricultural University.

Propagation and identification of isolates were done in accordance to the standard international procedure of WHO (2002) in specifically designed and isolated room with HEPA filtered inlet and outlet air. The room was equipped with negative pressured Biosafety Cabinet Class III (BSC-III) and utoclave. The isolates were propagated in specific pathogenic free chicken embryo of 9 days-old. Harvested allantoic fluids were tested using standard haemaglutination assay (HA), and haemaglutination inhibition (HI). The bird flu virus was confirmed using standard reverse-transcriptase-polymerase chain reaction (RT-PCR). The RT-PCR of polymerase genes were carried out using published universal forward primers (Hoffman *et. al.,* 2001) and specifically designed backward primers. The later was designed based on sequence database available in GeneBank. The length of the RT-PCR products were expected to be 300-400 bp or 500-600 bp, depended on the successful amplification.

Genomic RNA isolation from alantoic fluid was carried out using Trizol after proteinase K digestion. RT-PCR amplification was done using SuperScript™ III One-Step RT-PCR System with Platinum® Taq DNA Polymerase (Invitrogen). The program of RT-PCR was 60 minutes at 50ºC, seven minutes at 95ºC, and 45 cycles of 45 seconds at 94ºC, 45 seconds at 55ºC, and 90 seconds at 72ºC. Additional five minutes incubation at 72ºC was added at the end of the last cycle. Ten percents of the product were added with loading dye (Bromphenol-blue dan Cyline Cyanol) 1 µl, and electrophoreses in 1% agarose gel in TAE which was added with etidium bromide. The samples were run with 100-bp ladder (Invitrogen) as a marker. The DNA was visualized using ultraviolet transluminator and documented using Polaroid camera.

Positive and specific PCR products were sent to the Eijkman Institute for Molecular Biology, Jakarta. RT-PCR primers were also used for sequencing. PCR products were purified using QIAquick PCR Purification Kit (Qiagen). Sequencing reaction was done using Dye Terminator v3.1 Cycle Sequencing Kit and run in the Automatic DNA sequencer Applied Biosystem 3130 /3130x Genetic Analyzer. Sequence editing and alignment was done using Clusthal W of Mega 4 Software. Estimation of genetic distances and phylogenetic was carried out using Neighbor-Joining method (with Kimura-2 parameter (Tamura *et al*, 2007).

RESULTS AND DISCUSSION

Reverse Transcriptase Polymerase Chain Reaction results showed that some targeted fragments of various isolates could not be amplified successfully from 26 isolates under study, the amplification success of PB2 genes of chicken, duck, quail, and swine isolates were 100%, 28.5%, 60%, and 28.5%, respectively. The percentages of amplifiable PB1 fragment gene were 33.3 % of chicken, 100% of quail, 71.43% of duck, and 40% of swine isolates. In fragment gene of PA, the amplifiabelities of chicken, quail, duck, and swine isolates were 55.56 %, 80%, 85.71 %, and 80%, respectively. This result

showed that another primer sets were needed to amplify the NCR and CR of 5'-end of the polymerase genes. Sequence variation of each isolates seems to cause the unsuccessful amplification. NCR analysis result of PB2, PB1 and PA gene is presented in Table 2, 3 and 4.

Table 2. Analysis of non-coding region of PB2 gene

Isolates	Nukleotida position									
	-27								-1	123
Ck/Badung/2004	AGC	GAA	AGC	AGG	TCA	AAT	ATA	TTC	AAT	ATG
Ck/Kelungkung/2006	...	...	...	...	...	...	...	...	...	...
Ck/Kelungkung/2007	...	...	...	...	...	...	...	...	...	...
Ck/Nusa_Penida/2007	...	...	...	...	...	...	...	...	...	...
Ck/Badung/2007	...	...	...	...	...	...	...	...	...	...
Ck/Denpasar/2007	...	...	...	...	...	...	...	...	...	...
Ck/Klk/2006	...	...	...	...	...	...	...	...	...	...
Q/Solo/2006	...	...	...	...	...	...	...	...	...	...
Q/Klaten/2003	...	...	...	...	...	...	...	...	...	...
Q/DIY/2005	...	...	...	...	...	...	...	...	...	...
Dk/Badung/2006	...	...	...	...	...	...	...	...	...	.

Table 3. Analysis of non-coding region of PB1 gene

Kode Isolates	Nukleotida position									
	-24								-1	123
Ck/Badung/2004	A	GC	GAA	AGC	AGG	CAA	ACC	ATT	TGA	ATG
Ck/Denpasar/2007	.	..	...	...	...	...	...	...	...	...
CK/Kelungkung/2007	.	..	...	...	...	...	...	...	...	...
Q/Sleman/2006	.	..	...	...	...	...	...	...	...	...
Q/Solo/2006	.	..	...	...	...	...	...	...	...	...
Q/Demak/2006	.	..	...	...	...	...	...	...	...	...
Q/Klaten/2003	.	..	...	...	...	...	...	...	...	...
Q/DIY/2005	.	..	...	...	...	...	...	...	...	...
Dk/Badung/2006	.	..	...	...	...	...	..T	...	...	...
Dk/Tulikup/2006	.	..	...	...	...	...	...	...	...	...
Dk/IPB_3_RS/2006	.	..	...	...	...	...	...	...	...	...
Dk/IPB_6_RS/2006	.	..	...	...	...	...	...	...	...	...
Dk/IPB_8_RS/2006	.	..	...	...	...	...	...	...	...	...
SW/Surakarta_B3A/2006	.	..	...	...	...	...	...	...	...	...
Sw/Gianyar/2006	.	..	...	...	...	...	...	...	...	...

The readable sequence of NCR and CR of PB2 was from the position of – 27 to 171 or 442, PB1 from -24 to 234 or 390, and PA from -24 to 190 or 461. The minus (-) means the position before respective start codon of each gene. Alignments of PB2 and PA showed that both genes had a conserved NCR, while PB1 varies. Non-coding region sequence of PB2 gene was 5'-AGCGAAAGCAGGTCAAATATATTCAAT-3'. Non-coding region sequence of PA gene was 5'-AGCGAAAGCAGGTACTGATTCAAA-3'. Almost all isolates of avian influenza virus of H5N1 subtype pose NCR sequence of PB1 gene of 5'-AGCGAAAGCAGGCAAACCATTTGA-3'. However, one isolate, namely Duck/Badung/ 2006, posses an non-coding region (NCR) PB1 variant of 5'-AGCGAAAGCAGGCAAA C*T*ATTTGA-3' (polymorphic nucleic acid is italic and underlined).

Table 4. Analysis of non-coding region of PA gene

Isolates	Nukleotida position												
	-24											-1	123
Ck/Badung/2004	A	G	C	GAA	AGC	AGG	TAC	TGA	TTC	AAA	ATG		
Ck/Nusa_Penida/2007	.	.	.	...	...	...	...	...	...	...	...		
Ck/Kelungkung/2007	.	.	.	...	...	...	...	...	...	...	...		
Q/Solo/2006	.	.	.	...	...	...	...	...	...	...	...		
Q/Demak/2006	.	.	.	...	...	...	...	...	...	...	...		
Q/Klaten/2003	.	.	.	...	...	...	...	...	...	...	...		
Q/DIY/2005	.	.	.	...	...	...	...	...	...	...	...		
Dk/Kelungkung/2006	.	.	.	...	...	...	...	...	...	...	...		
Dk/Tulikup/2006	.	.	.	...	...	...	...	...	...	...	...		
Dk/IPB_3_RS/2006	.	.	.	...	...	...	...	...	...	...	...		
Dk/IPB_6_RS/2006	.	.	.	...	...	...	...	...	...	...	...		
Dk/IPB_8_RS/2006	.	.	.	...	...	...	...	...	...	...	...		
Sw/Surakarta_B3A/2006	.	.	.	...	...	...	...	...	...	...	...		
Sw/Surakarta_B3A1/2006	.	.	.	...	...	...	...	...	...	...	...		
Sw/Kranganyar_B1W28/2006.	.	.	...	...	...	...	...	...	...	...			

Host diversity and geographical distribution did not influence the pattern of the 5'- end CR secuences and the mean genetic distance. Furthermore, the 5- end CR PB2, PB1, and PA genes of all isolates under study did not show any specific geographical and species pattern.

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EMBRYOGENIC CALLUS INDUCTION FROM MALE INFLORESCENCE OF LOCAL BANANA CULTIVARS WITH A VIEW TO PRODUCE *FUSARIUM* WILT RESISTANT PLANT *VIA IN VITRO* SELECTION [2]

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ABSTRACT

This I-MHERE funded research is the first step of a research program to produce *Fusarium* wilt resistant of local banana cultivars using in vitro selection approach. The objectives of this first research step are to produce sufficient material for in vitro selection. The specific objectives of this experiment was to study the influence of auxin (2,4-D and IAA) on embryogenic callus formation from male inflorescence of four local banana cultivars (Raja, Ambon, Ambon Nangka, Kapok Kuning). An experimental research method on a *split-split plot design* has been used. The main plot was local banana cultivars (Raja, Ambon, Ambon Nangka, Kapok Kuning), the sub plot was the kind of auxin (2,4-D, IAA), and the sub-sub plot was auxin concentration (0; 5; 10; 15 µM). All treatment combinations were replicated 3 times. The nutrient medium used was Murashige and Skoog (MS-1962) supplemented with 7.5 µM BAP and solidified with 0.8% agar. The cultures were kept in dark condition at 24 °C for six weeks. The parameters measured include the percentage of callus formation, callus formation time, and the type of callus formed. The research results showed that male inflorescence can be used as explants for mass production of embryogenic calli of 4 local banana cultivars (Raja, Ambon, Ambon Nangka, Kapok Kuning). "Raja" cultivar was found to be the most responsive cultivar leading to the highest percentage of callus formation and short callus formation time. In addition, it was also found that no significance difference between 2,4-D and IAA on both callus formation time and the percentage explants which produced callus, however 2,4-D resulted in better embryogenic calli formation than IAA. Callus formation was controlled by the concentration of auxin applied. The higher the concentration applied, the higher the percentage of explants which produced callus.

Keywords: embryogenic callus, 2,4-D, IAA, Banana

INTRODUCTION

Indonesia is one of most important banana genetic variance centres in the world. Indonesian banana production has increased in the last 10 years from 3.3 million tones in 1999 to 6.0 million tones in 2008. However, its producing area continuously decreases, especially in Sumatra, Sulawesi, Central and east Java (Laporan Akhir Riset Unggulan Strategis Nasional 2003; Dimyati, 2004). The decrease in banana plantation area is mainly caused by pest and disease such as Panama (*Fusarium* wilt), blood, Moko, sigatoka, BBTV, CMV, stem borer and nematode (Dimyati, 2004).

Fusarium wilt disease caused by *Fusarium oxysporum* Schlecht sp cubense (Panama disease) is one of the most dangerous banana diseases. This soil-borne disease can attack banana at all banana growth phase, from seedling to adult plant, and also easily distributed (Cahyono, 1995; Balai Penelitian Tanaman Buah, 2004; Purwati *et al*., 2007). Today those to pathogens have spreaded to almost all banana plantations in Indonesia, which resulted in the destruction of almost 8 million clump of banana in just 5 years period (Companioni *et al*., 2003; Widodo *et al*., 2003; Balai Penelitian Tanaman Buah, 2004; Purwati *et al*., 2007)).

Control of *Fusarium* wilt disease is extremely difficult since this pathogen form chlamidospora which able to live in the soil for a long period of time (Widodo, *et al*., 2003). Furthermore, chemical control of this pathogen is not economically feasible. Therefore efforts to produce *Fusarium* resistance plants are paramount (Companioni *et al*., 2003; Purwati *et al*., 2007).

Somaclonal variation and genetic transformation are two available approaches which can be used to speed up banana improvement program. Callus culture is one of the most important steps in the induction of somaclonal variation and genetic transformation (Da Silva Conceicao *et al*., 1998). Callus culture includes induction and culture of callus *in vitro* with a view to improved plant quality or to obtain plant secondary metabolic product (Adkins *et al*, 1990). The advantage of callus culture is the simplicity in controlling its environment both physically and chemically (Allan, 1991). It can be expected that from this type of culture genetic variant(s) can be obtained trough *somaclonal variation,* or an *in vitro* selection can be carried out to produce a desired character (Adkins, *et al*, 1990). The success of a callus culture depends on the induction stage.

With a view to produce banana embryogenic callus, various explants can be used such as immature male inflorescence (Escalant *et al*., 1994; Ganapathi *et al*., 1999; Gomez Kosky *et al*., 2002; Khalil *et al*, 2002; Sidha *et al*., 2007; Wirakarnain *et al*., 2008), young leaves (Da Silva Conceicao *et al*., 1998), or shoot (Srangsam and Kanchanapoom, 2003; Ramírez-Villalobos and de García, 2008).

Moreover, somaclonal variation to produce *Fusarium* wilt resistance banana can be induced by irradiation (Kosmiatin *et al*., 2006; Zarmiyeni, 2007), *in vitro* selection with toxin such as fusaric acid ($C_{10}H_{13}O_2N$) (Matsumoto *et al*., 1995;; Purwati *et al*., 2007; Zarmiyeni, 2007;) or *Fusarium* culture filtrate (Lestari, 2006).

MATERIALS AND METHODS

The materials used are immature male inflorescence of 4 local banana cultivar (Raja, Ambon, Ambon Nangka, Kapok Kuning), Murashige and Skoog (MS-1962) medium , 2,4 Dichlorophenoxy Acetic Acid (2,4-D), Indole-3-aceticacid (IAA), 6-Benzylaminopurine (BAP), *casein hydrolyzate*, sucrose, agar, $HgCl_2$ 0,2%, *sterril destilate water* (SDW), ethanol 70 % and 96%.

An experimental research method on a *split-split plot design* has been used. The main plot was local banana cultivars (Raja, Ambon, Ambon Nangka, Kapok Kuning), the sub plot was the kind of auxin (2,4-D, IAA), and the sub-sub plot was auxin concentration (0; 5; 10; 15 µM). All treatment combinations were replicated 3 times. The nutrient medium used was Murashige and Skoog (MS-1962) supplemented with 7.5 µM BAP and solidified with 0.8% agar. The cultures were kept in dark condition at 24 ^oC for six weeks. The parameters measured include the percentage of callus formation, callus formation time, and the type of callus formed.

RESULTS AND DISCUSSION

After six weeks of culture under dark conditions, it was observed that most explants were able to dedifferentiate to produce callus/calli (Figure 1). Callus emerged from the surface of explants as early as 17 days after culture. The time needed for callus formation was cultivar dependence. It was found that in general the fastest callus formation was observed in "Ambon Nangka" cultivar (Figure 2). Furthermore, the percentage of explants forming callus ranged from 56.77% to 92.71%. It was also found that "Raja" cultivar had the highest percentage of callus formation, in contrast "Kapok kuning" cultivar produced the lowest percentage of callus formation (Figure 3).

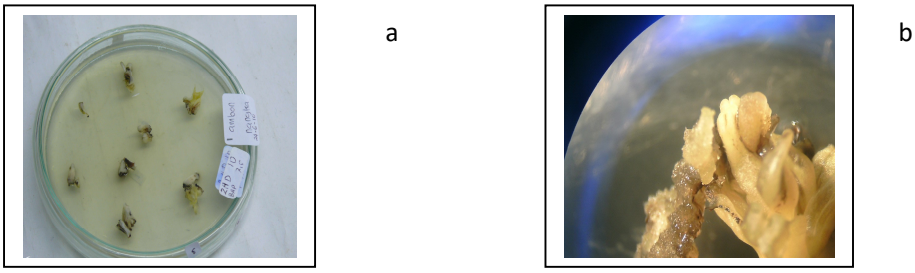

Figure 1. Callus formation: a) explants; b) callus formed on the explants surface

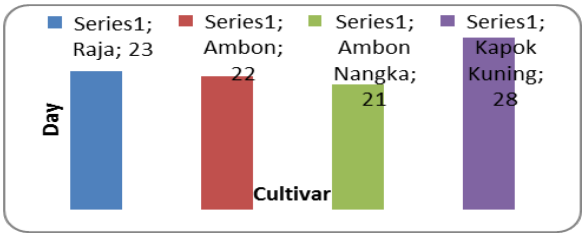

Figure 2. Callus formation time

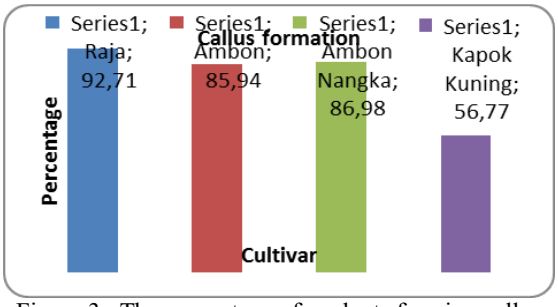

Figure 3. The percentage of explants forming callus

It was also found that both auxins (2,4-D and IAA) can be used to induced callus formation from immature male inflorescence of banana with no significance difference on both callus formation time and the percentage explants which produced callus. However, callus formation was controlled by the concentration of auxin applied. The higher the concentration applied the higher the percentage of explants which produced callus (Figure 4). In addition, no significance difference was observed on the effect of auxin concentration on callus emergence.

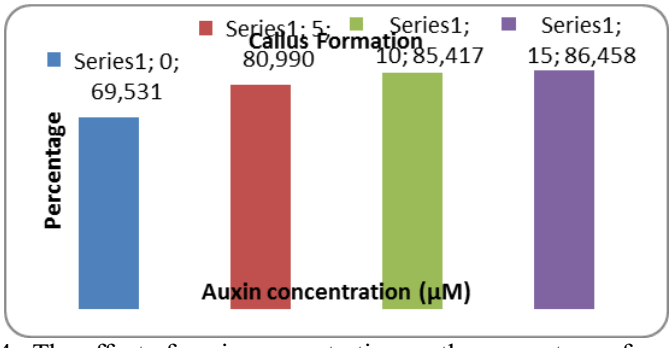

Figure 4. The effect of auxin concentration on the percentage of explants forming callus

Further examination on the type of callus formed, it was found that all three types of callus i.e. embryogenic, proliferative, and senescence were formed (Figure 5). Further data analysis showed that 2,4-D application resulted in higher percentage of embryogenic callus formation. In contrast, IAA application resulted in high percentage of senescence callus formation (Figure 6). However, the high percentage of proliferative and senescence callus formation was not expected.

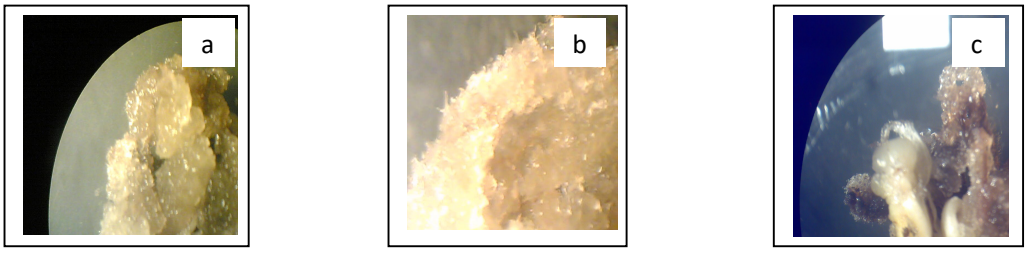

Figure 5. Banana callus type: a) embryogenic; b) proliferative; c) senescence

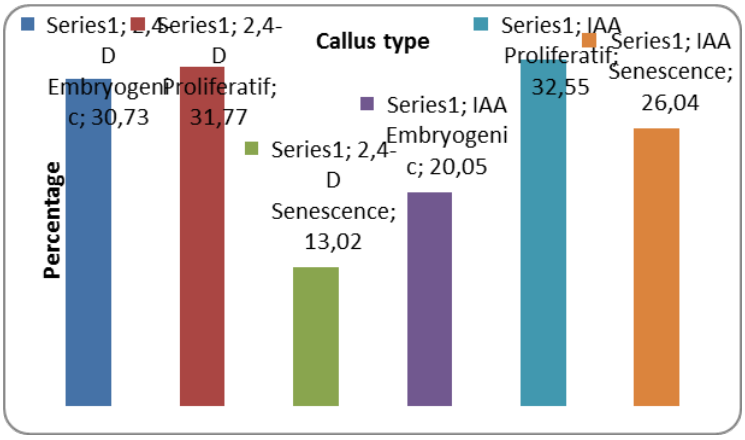

Figure 6. The effect of auxin concentration on the type of callus formed

The successful callus induction from immature male inflorescence of five local banana cultivar implied that banana male inflorescence can be used as explants for mass production of embryogenic calli. Callus induction from immature male inflorescence of banana has also been reported by Escalant *et al*., (1994); Ganapathi *et al*., (1999); Gomez Kosky *et al*., (2002); Khalil *et al*, (2002); Sidha *et al*., (2007); and Wirakarnain *et al*., (2008).

Banana callus induction was carried out on Murashige and Skoog media supplemented with auxin and cytokinin. The used of MS medium, auxin and cytokinin for banana callus induction from immature male inflorescence have been reported elsewhere (Da Silva Conceicao *et al*., 1998; Ganapathi *et al.*, 1999; Gomez Kosky *et al*., 2002; Srangsam and Kanchanapoom, 2003; and Wirakarnain *et al*., 2008). The used of MS media resulted in better embryogenic callus formation that that of White medium (Ganapathi *et al.*, 1999).

In addition, the least expected high percentage of proliferative and senescence callus formation might have been caused by high cytokinin application in callus induction medium. 7.5 µM 6-benzylaminopurine (BAP) was supplemented in all media used. High cytokinin concentration will induce high cell multiplication leading to proliferative and senescence callus formation.

Callus morphology in callus culture can be classified into 3 types (Kesee *et al*, 1991):

a. Developmental/embryogenic callus, i.e. callus which capable of developing into somatic embryogenesis or somatic organogenesis. This type of callus is characterised by the formation of chlorophyll, green in colour, compact and fast growing.
b. Proliferative callus i.e. callus which has a very high multiplication rate leading to the formation of a massive amount of cells but very small in size. This type of callus has very limited cytoplasm which also incapable of regenerating.
c. Senescence callus i.e. a very slow growing callus and shows no symptom of development. This type of callus is characterised by the absence of chlorophyll, brownish in colour, watery, and polyhedral cell form.`

CONCLUSIONS

It can be concluded that male inflorescence can be used as explants for mass production of embryogenic calli of 4 local banana cultivars (Raja, Ambon, Ambon Nangka, Kapok Kuning). "Raja" cultivar was found to be the most responsive cultivar leading to the highest percentage of callus formation and short callus formation time. In addition, it was also found that no significance difference between 2,4-D and IAA on both callus formation time and the percentage explants which produced callus, however 2,4-D resulted in better embryogenic calli formation than IAA. Callus formation was controlled by the concentration of auxin applied. The higher the concentration applied, the higher the percentage of explants which produced callus.

ACKNOWLEDMENTS

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BRINGING DOWN POTATO CROPS TO LOWER ELEVATIONS IN INDONESIA

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ABSTRACT

The objective of this experiment is to promote tuber production at the low (10 m ASL) and medium (600 m ASL) elevations by watering potato crops with cold water (15° C) and by creating mutants fit to the low and medium elevations. Granola and Atlantic grown at the low and medium elevations were evaluated for its ability to produce tubers. Granola and Atlantic were grown at low elevations and were watered with regular (22° C) and cold (15° C) waters. Granola irradiated with 0, 30, and 60 Gy gamma rays were grown in low and medium elevations. Atlantic irradiated with 0, 30, and 60 Gy were grown at low elevation and watered with regular and cold water. The results showed that Atlantic is a prospective cultivar to be promoted at the low elevation, especially when cold watering is applied to the crops. On the other hand, Granola is a prospective mutant to be promoted at the medium elevation after being irradiated with 30 Gy and 60 Gy gamma rays.

Keywords: Potato crops, low elevations, high temperature.

INTRODUCTION

Due to many problems caused by potato growing activities at high elevation, the National Research Council (*Dewan Research National*, DRN) of the Republic of Indonesia has launched a program called *Tropikasi Tanaman Kentang* since 2004 (DRN, 2008). It is basically promoting the practice of growing potatoes at lower elevations. In accordance with the program, *Badan Litbang Pertanian* has adopted the program into BALITBANGTAN's research agenda since 2005 (BALITBANGTAN, 2008).

In Indonesia, potato crops are grown at high elevations, such as the Dieng Plateau and Brastagi, due to its requirement for low temperature to produce maximum yield (Stark and Love, 2003). Growing potato crops at lower elevations will face many problems related to high temperature, such as the increase in disease incidence, in physiological disorder of the tubers (Gawronska *et al.*, 1992; Stark and Love, 2003), in respiration rate (Sarquis *et al*., 1996), and in gibber relic acid (GA_3) biosynthesis (Menzel, 1983). The last two factors will lead to smaller number of tubers (Azhari, 2008) and smaller size of tubers (Popi, 2008). Because of which, a strategic approach needs to be developed to enable farmers to grow potatoes at lower elevations without significant yield lost. The strategy in question would be creating a sound technology for growing potato at lower elevations and creating a new high yielding potato variety adapted to local lower elevations, as presented in more detail in Figure 1 and Figure 2.

Producing Sound Technology

Menzel (1983) reported that the biosynthesis of GA_3 at the potato auxiliary buds increased significantly from 4.0 to 71.8 µg per kg fresh weight when the temperature increases from 20/15 to 35/30° C. Increasing GA_3 biosynthesis was followed by the reduction in tuber formation as shown by the following table (Menzel, 1980).

This report showed that tuber production at high temperature is inhibited by GA_3; however, the negative effect of GA_3 could be overcome by the application of CCC (retardant) and the effect was completely nullified at high concentration of CCC (4 g/L).

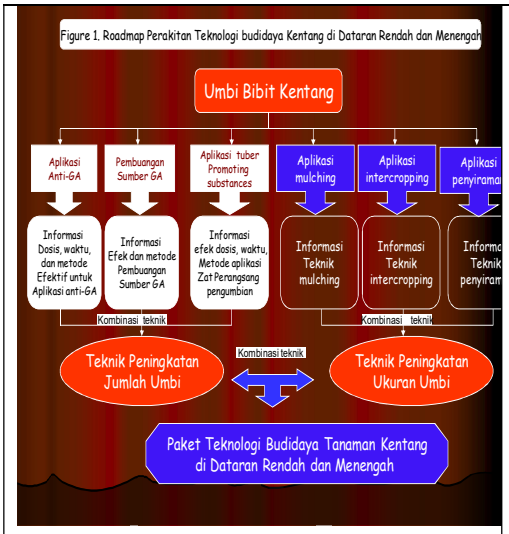

Figure 1. Roadmap Perakitan Teknologi Budidaya Kentang di Dataran Rendah dan Menengah

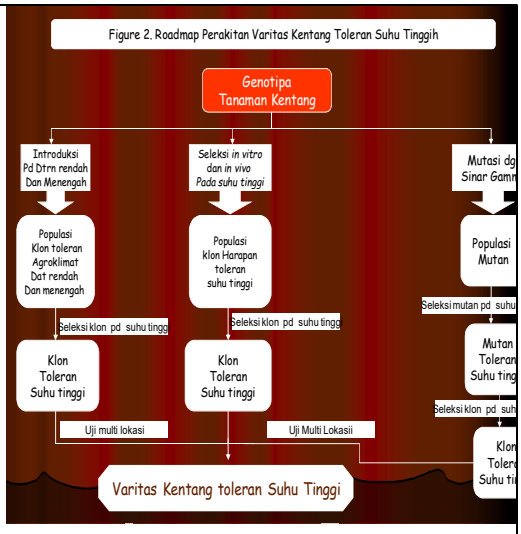

Figure 2. Roadmap Perakitan Varietas Kentang Toleran Suhu Tinggi

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In regard to those findings, since 2006, Suharjo *et al.* (2007) have been working on creating a package of technology for growing potato crops at 10 m and 600 m elevation above sea level (ASL) by (i) Removing the source of GA_3 synthesis, (ii) Reducing or inhibiting GA_3 biosynthesis by applying various retardants, acting as anti-GAs, and (iii) Reducing the rhizosfer temperatures by mulching, intercropping, watering the crops with cold water.

Buds Removal. Our results showed that while removing auxiliary buds, the source of GA_3 biosynthesis, at 600 m elevation gave no significant results compared to the Control treatment (no bud removal), buds removal at 10 m elevation gave very significant results, compared to the Control treatment. At 600 m elevation both treatments produced 8 tubers per hill while there was no tuber produced at 10 m for Control and produced 5 tubers for buds removal (Suharjo *et al*, 2010).

Effective Concentration of Retardants. Suharjo *et al*. (2007) reports that some retardant (Ancymidol, Paclobutrazol, CCC, and Coumarin) have been proven to be effective to promote tuber formation at high temperature (30/25° C). Each retardant has its own effective concentration, such as 4 ppm for Ancymidol, 4000 ppm for Paclobutrazol, 1200 ppm CCC, and 50 ppm Coumarin.

Application Time of Retardant. Grower should pay close attention in regard to the time for applying effective retardants, because of its effect on plant growth. Some retardants significantly inhibit crops growth when applied at the wrong time (Suharjo *et al.*, 2008), as shown in Figure 3.

Watering. Suharjo *et al*. (2008) reports that watering time did not reduce soil temperature (data not shown). However, it significantly affected the number of tuber formed and its size (Figure 4).

Others. More results are to be reported, such as the effect of mulching and intercropping in promoting tuber growth. However, the experiments are not done yet at this very moment.

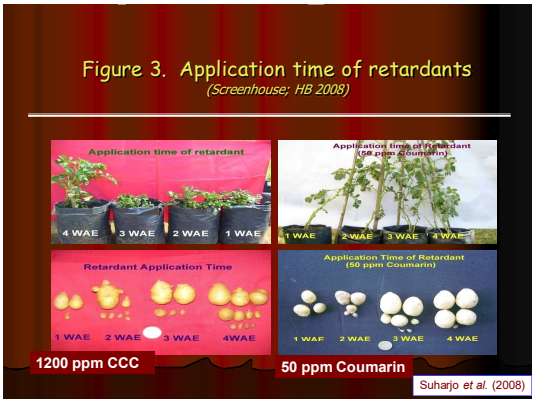

Figure 3. Application time of retardants (Screenhouse HB 2008)

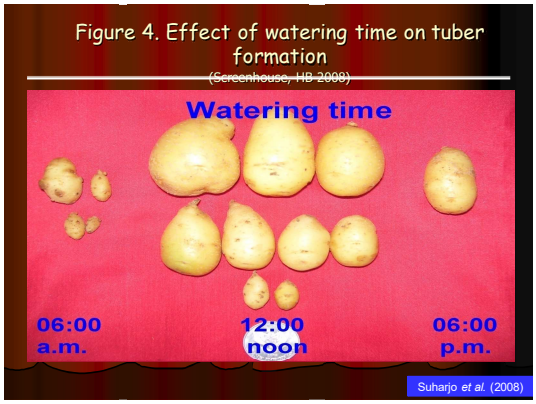

Figure 4. Effect of watering on tuber formation (Screenhouse HB 2008)

Creating New Potato Variety

Creating new potato varieties might be done by introduction, hybridization, selection, and mutation (Suharjo *et al.*, 2009). Among those techniques, selection and mutation are chosen for our purposes, due to very simple reasons. They are doable, faster, and suitable for Indonesian researchers. Due to the nature of genetic characters of potato crops, being polyploidy and therefore more complicated for hybridization (Manrique *et al.*, 1996), as most offspring will be unfertile, mutation is chosen as the alternative method.

Mutation is believed to be an effective method for creating genetic variations (Koornneef, 1991; Micke dan Donini, 1993) although the results are unpredictable (Ismachin, 1988) and very much depends on the irradiation dosage (Al-Saladi *et al*., 2000).

Potato seeds were irradiated with 0, 30, and 60 Gy of Gamma rays and then challenged with high temperature, by growing them at 10 m and 600 m elevations in the Province of Bengkulu. The results show that high level of irradiation delayed plant emergence, reduced seed survival, and improved tolerance to high temperature especially when the crops grown at 600 m elevation (Figure 5).

A. 10 m dpl	B. 600 m dpl

Figure 5. The performance of potato crops at 0 and 600 m elevation following irradiation with gamma rays at 0, 30, and 60 Grays, from left to right (Suharjo *et al*., 2009).

Closing Remarks

Attempts to bring down potato crops to lower elevations have been entering new phase of research strategy, from studying the growth of the crops to designing technology for growing potato at lower elevations combined with finding a new potato variety suitable for local environment.

Our lab has successfully demonstrated that potato could be produced at high temperatures by applying some growth retardants at proper time and by suitable methods. In addition, we also demonstrated that potato mutants could be produced by irradiating potato seed tubers with gamma rays.

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SUSTAINABLE MANAGEMENT OF LAND AGRICULTURE IN BALI BASED ON SOIL HEALTH

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ABSTRACT

Soil health (also called soil quality) is soil conditions that indicate those soil in *health* state has a good characteristics (physically, chemically, and biologically) and sustainable for high soil productivity. When the soil is healthy, roots can explore soil pores to find the water and nutrients needed by the plant. The indicators of physical soil health are the proportion of soil particles, i.e. sand, silt, and clay in top soil and sub soil, whereas the indicators of chemistry soil health are P (phosphorous) and K (potassium) contents, Cation Exchange Capacity (CEC), Base Saturation, and Organic Matter. The activity of the soil micro organism as indicators of biological soil health are biomass, biodiversities, mineralization process, and the of amount organic matter in the soil. Generally, the physical health of soil in Bali was good (its had a medium texture with clay content < 35%); but at some locations like a long the coast area is bad (because of sand content > 70%). The chemistry health of soil is low to medium (it's had high of CEC and Base Saturation, low of Phosphorous and Organic Matter, medium to high of Potassium) ; and the biological health of soil was bad because of low substrate and energy for microorganism which is come from organic matter. The limitation factors of paddy soil in Bali were low of organic matter, nitrogen and phosphorous contents. The dray land (upland) area, generally, managed by traditional system using imbalance chemical fertilizer. To support sustainable management of agricultural land in Bali some actions showed done such as increasing the soil quality (physical, chemistry, and biological) by adding organic matter, using a balance of N, P, K fertilizers, and rotate plantation using alternative agriculture commodities.

INTRODUCTION

In the future, soil will become even more crucial, because of its functions for agriculture, human generation, and animal living, and it has important ecological function in recycling resources needed for maintaining plant, animal, and human health. Environmental protection and food quality become the focus on agriculture management because that factors influence the soil health.

Soil health, also called soil quality, is the capacity of specific kind of soil to function, within natural or managed ecosystem boundaries, to sustain plant and animal productivity, maintain or enhance water and air quality, and support human health and habitation (Karlen *et al*., 1997). Classification of soil health is concerned with soil function to biological productivity, the environment, and different expressions of plant, animal, and/or human health (Doran, 2002). Soil health can be stated in good/bad or low/high. It indicates that the soil has good characteristics (physically, chemically, and biologically) and sustainable for high soil productivity.

Physical and chemistry of soil health can be measured by soil texture and soil fertility status respectively; whereas the biological of soil health can be predicted by soil organic matter in soil, or analyzing the soil biomass and biodiversity. Soil productivity is the capacity of specific soil to plant growth, it's changing greatly on management system, plant production, and soil condition. Soil health can maintain plant and animal growth and productivity, maintain air and water quality, and support human life.

Soil health can be lost by soil degradation, such as: erosion, pollution, desertification, changes in soil chemistry, salination, and loss of soil organic matter (Edward, 2003). Preserving soil health involves understanding the soil function and soil management. The function and services of soil to agriculture, are: (i) the production of biomass; (ii) the use of soil for filtering, buffering, and transforming action, and (iii) the

provision of gene reserve for plant and animal organism (Blum, 1998). A large biomass and a high biodiversity of soil organism may link to the degree of sustainable soil health.

Smyth and Dumanski (1993) stated that sustainable land management combines technologies, policies and activity aimed at integrating socio-economic principles with environmental concerns so as to simultaneously: (i) maintain or enhance production and services, (ii) reduce the level of production risk, (iii) protect the potential of natural resources and prevent degradation of soil and water quality, (iv) be economically viable, and (v) socially acceptable.

Soil health indicators would be too complex to be used by land managers. To identify the most adequate indicators, 7 key parameters were be used such as soil texture (at top soil and subsoil), P (Phosphorous) content, K (Potassium) content, Cation Exchange Capacity (CEC), Base Saturation, Organic Matter, and Biodiversities of soil organism.

METHODS

Secondary data base collected from Soil Science Department, Faculty of Agriculture, Udayana University, and reviews of some other references. The Physical and Chemical health of soil adapted from Sanchez and Buol (1985 *In* Puslittanak, 1985) and Puslittanak (1983) respectively. The biomass and biodiversity of microorganism as biological soil health confirmed by soil organic matter content and standard spread-plate dilution method (Seely and VanDemark, 1981) was used for the estimation.

RESULTS AND DISCUSSION

Soil Health in Bali

1. Physical Soil Health.

Generally, the physical health of soil in Bali was good, it had a medium texture with clay content < 35%; but at some locations like the coastal area was bad, because of sand content > 70% (Table 1).

2. Chemical Soil Health

The chemical health of soil was low to medium, because of high Cation Exchange Capacity (CEC), and Base Saturation (BS), low of Phosphorous and Organic Matter, and medium to high of Potassium (Table 2).

3. Biological Soil Health

The biological health of soil was bad, because of low substrate and energy for microorganism that's come from organic matter. Generally, the organic matter content of soil in Bali was low. Microorganism play key roles in organic matter decomposition, decreasing plant diseases, and increasing plant growth. The mean total of microorganism (biodiversity) depend on soil health (Table 3). Numbers of bacterial, actinomycetes, and fungi in healthy soil (suppressive soil) were more than in unhealthy soil (conducice soil).

Table 1. Physical soil health at some locations in Bali

No	Location	Soil texture						Physical quality/ Physical soil health
		Top soil (0-20 cm)			Subsoil (20-60 cm)			
		Sand (%)	Silt (%)	Clay (%)	Sand (%)	Silt (%)	Clay (%)	
Tabanan Regency								
1	Bangkiangsidem, Selemadeg	48	28	24	54	14	32	**Good** [a)]
2	Perean, Baturiti	50	44	6	42	52	6	**Good**
3	Dukuh, Marga	58	36	6	50	28	22	**Good**
4	Senganankangin, Penebel	74	20	6	71	23	6	**Bad**
Buleleng Regency								
5	Bengkel, Busungbiu	38	60	6	58	36	6	**Good**
6	Ambengan, Sukasada	38	24	38	38	26	36	**Medium**
Gianyar Regency								
7	Sebatu, Tegalalang	58	32	10	44	38	18	**Good**
8	Padangsigi, Tampaksiring	38	52	10	48	42	10	**Good**
9	Siangan, Gianyar	34	60	6	54	40	6	**Good**
Bangli Regency								
10	Susut	48	40	12	44	40	16	**Good**
11	Apuan, Susut	58	32	10	58	28	14	**Good**
Klungkung Regency								
12	Takmung	50	38	12	50	38	12	**Good**
13	Sidayu	54	40	6	54	36	10	**Good**
Karangasem Regency								
14	Sidemen	48	40	12	44	41	15	**Good**
15	**Rendang**	**45**	**40**	**15**	**42**	**43**	**15**	**Good**

Source : Soil Deparment, Faculty of Agriculture, Udayana University. [a)] Data analysis

Table 2. Chemical soil health at some locations in Jembrana Regency of Bali [a)]

Land unit	Site	CEC (me/100g)	BS (%)	P (me/100g)	K (me/100g)	C-org (%)	Soil fertility status	Chemical quality of soil
1	Perancak1	26,37 h	120 vh	16,01 l	35,13 m	1,74 l	Low	**Low**
2	Perancak2	12,27 l	44,45 m	1,97 l	32,06 m	1,33 l	Low	**Low**
3	Baluk 1	20,98 m	115,46 vh	7,88 vl	68,22 vh	0,42 vl	Low	**Low**
4	Baluk 2	39,38 h	98,82 vh	9,84 vl	49,86 t	0,45 vl	Medium	**Medium**
5	Tegal badeng 1	28,57 h	81,25 vh	11,58 l	59,28 t	2,11 m	Medium	**Medium**
6	Tegal badeng 2	35,67 h	109,80 vh	10,51 l	67,73 vh	0,91 vl	Medium	**Medium**
7	Cupel 1	38,97 h	104,55 vh	10,90 l	45,95 t	0,86 vl	Medium	**Medium**
8	Cupel 2	23,51 m	121,90 vh	14,04 l	39,03 m	0,87 vl	Low	**Low**
9	Kaliakah	31,86 h	116,67 vh	3,83 vl	73,46 vh	0,86 vl	Medium	**Medium**
10	Pendem 1	29,36 h	95,39 vh	3,00 vl	18,75 l	1,76 l	Medium	**Medium**
11	Pendem 2	30,81 h	98,46 vh	4,61 vl	29,51 m	1,39 l	Low	**Low**
12	Batu agung	36,82 h	83,95 vh	4,61 vl	64,14 vh	0,45 vl	Medium	**Medium**
13	Berangban1	31,13 h	101,41 vh	10,97 l	21,84 m	2,21 m	Medium	**Medium**
14	Tukad daya	32,17 h	100,70 vh	9,17 vl	70,96 vh	0,85 vl	Medium	**Medium**
15	Dauhwaru 1	32,24 h	112,59 vh	3,25 vl	29,73 m	2,19 m	Medium	**Medium**
16	Dauhwaru 2	32,42 h	109,33 vh	14,88 l	109,42 vh	0,47 vl	Medium	**Medium**
17	Berangban2	42,86 h	82,02 vh	8,32 vl	40,17 m	1,68 m	Medium	**Medium**
18	**Dauhwaru 3**	**27,49 h**	**85,04 vh**	**8,57 vl**	**93,80 vh**	**0,45 vl**	**Medium**	**Medium**

[a)]Adapted from Ninik (2006); l=low, vl=very low, m=medium, h=high, vh=very high

Table 3. The mean total of bacterial, actinomycetes, and fungal in soil (Sudarma *et el.,* 2009)

Soil condition	Disease incidence (%)	Bacteria (cfu)	Actinomycetes (cfu)	Fungi (cfu)
Suppresive soil	0	$1.2x10^6$	$1.5x10^5$	**$3.1x10^4$**
Conducice soil 1	50	$5.9x10^5$	$7.5x10^4$	**$2.8x10^3$**
Conducice soil 2	25	$2.6x10^4$	$5.3x10^4$	**$1.7x10^4$**
Conducice soil 3	37	$2.0x10^5$	$7.3x10^4$	**$3.0x10^3$**
Conducice soil 4	**40**	**$3.9x10^5$**	**$3.7x10^4$**	**$2.3x10^4$**

SUSTAINABLE MANAGEMENT OF LAND AGRICULTURE

The limiting factors of paddy soil in Bali were low of organic matter, Nitrogen and Phosphorous contents. The dry land (upland), generally, managed by traditional system using inbalance chemical fertilizers. The sustainable management of land agriculture in Bali can be done by increasing the soil quality (physical, chemistry, and biological)a adding organic matter, balance of N, P, K fertilizers, and suitable alternative agriculture commodities. These parameters are useful indicators of soil health, because they react sensitively to changes in agriculture management. In the other hand, those parameters also influence the activity and population dynamics of microorganisms in soil. Organic matter contained about 15% of microorganisms cells. Biomass of microorganisms on fertile soil with enough organic matter content was 20 ton/ha approximately. The amount of organic matter associated with the clay plus silt, whereas microbial biomass carbon within those particles highest than sand particle. The groups of soil microorganisms were principally responsible for biological functions and management practices to sustain soil health (Table 4).

Table 4. Biological function, biological groups, and management practices to sustain soil health [a)]

Biological functions	Biological groups	Management practices
Residue decomposition	Residue-borne microorganisms, meso/macrofauna	Suppressing, soil tillage, biopesticide applications
Carbon sequestration	Microbial biomass (fungi), macrofauna building compact structure	Suppressing, shortening, soil tillage
Nitrogen fixation	Free and symbiotic nitrogen fixer	Reduction in crop diversity, fertilization
Organic matter/ nutrient redistribution	Roots, mycorrhizas, soil macrofauna	Reduction in crop diversity, soil tillage, fertilization
Nutrient cycling, mineralization/ immobilization	Soil microorganism	Soil tillage, irrigation, fertilization, biopesticide application, Suppressing
Bioturbation	Roots, soil macrofauna	Soil tillage, irrigation, biopesticide application
Soil aggregation	Roots, fungal hyphae, soil macrofauna, soil mesofauna, predators, parasites, pathogens	Fertilization, biopesticide application, reduction in crop diversity, soil tillage

[a)] Adapted from Giller *at al*., (1997) *In* Brussard, *et al*., (2004).

However, it can be argued that one of the most important features of a soil, and the organic matter (also nutrients) content, is the ability to act as a sustainable living system.

The physical health of soil in Bali was good, with clay content < 35%; however the chemical health was low to medium with low contents of Phosphorous and Organic Matter. The biological health was bad, with low of substrate and energy for microorganism which is come from organic matter. The numbers of bacterial, actinomycetes, and fungi in healthy soil were more than in infected soil. The sustainable management of agriculture land in Bali can be done by increasing the soil health (physical, chemistry, and biological), adding organic matter using balance of N, P, K fertilizers, and selecting for suitable alternative agriculture commodities. The groups of soil microorganisms have principal and basic role for biological functions and management practices to maintain soil health.

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THE EFFECT OF "EFFECTIVE MICROORGANISMS-4" (EM4™) AND STARBIO ON THE PERFORMANCE OF CHERRY VALLEY (CV) 2000 DUCK AGED OF 0 – 4 WEEKS OLD

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ABSTRACT

An experiment was carried out in Faculty of Animal Husbandry Udayana University Farm to study the effect of EM4™ in drinking water and Starbio in the ration of CV 2000 duck aged of 0 – 4 weeks old. The experiment design used was Randomized Block Design consisting of seven treatments and four blocks with five birds each, so the total CV 2000 ducks used in this experiment were 140 birds. The treatments consisted of E1 (1 cc of EM4), E2 (2 cc of EM4), E3 (3 cc of EM4) in one litre drinking water respectively, while S1 (0,5 g), S2 (1,0 g), S3 (1,5 g) Starbio in one kg ration and the control K is ration without either EM4 in drinking water nor Starbio in the ration. Variable observed were the body weight gain, feed consumption, feed efficiency and the final body weight. Results of this experiment showed that there was no significant difference of EM4 levels neither of starbio on body weight gain, feed consumption, feed efficiency neither in the final body weight ($P > 0,05$).

Keywords: Performance, Cherry Valley 2000 Duck, aged of 0 – 4 Weeks Old, EM4™. and Starbio

INTRODUCTION

Poultry enterprise is growing fast in Indonesia. This shows that this enterprise has been developing very well for its contribution spreads widely in improving economy, giving job opportunity, and also in providing protein sources (Murtidjo, 1988). One of duck breeds in Indonesia that is potential to produce more eggs and meat is CV 2000. Besides that, this duck is more resistant to diseases, riskless and it is easy in rearing, CV 2000 was originally came from Cherry Valley Farm in England and was firstly introduced with Indonesian climate in a poultry farm in Bogor in November 1993. Its first commercial breed was launched with a name of CV 2000-INA. In its original country these CV 2000 ducks have been observed on its genetics engineering over 25 years. This genetics engineering created the best duck breed that was highly producing eggs (Windhyarti, 1999).

Feed resources are needed in surrounding poultry farms to make sure that it provides an optimal growth to ducks. In intensive rearing, ducks need a quality and quantity of feed about 60-70% from total production cost (Rasyaf, 1982). Probiotic such as Starbio is important mix in the feed to increase digestibility of duck. Starbio may help in digestibility as its feed nutrients will be metabolized into nutrient enzymatically, so may be directly absorbed and transported into body cells (Anonim, 1999). Starbio is brown powder that consists of lignolitic, cellulitic, proteolitic and symbiotic non nitrogen fixation microbial. To reach the optimal result of starbio usage, it is suggested that the use is 2 – 2,5 g/1 kg feed in broiler, 1 – 1,5 g/1 kg feed in layer and 2 – 2,5 g/1 kg feed in local hens meat producing.

Effective Microorganisms-4 or EM4 that is mixed up in drinking water and feed will improve microorganism composition in digestive tract of duck (Wididana, 1996) and hence will improve their growth. EM4 consists of four components of microorganism, i.e. photosynthetic bacteria, Actinomycetes, Lactobacillus and yeast. Different bacteria have different roles and functions. Photosynthetic bacteria are produced to consume the poisonous gas and heat from fermentation process; *Actinomycetes* produce antibiotic compounds that is toxic to pathogen microorganisms and may dilute phosphate ions and

also other micro ions; *Lactobacillus* ferments organic into lactate compounds; and yeast to ferment organic that is diluted in alcohol forms, sugar and also amino acids.

The purpose of this study is to observe dose usage of *Effective Microorganisms-4* or EM4 as well as starbio that will give the best performance to female ducks generated from CV2000 aged of 0-4 weeks old.

MATERIALS AND METHODS

140 of day old female ducks generated from CV 2000 were used to study the effect of EM4 and Starbio on their performance until age of 4 weeks. The early body weight in control was 40,95 g, while treatment of S1, S2, S3, E1, E2, and E3 were 40,77 g; 40,96 g; 40,56 g; 40,84 g; 40,78 g; and 40,90 g respectively. All ducks were purchased from Mengwi Village, Badung Residence.

Battery cages used have the length x width x height of (76 x 40 x 39) cm^3 of each unit and 56 cm height from floor surface. Every unit of battery cages were provided with feed and water troughs of 25cm square each. The feed trough was made from bamboo whilst water trough from plastic and then a piece of plastic paper was put right underneath every cage to collect feed split. Electric ball was put in the centre of each unit of battery cages as light and heat sources.

Feed was formulated by Farrel Standard (1995) and the composition of feed ingredients was shown in Table 1.

Table 1 Ingredient of starter 0-4 weeks

Ingredients	Composition (%)
Corn	50.5
Fine rice mills	21.1
Soybean by-products	9.5
Fish meal	11.4
Meat meal	6.0
Coconut oil	1.5

Table 2 The composition of nutrient in feed

Nutrients	Composition	Standard *)
ME (kcal/kg)	2,846.96	2,799 – 2,899
Protein (%)	20.064	20
Fat (%)	5.936	5
Crude Fibre (%)	3.873	Maximal 5
Ca (%)	10.097	(9.12)
P (%)	0.86	(0.4)

Note : *) Starter Feed Standard by Farrel (1995)

"Sartorius" weight measurement that has 2 kg capacity and 1 g sensitiveness was used to weigh feed fed, whereas Oertling with has 1.5 kg capacity and 0.01 g sensitiveness was used to weigh ducks and feed left. Volume glass 1000 ml was used to measure the mixture of EM4, and thermometer was used to measure temperature and humidity.

Ducks were selected randomly based on homogen average body weight and then band were put on each wing before releasing into cages. Treatment used was randomized block design (RBD) with 6 treatments and 1 control that was divided into 4 groups with five ducks in each treatment. In EM4 treatments, water was mixed up with *Effective Microorganisms-4* (EM4) with dosage of 1 cc/L water (E1), 2 cc/L water (E2), and 3 cc/ L water (E3). In Starbio treatment, Starbio was mixed up with feed with doses as: 0,5 g/1 kg feed (S1), 1,0 g/kg feed (S2), 1,5 g/kg feed (S3). Water and feed were provided

adlibitum and cleaning up all water and feed trough were done every day. Temperature recording was done three times daily, i.e. morning, noon and afternoon.

This research was completed in four weeks and the parameters were body weight gain, final body weight, feed consumption and feed conversion that were recorded weekly. All the results were analysed with variance analysis and when there were significantly differences among treatments, then it tested with Double Space from Duncan Test (Steel & Torrie, 1993)

RESULTS AND DISCUSSION

The body weight gain of CV 2000 ducks in control was 90.98 g/bird/week and in S1, S2, S3, E1, E2 and E3 treatments were 18.89%; 9.22%; 0.11%; 3.80%; 4.82% and 7.77% respectively. The body weight gain of CV200 in treatments were higher than those in control but not statistically different (P > 0.05). Feed consumption in control was 233.19 g/bird/week. Ducks in S1, S3 and E3 treatments were 22.84 %; 29.12 % and 1.37 % respectively, and were higher than those in control and not significantly different (P > 0.05) either. Feed consumption of those ducks in S2, E1 and E2 treatments were 2.56 %; 1.13 % and 9.12 % respectively and were less than those in control and was not statistically different (P > 0.05).

Table 3. The effect of EM4 and Starbio on the performance of female ducks generated from CV2000

Parameter	Treatments							SEM
	K	E1	E2	E3	S1	S2	S3	
Mean of early body weight (g)	40.95*	40.84*	40.78*	40.90*	40.77*	40.96*	40.56*	0.19
Mean of final body weight (g)	404.86*	418.62*	422.24*	433.09*	473.45*	438.45*	404.86*	39.98
Weekly weight gain (g/bird/week)	90.98*	94.44*	95.37*	98.05*	108.17*	99.37*	91.08*	7.59
Feed consumption (g/bird/week)	233.19*	230.56*	211.93*	236.39*	286.46*	227.22*	301.09*	24.41
Feed conversion	2.56*	2.44*	2.23*	2.41*	2.65*	2.28*	3.30*	0.31

Mean of feed conversion of CV 2000 in control was 2.56 while those in S2, E1, E2 and E3treatment were 10.94%; 4.69%; 12.89% and 5.86 % respectively and they were not significantly different (P > 0.05), but more efficient compared to controls. Ducks in S1 and S3 treatments, their feed conversion were 3.52% and 28.91% and was not significantly different (P > 0.05), and were less efficient compared to control. The final body weight of CV 2000 ducks in control were 404.858 g. Ducks that were in S1, S2, S3, E1, E2 and E3 treatments have body weight of 16.94%; 8.30%; 0.005%; 3.40%; 4.29% and 6.97% respectively and they were not significantly different (P > 0.05) and heavier than those in control (Table 3). Feed consumption of CV 2000 ducks either in control nor in treatments of *Effective Microorganisms-4* were not significantly different (P > 0.05). It may be probably that *Effective Microorganisms-4* had not optimized its function in ileum yet, so their population may not be maximized either. *Effective Microorganisms-4* consisted of *Actinomycetes* and yeast. *Actinomycetes* functioned to produce toxically antibiotic compounds over pathogen bacteria, so it caused thickness in ileum, and thus negatively affected the nutrients absorption. Yeast had role in fermentation of organic to be alcohol, amino acid sugar compounds and also produce B12 vitamins that trigger appetite and raised feed consumption (Tillman *et al.*, 1986). Due to *Effective Microorganisms-4* did not grow optimally in ileum of ducks; it did not improve digestibility and absorption of nutrients. As it also did not trigger appetite, therefore the feed consumption was not affected. The result of this research was different compared to those male layers aged 0 – 8 weeks that had higher feed consumption (Sudiastra, 1999).

Ducks that fed Starbio, their feed consumption was not significantly different (P > 0.05) compared to control. This was probably due to the microbe in Starbio such as lygolitic, amylotick, proteolitic and cellulitic had not grown optimally in digestive tracts of ducks. Therefore they were not able to metabolize organic compounds of feed into simple compounds to be easily absorbed and thus feed consumption was not affected. This result was relevant to what Rai Yasa *et al.* (1999) found that adding Starbio probiotic 0.25% did not affect on feed consumption.

Body weight gain and final body weight in ducks that fed *Effective Microorganisms-4* and Starbio were not significantly different (P > 0.05) compared to control. It was due to that feed consumption in *Effective Microorganisms-4* and Starbio treatments were same. Matram (1984) stated that generally growth has correlation with genetic interaction ability with feed and environment. The level of animal's growth was influenced by species, age, quality and quantity of feed.

Feed conversion of CV 2000 ducks that fed *Effective Microorganisms-4* and Starbio was not significantly different (P > 0.05). It was due to feed consumption and body weight gain was not significantly different. This result was relevant to Haryanto (1985) who stated that feed conversion was total feed consumption compared to body weight gain of an animal during a certain period.

CONCLUSIONS

Adding the *Effective Microorganisms-4* in drinking water in doses of 1 cc/L, 2 cc/L and 3 cc/L did not affect the performance of female ducks generated from CV 2000 aged 0 – 4 weeks. Adding Starbio in doses of 0.5 g, 1.0 g and 1.5 g in 1 kg feed did not affect the performance of female ducks generated from CV 2000 aged 0 – 4 weeks. It was suggested that those CV 2000 ducks reared on aged 0 to 4 weeks did not necessarily need *Effective Microorganisms-4* or Starbio, as it increases feed cost. Further research on the usage of *Effective Microorganisms-4* and Starbio on ducks performance for longer periods was needed.

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SMALL-SCALE ORGANIC FARMING EMPOWERMENT FOR LOWER-MIDDLE INCOME COMMUNITY (A SYSTEMATIC APPROACH FOR NATIONAL FOOD SECURITY AND POVERTY REDUCTION)

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ABSTRACT

The small scale organic farming (SSOF) has a potential power to improve or even to establish the national food security on the community basis. The synergic and holistic combination of both the households and local farmer groups SSOF will lead lower-middle income community into food security. This combination promotes food availability, food stability, food accessibility, and food safety among poor community. Indeed, this is also a potential way to generate more incomes and to decrease the rate of dependency to newly form of agricultural technology which is benefit to establish national food dignity. The small scale organic farming can be implemented as an effective approach to overcome the poverty on the community basis by generating income through organic agriculture. This strategy will be in line with poverty reduction programs which are implemented by Indonesian government. This strategy could be more effective due to directly related to household food security. The SSOF could be an effective and a practical solution for poverty reduction at least due to 5 specific reasons: (1) the organic farming in the household levels reduce cost for daily foods, since they are producing by them self, (2) the organic farming in the local farmer groups effectively increase their income and prosperity since this method bring many advantages as mentioned above, (3) the SSOF might be able to create job opportunities in the lower to middle income community, (4) the SSOF promotes sustainable chain agriculture which are benefited for small scale farmers, (5) the SSOF promotes food security as well as food safety for poor community, which leads the increasing of health and nutritional status, reduce the morbidity, reduce the mortality, and effectively increase the productivity of the community. The implementation of the small scale organic faming in Indonesia will face several obstacles including financial aids, skills improvement of the community, and marketing strategy to distribute the products. Developing public and private partnership and providing mentoring for both skill/knowledge and marketing/distribution are practical solutions to succeed the implementation of the SSOF in Indonesia.

Keywords: organic farming, food security, poverty, implementation, agriculture, health

ORAL PRESENTATIONS:

AGRITECH AND FOOD

STRUCTURE AND ABSOLUTE CONFIGURATION OF BIOACTIVE 3 ALKYLPIPERIDINE ALKALOIDS FROM A BALINESE MARINE SPONGE OF THE GENUS *HALICHONDRIA*

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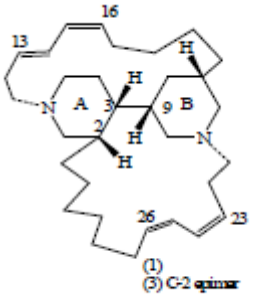
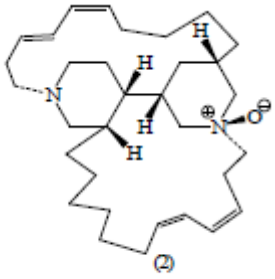
ABSTRACT

Chemical analysis of a Balinese marine sponge has provided three new 3- alkylpiperidine alkaloids, tetradehydrohaliclonacyclamine A (**1**), its mono-N-oxide derivative (**2**), and a 2-epi isomer (**3**). The absolute structure of tetradehydrohaliclonacyclamine A has been established by X-ray crystallography from anomalous dispersion effects using Cu radiation, which established that the absolute configuration is 2S, 3S, 7S, 9S while an HPLC study revealed that the alkaloid is enantiomerically pure. When these metabolites were evaluated for antitumour activity against the P388 cell line, compound **1** exhibited an IC50 of 1.8 2g/mL whereas **2** was not active at the concentrations tested.





INTRODUCTION

Over thirty different 3-alkylpiperidine-based carbon frameworks have been discovered in marine sponge metabolites isolated from sponges of the order *Haplosclerida*. Representatives include the haliclamine/cyclostellettamine, ingenamine, madangamine, ircinal, manzamine, halicyclamine, sarain, petrosin, xestospongin/araguspongine and aragupetrosine skeletons.1,2 Previously we reported the isolation of four bioactive alkylpiperidine alkaloids, the haliclonacyclamines A to D, from a Haliclona species collected at Heron Island (Australia);3,4 our X-ray crystallographic studies have recently established that (-)-haliclonacyclamine A and (+)- haliclonacyclamine B each have the absolute configuration 2R, 3R, 7R, 9R.5 Two additional haliclonacyclamine metabolites, E and F , and a series of C-22 hydroxylated metabolites known as the arenosclerins have been isolated from the Brazilian sponges Arenosclera braziliensis6 and Pachychalina alcaloidifera.7 All of these metabolites share two connecting chains ('spacer groups') of ten and twelve carbons, respectively, in length, but showvariation in double bond locations. The identical carbon skeleton is present in halicyclamine A, isolated by two separate research groups from an Indonesian Haliclona sp.8 and a Japanese Amphimedon sp.,9 and in halichondramine, isolated from the Red Sea sponge Halichondria sp. The related halicyclamine B, whose relative stereochemistry has been secured by an X-ray

analysis, possesses two spacer groups that each contain only eight carbon atoms. Differences in the relative configuration at the methine centres of these various alkaloids may be of biosynthetic significance. We now report the isolation and characterization of a new alkaloid tetradehydrohaliclonacyclamine A (1) from the Indonesian sponge Halichondria sp., together with its N-oxide derivative (2) and the 2-epi isomer (3). X-ray crystallographic determination of the absolute configuration of **1** was undertaken using anomalous dispersion effects, while an HPLC study has confirmed that **1** is present in a single enantiomeric form in the sponge.

MATERIALS AND METHODS

Biological material

A sponge sample provisionally identified as *Halichondria* sp. was collected from Tulamben Bay, Bali, using SCUBA at a depth of 20 m on 15 January 2008. The sample was taken back to the laboratory and stored at -20 0C until extraction.

Extraction and isolation of alkaloids

The specimen of Halichondria sp. (frozen weight 500 g) was extracted exhaustively with DCM/MeOH (1:1), and the combined extracts (13.7 g) dried under vacuum, then sequentially partitioned into hexanes, DCM, and EtOAc fractions. A portion (2.7 g) of the DCM extract was subjected to SiO2 flash chromatography with gradient elution (hexanes→EtOAc; plus 1% Et3N) to give fifteen fractions. Fraction 10 (145 mg) eluting in hexanes/EtOAc (1:1) was subjected to NP HPLC (hexanes/EtOAc/Et3N 60:35:5) to give compound **1** (56 mg). Alkaloid-containing fractions eluting in 100% EtOAc or EtOAc/MeOH (1:1) were combined and subjected to a DCM/1 M aq HCl solution (10 mL each) partition. The aqueous layer was then basified to pH 10 with K2CO3 and extracted three times with DCM (10 mL). The organic layer was separated, dried over Mg2SO4, filtered and evaporated to yield additional alkaloid (43.5 mg). A portion of this sample (22 mg) was subjected to SiO2 flash chromatography with isocratic elution (DCM/MeOH/Et3N 80:15:5) to yield additional tetradehydrohaliclonacyclamine A (**1**) (13 mg) and tetradehydrohaliclonacyclamine A mono-N-oxide (**2**) (0.7 mg). The 2-epi isomer (**3**) was isolated from another portion of the DCM extract (480 mg) using SiO2 flash chromatography eluting with 75:20:5 hexane/EtOAc/Et3N. The fractions containing **3** eluted before the fractions containing **1** and were combined (6.3 mg) and subjected to repetitive NP HPLC (hexanes/EtOAc/Et3N 60:35:5), which yielded compound 12 (1.1 mg). Crystals of 10 were grown slowly from hexanes/EtOAc (1:3) at -4 0C using the vapour diffusion method. Before inducing crystallization, it was essential to remove any residual traces of halogenated solvents
remaining from the earlier NMR studies.

RESULTS AND DISCUSSION

The NMR assignments for tetradehydrohaliclonacyclamine A (**1**), its N-oxide (**2**), and 2-epi isomer (**3**) is shown in Table 1. It is quite impossible to elaborate the full structure elucidation steps in this limited space. The full discussion is available soon from the tetrahedron journal. However, data presented in Table 1 will guide the readers to the final structure of the isolated compounds. The X-ray crystallography data are also excluded from this discussion for the similar reason.

Table 1. NMR assignments for tetradehydrohaliclonacyclamine A (1), its N-oxide (2), and 2-epi isomer (3)

C	Tetradehydrohaliclonacyclamine A (1)				N-oxide (2)		2-epi isomer (3)	
	^{1}H (mult., *J*, Hz)[a,b]	^{13}C, ppm[c]	HMBC[d,e]	DQFCOSY	^{1}H (mult., *J*, Hz)[a,b]	^{13}C, ppm[f]	^{1}H (mult., *J*, Hz)[a,b]	^{13}C, ppm[g]
1	2.62 (t, 11.6) 2.46 (brd, 11.6)	52.9	3, 11, 32	2	2.64 (t, 11.6) 2.48 (m)	52.9	2.85 (m) 2.22 (m)	55.0
2	1.63 (m)	42.7	1a, 1b, 32	1, 32	1.72 (m)	43.0	1.46 (m)	33.4
3	1.55 (m)	38.8	1a, 1b, 2, 3, 4a, 4b	4a	1.53 (m)	38.8	1.48 (m)	37.6
4	1.85 (m) 1.56 (m)	37.0	5a,5b	3, 5a,	1.87 (m)	37.6	1.80 (m) 1.22 (m)	29.1
5	2.92 (m) 2.46 (m)	47.8	1a, 1b, 3, 4a, 4b, 11	4a	2.96 (m) 2.51 (m)	47.8	2.88 (m) 2.41 (dt, 5.0, 10.7)	50.3
6	2.53 (m) 2.01 (t, 11.0)	57.7	8a, 8b, 10a, 10b, 20b, 21	7	2.71 (t, 11.7) 2.88 (m)	69.0	2.65 (m) 2.07 (t, 10.7)	58.6
7	1.41 (m)	38.3	6a, 6b, 8a, 8b, 9, 20a, 20b	6a, 6b, 8a, 8b	2.50 (m)	32.7	1.25 (m)	38.2
8	2.03 (dd, 12.3, 10.8) 0.77 (q, 12.3)	36.6	3, 6a, 6b, 10a, 10b, 20a, 20b	7, 9	2.14 (m) 0.88 (q, 12.3)	35.5	1.95 (m) 0.65 (q, 12.4)	34.3
9	1.59 (t, 12.3)	46.6	4a, 4b, 8a, 8b, 10a, 10b	8a, 8b, 10a, 10b	2.77 (br t 12.3)	38.0	1.48 (m)	44.2
10	2.52 (m) 2.49 (m)	61.1	3, 6a, 6b, 8a, 8b, 21	9	2.82 (m) 3.09 (t, 12.3)	72.0	2.63 (m) 1.91 (t, 10.7)	59.9
11	3.00 (m)	56.5	5a,5b, 13	12	3.05 (m) 3.03 (m)	57.0	2.85 (m) 2.75 (m)	55.9
12	2.42 (m) 2.29 (m)	29.5	11, 13	11, 13	2.48 (m) 2.29 (m)	29.5	2.29 (m) 2.24 (m)	29.7
13	5.65 (dt, 7.5, 14.1)	135.5	11, 12, 15	12, 14	5.71 (dt, 7.1, 14.6)	136.2	5.69 (dt, 7.2, 14.8)	135.0
14	6.32 (dd, 14.1, 11.5)	125.5	12, 15, 16	13, 15	6.34 (dd, 14.6, 11.4)	125.3	6.35 (dd, 11.0, 14.8)	126.2
15	5.99 (dd, 11.5, 10,6)	129.9	13, 14, 17, 18	14, 16	6.03 (dd, 11.4, 10.4)	129.7	5.99 (dd, 11.0, 10.7)	129.4
16	5.23 (dt, 8.6, 10.6)	129.4	14, 17, 18	15, 17	5.26 (dt, 8.3, 10.4)	129.5	5.22 (dt, 8.8, 10.7)	129.8
17	2.12 (q, 7.9)	26.4	15, 18, 19	16, 18	2.16 (m) 2.19 (m)	25.5	2.18 (m)	27.0
18	1.44 (m)	28.0	16, 19a, 19b		1.45 (m)	27.5	1.33 (m)	27.9
	1.30 (m)				1.25 (m)		1.25 (m)	
19	1.29 (m) 1.12 (m)	24.6	17, 20a, 20b		1.28 (m) 1.22 (m)	23.7	1.45-1.20 (m)[j]	[j]
20	1.30 (m) 1.04 (m)	33.1	6b, 7, 8a, 8b, 18a, 18b		1.34 (m) 1.06 (m)	32.0	1.33 (m) 1.04 (m)	33.1
21	2.93 (m)	57.0	6a, 6b		3.52 (br)	73.8	2.98 (dt, 3.0, 12.0) 2.65 (m)	57.5
22	2.86 (m) 1.81 (brd, 14.2)	23.4	23, 24	23	2.68 (m) 2.77 (brt, 12.2)	23.5	2.15 (m)	25.9
23	5.45 (dt, 4.2, 10.6)	132.9	25, 22, 21	22, 24	5.50 (m)	128.3	5.74 (m)	132.5
24	6.11 (dd, 11.7, 10.6)	122.6	22, 25, 26,	23, 25	6.23 (t, 11.3)	123.7	6.17 (t, 11.0)	123.3
25	6.32 (dd, 11.7, 10.9)	124.5	23, 24, 27	24, 26	6.34 (t, 11.3)	125.3	6.29 (t, 11.0)	123.9
26	5.35 (dt, 5.4, 10.9)	132.2	24, 27, 28,	25, 27	5.50 (m)	134.3	5.47 (m)	132.4
27	2.33 (q, 11.7) 1.95 (m)	25.6	25, 26, 28, 29, 30	26, 28	2.40 (m) 2.04 (m)	24.8	2.58 (m) 2.22 (m)	24.7
28	1.45 (m)	28.1	27, 29, 30	27a	1.50 (m) 1.40 (m)	27.5	1.45-1.20 (m)[h]	[j]
29	1.21-1.14 (m)[i]	28.6[g]	28, 31		1.22 (m)[h] 1.40 (m)	29.1[i]	1.45-1.20 (m)[h]	[j]
30	1.35-1.27 (m)[i]	28.7[g]	28, 31, 32		1.22 (m) 1.35 (m)[h]	28.5[i]	1.45-1.20 (m)[h]	[j]
31	1.17 (m)	25.7	29, 30		1.19 (m) 1.16 (m)	25.2	1.45-1.20 (m)[h]	[j]
32	1.15 (m)	32.3	1a, 1b, 2, 3	2	1.15 (m) 1.04 (m)	32.3	1.33 (m) 1.22 (m)	33.0

[a]500 MHz, $CDCl_3$ referenced to ^{1}H at δ 7.24 ppm; [b]coupling constant in Hz; [c]100 MHz, $CHCl_3$ referenced to ^{13}C at δ 77.0 ppm [d] HMBC connectivity from C to H; [e] correlations observed for one bond J_{C-H} of 145 Hz and long range J_{C-H} of 8 Hz; [f]187.5 MHz, $CHCl_3$ referenced to ^{13}C at δ 77.0 ppm; [g]125 MHz, $CHCl_3$ referenced to ^{13}C at δ 77.0 ppm; [h]Signals may be interchangeable; [i]Signals may be interchangeable; jThe three signals at 28.8, 28.7, and 27.9 ppm and the two signals at 26.4 and 25.0 ppm could not be individually assigned

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EFFECT OF AMYLOSE CONTENT AND TEMPERING TIME ON CHARACTERISTICS OF FRESH RICE FLOUR-BASED SPRING ROLL WRAPPERS

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ABSTRACT

The effects of amylose content and tempering time on characteristics of fresh rice flour-based spring roll wrappers were investigated by using added free amylose of cassava to rice flour. The used rice flour in this research was from variety Mentik (an Indonesian local rice variety). Amylose content of blended rice flour ranged from 25% up to 40%. The fresh rice flour-based spring roll wrappers were made without frying oil on Teflon frying pan at 72°C during 4 minutes. After heating, the product was tempered for 30, 45 and 60 minutes at 25°C. The product was evaluated for rice starch granules size, moisture content, water activity and elongation at break. Each experiment was conducted by three replications. All of the data were analyzed by analysis of variance (α 5%). Duncan multiple range test (α 5%) was used to determine the significant difference among the treatments. The result showed that free amylose adding to rice flour blends homogenized the swelling of rice starch granules. The increasing of amylose content more than 34% increased water activity. The amylose content from 31 % up to 40% increased the moisture content but tempering time from 30 up to 60 minutes did not affect moisture content and elongation significantly. Increasing amylose content decreased elongation at break.

Keywords: *amylose content, tempering time, fresh spring roll, rice flour, characteristics.*

INTRODUCTION

Tempering time of fresh spring roll wrapper is a given time to the product after heating until it can be removed from the frying-pan at room temperature. In cooling process still occur water vapor evaporation and water migration through the system slowly (Anonymous, 2007).During the process of heating and tempering occur evaporation of water to produce solid material which is a group of polymers of inter-connected polymer chains (Andersen et al., 2000).This change resulted in a drop of temperature and provided the product was in rubbery state and then became the glassy state (Moraru and Kokini, 2003). In these conditions the product will become more cohesive and it could be removed from the frying-pan easily.

Starches with higher amylose content will form stronger gel and will be more difficult to damage. Increasing of amylose content will inhibit the swelling of the granules thus maintained the integrity of the swollen starch granules.Too short tempering time will produce a sticky product which is related to high amount of surface water as a result of insufficient water migration from the surface to interior parts of the product. In contrary long tempering time will dehydrate the product (Anonymous, 2007). Longer time of tempering may increase the alignment of free amylose molecules and starch crystallization which lead to decrease of water binding ability of the system. It resulted in increasing of free water molecules that make increasing Aw (Yao et al., 2003). The purpose of this study is to investigate the influence of amylose content and tempering time on the characteristics of fresh rice flour-based spring roll wrappers.

MATERIALS AND METHODS

Materials. Mentik rice from Candi, Nglames, Madiun, obtained from the UD. Eka Jaya rice mill, Surabaya. Rice flour obtained by grinding the rice in dry process (without

soaking) and sifted with a 80 mesh sieve size. Amylose extraction from tapioca used modified method of Takeda et al. (1986) and Patindol et al. (2003). Leghorn chicken eggs obtained from a local shop in Surabaya.

Methods. The research design was factorial experiment with randomized completely block design. Various factors is the amylose content consists of six levels, namely: 25%; 28%; 31%; 34% ; 37% and 40% (w / w); while tempering time with three levels of factors (30, 45 and 60 minutes) at 25ºC. The observed dependent variables are starch granule size, $a_{w,}$ moisture content and elongation at break. The data were processed by analysis of variance, the difference of among treatments were tested by Duncan Multiple Range Test with α = 5%. Starch granules size was measured by using Olympus DP 20 Digital Camera Microscope. Water activity was measured with a Rotronic hygrometer AW1 Hygro Palm at 85% RH + / - 1% at temperature of 25 º C + /-2 º C. Moisture content was measured by gravimetric method (AOAC, 2000). Elongation at break was measured by Shimadzu Autograph. The batter has been mixed to be homogeneous by placing the mixture on a magnetic stirrer with a speed of 100 rpm for 2 minutes, then placed on a Teflon material frying pan (diameter 10 cm). Heating was held at 72 º C for 4 minutes.

Table 1. Formula of Fresh Rice flour-based Spring Roll Wrapper

Ingredients (g)	Amylose Content (%)					
	25	28	31	34	37	40
Rice Flour	3.00	2.85	2.70	2.55	2.40	225
Crude amylose of 85% purity*	0.00	0.15	0.30	0.45	0.60	075
White Egg	3.50	3.50	3.50	3.50	3.50	3.50
Water	6.00	6.00	6.00	6.00	6.00	6.00
Tapioca	0.50	0.50	0.50	0.50	0.50	0.50
Total (g)	13.00	13.00	13.00	13.00	13.00	13.00

RESULTS AND DISCUSSION

Rice Starch granules size

Data in Table 2. showed a significant difference in the effects of amylose content and there was interaction between two factors to the size of rice starch granules.

Table 2. Rice Starch Granule Size of Fresh Rice Flour-based Spring Roll Wrappers on Different Levels of Amylose and Tempering Time

Tempering Time (minutes)	Rice Starch Granule Size (µm2)*					
	Amylose Content (%)					
	25	28	31	34	37	40
30	1596.59 h	1531.39 ef	1476.12 d	1408.92 c	1323.34b	1271.57 a
45	1568.19 g	1515.53 e	1470.49 d	1403.02 c	1321.74b	1261.59a
60	1539.75 f	1512.84 e	1468.43 d	1402.06 c	1321.91b	1260.12a
DMRT 5%	18.23 - 20.51					

*Values in same column with different letter are significantly different based on DMRT test with α =5%

It also showed that starch granules size decreased significantly with increasing amylose content on tempering time for 30, 45 and 60 minutes.

Water activity (a_w)

Data showed a trend of increasing in $\mathbf{a_w}$ as levels of amylose increasing. This phenomena could be influenced by amylose alignment molecules, the freed water molecules will lead increasing of $\mathbf{a_w}$.

Table 3. A_w of Fresh Rice Flour-based Spring Roll Wrappers on Different Levels of Amylose

Amylose Content (%)	a_w *
25	0.516 **a**
28	0.518 **a**
31	0.521 **a**
34	0.524 **ab**
37	0.529 **b**
40	0.539 **c**
DMRT 5%	0.0075 – 0.0084

*Values with different letter are significantly different based on DMRT test with α =5%

Table 4. A_w of Fresh Rice Flour-based Spring Roll Wrappers on Different Time of Tempering

Time of Tempering (minutes)	A_w *
30	0.520 **a**
45	0.526 **ab**
60	0.529 **b**

*Values with different letter are significantly different based on DMRT test with α =5%

Moisture Content

The average moisture content showed a trend of increasing water content as increasing levels of amylose.This phenomenon is caused by the amount of water entrapped in the gel system will be more and more with the increased amylose content. This deals with the role of amylose on gel formation (Gimeno, et al., 2004).

Table 5. Water Content of Fresh Rice Flour-based Spring Roll Wrappers on Different Levels of Amylose Content

Amylose content (%)	Water content (%)*
25 %	36.30 a
28 %	36.83 a
31 %	39.25 b
34 %	40.24 b
37 %	41.27 b
40 %	41.29 b
DMRT 5%	2.2834 - 2.5690

*Values with different letter are significantly different based on DMRT test with α =5%

Elongation

The result in Table 6 showed increasing of amylose content and it affected the distance of molecular components which decreased cohesiveness. In this condition water in the system acted as a plasticizer materials (Chang et al., 2006).

Table 6. Elongation at Break of of Fresh Rice Flour-based Spring Roll Wrappers on Different Levels of Amylose Content

Amylose Content (%)	Elongation at Break (%)*
25	16.07 **e**
28	14.52 **d**
31	14.27 **cd**
34	13.71 **c**
37	12.03 **b**
40	10.96 **a**
DMRT 5%	0.6835 – 07690

*Values with different letter are significantly different based on DMRT test with α =5%

CONCLUSION

Based on the study of all the response of depended variables, it can be concluded that the treatment of amylose content of rice flour and long of tempering time influenced the characteristics of fresh rice flour-based spring roll wrappers. Amylose content in the range of 25% to 40% tend to increase the moisture content of product. Tempering is longer than 60 minutes is not recommended in relation to the decrease of elongation.

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SYNERGISTIC SACCHARIFICATION PROCESS OF DIFFERENT SOURCES OF STARCH BY GLUCOAMYLASE AND PULLULANASE IN THE GLUCOSE SYRUP PRODUCTION

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ABSTRACT

The high demands for sugars and the development of enzymatic technology have increased the production of sweeteners, especially for glucose syrups. Glucose syrup can be produced enzymatically by liquefaction and saccharification of starch. This research was to elaborate the role of dextrozyme (a mixture of glucoamylase (an exoamylase) and pullulanase (a debranching enzyme) during the saccharification process of different sources of Indonesian indigenous starches i.e gembili, arenga, arrowroot and taro starches for glucose syrup production. Dextrozyme was applied in 30% w/w of starch suspension after liquefaction process using α-amylase, where 0.04%, 0.06% and 0.08% w/w of dextrozyme were used for arrowroot, gembili and taro starches and 0.08%, 0.12%, 0.16% w/w of dextrozyme were used for arenga starch. This enzyme concentration factor was combined with incubation time factor including 24, 48 and 72 hours. Based on dextrose equivalent values, it was shown that dextrozyme could hydrolysed arrowroot starch more easily into dextrose than for gembili and taro starch, respectively. It was found that the saccharification of arrowroot starch by 0.06% w/w of dextrozyme concentration during 72 hours incubation and the saccharification in 0.08%w/w of dextrozyme during 24 and 48 hours incubation showed as the best treatment where the dextrose equivalent of glucose syrup of arrowroot were 91.44; 92.14 and 91.66, respectively. For gembili, 24 and 48 hours of incubation time in 0.08%w/w of dextrozyme yield 89.43 and 88.11 of dextrose equivalent, respectively. The saccharification of taro starch (48 hours of incubation time and 0.08% w/w of dextrozyme) gave 87.08 DE. Meanwhile, arenga starch was the most difficult substrate for glucoamylase, where the use of 0.16% w/w of dextrozyme could only yield 82.85 of dextrose equivalent at 48 hours of saccharification process.

Keywords : Dextrozyme, saccharification process, dextrose equivalent, glucose syrup.

INTRODUCTION

The high demands for sugars and the development of enzymatic technology have increased the production of sweeteners from starch, especially for glucose syrup. The enzymatic process became economically attractive for biotechnological industries. Furthermore, with the current worldwide increased sugar demand, a new research era in enzymatic technology has concentrated on decreasing the high costs of syrup production (Sanjust, 2004; Voragen, 1988). Indonesia has a potential sources of starch for glucose syrup production for instance arrowroot, gembili, taro, sago and arenga starch.

Raw starches are granules that are spherical, polyhedral, or lenticular. Most starches are triphasic with concentric alternating growth rings or lamella of amorphous and semi crystalline character (Oostergetel and van Bruggen, 1993). Crystalline units are more resistant to treatment of acid and enzyme than amorphous unit (Hodge dan Osman (1978). The amorphous unit can absorb up to 30% of water without any effect to strach granules. The form and size of starch granules of gembili, arrowroot, taro, sago, arenga are different each other. The size of starch granule have an influence to enzyme activity during glucose syrup production. A small size of granule will increase the activity of enzyme during hydrolysis process (Satin, 2000).

The percentage ratios of amylose to amylopectin of gembili, arrowroot, taro and arenga starches are 12.87:87.13; 23.95:76.05; 17,60: 87,54 and 14,91:85,09, respectively. The gelatinization temperatures of gembili, arrowroot and taro starches were in the range

of 69.18-81.5°C (Aprianita *et. al*., 2009); 70°C (Villamajor dan Jurkema, 1996); 70.95-84.67 (Aprianita *et. al*., 2009), respectively.

Several enzymatic synergies have been reported for starch digestion. Most common are synergies by endo- and exo-acting amylases. When these amylases act in concert, each endo-catalytic event also increases the number of substrate sites for the exo-acting enzymes, leading to an enhanced rate of conversion (Wang, *et al*. , 1996). Exo- and endo-amylase, raw-starch digestion synergies, have been reported for α-amylase and glucoamylase (Abe *et al*, 1988; Arasaratnam and Balasubramaniam, 1992; Matsubara *et al*. , 2004; Monma *et al*. 1989), α-amylases and glucosidases (Sun and Henson, 1990).

Synergies have been reported for two endoamylases: α-amylase and pullulanase. The pullulanase, acting on α-1,6-branches may expose new sites for α-amylase that previously had been inaccessible because of steric factors introduced by the branch. The combination between glucoamylase and pullulanase in a mixture is called dextrozyme (Whitehurst dan Law, 2002) and can increase glucose yield from 94% to 95% (Crabb and Mitchinson, 1997).

Yunianta *et al.* (2010) have studied the synergistic effect of α-amylase, glukoamylase and pullulanase activities on arrowroot starch. Gorinstein (1994) have found that the difference in incubation time of liquefaction could influenced to the characteristic of glucose syrup. This research was to investigate the effect of dextrozyme concentration (a mixture of glucoamylase and pullulanase) and incubation time of saccharification process to glucose syrup characteristics of different sources of starch.

MATERIALS AND METHODS

Substrate used in this experiment is starch from gembili, arrowroot, taro and arenga purchased from Indonesian market. α-amylase (Liquozyme Supra®) from *Bacillus licheneformis with* 90 KNU/g activity was used for liquefaction process and dextrozyme (Optimax 4060 VHP®). This enzyme is a mixture of glucomylase from *Aspergillus niger* (260 GAU/g enzyme activity) and pullulanase *from Bacillus licheneformis* (390 ASPU/g enzyme activity) was used for saccharification process.

Factorial block randomized design was carried out for this experiment, where two factors were studied. The first factor was dextrozyme concentration including three levels (0.04%, 0.06% and 0.08% w/w) for gembili, arrowroot and taro starches and (0.08%, 0.12% and 0.16% w/w) for arenga starch. The second was incubation time for saccharification process i.e : 24, 48 and 72 hours.

Glucose syrup production

Enzymatic glucose syrup production was started by preparing substrate, where 30% w/v of starch suspension was added with 20 ppm of CaCO3. pH was adjusted to 5,3 by addition of 1 N hydrochloric acid. After gelatinization at 75ºC, 0,045%w/w of α-amylase was added for liquefaction process at 95ºC for 1,5 hours incubation time. Liquefied starch then was cooled at room temperature. pH was adjusted to 4,5 by addition of hydrochloric acid. The liquefied starch was saccharified by dextrozyme (a mixture of glucoamylase and pullulanase) where 0.04%, 0.06% and 0.08% w/w of dextrozyme was used for gembili, arrowroot, taro starches and 0.08%, 0.12% and 0.16% w/w for arenga starch. Samples were incubated at 60ºC for 24, 48 and 72 hours. Saccharification process was stopped by heating all of samples at 105ºC for 15 minutes. Dextrose equivalent and reducing sugar were measured. Reducing sugar was determined by Nelson-Somogyi (AOAC, 1970) and dextrose equivalent according to anonym, 2002. Data were analyzed statistically using analysis of variance and continued by LSD test and DMRT test (Yitnosumarto, 1991).

RESULTS AND DISCUSSION

Analysis of raw material showed that taro and gembili have relatively the same composition of amylose and amylopectin in their starches. Arrowroot starch has the highest amylose content and the lowest amylopectin content compared to gembili, taro and arenga starches, where the amylose:amylopectin ratio of arrowroot, arenga, gembili and taro starch is 23.95:76.05; 14.91:85.09; 12,87:87.13 and 12.46:87.54, respectively. The usual starch used for glucose syrup, cassava, has 17.60:82.40 ratio of amylose:amylopectin.

Data in Table 1 showed the reducing sugar of glucose syrup obtained from hydrolisis of gembili, arrowroot, taro and arenga starches. The increase of enzyme concentration was followed by the increase of reducing sugar. The highest reducing sugar content was found in gembili syrup followed by arrowroot, taro and arenga syrups. It was shown that the reducing sugar content of arrowroot and gembili syrups was similar. For glucose syrup of gembili, the maximum enzyme activity was found in the treatment of 0.08% w/w of enzyme concentration and 24 hours of incubation time. The prolongation of incubation time did not increase the yield of reducing sugar, but in contraire, the yield of reducing sugar decreased from 28.58% to 23.70%, during the prolongation from 24 to 72 hours. This was estimated by the decreased of substrate / starch concentration due to the hydrolytic activity of alpha amylase, glucoamylase and pullulanase.

Table 1: Percentage of reducing sugar in glucose syrup from different starches.

Dextrozyme (%)*	Saccharification time(hours)	% reducing sugar			
		Gembili	Arrowroot	Taro	Arenga*)
0.04	24	25,407 d	22.65 a	20,47 a	22.687 a
0.04	48	23,368bc	23.22 b	21,73 b	22.996 b
0.04	72	20,544 a	23.45 b	23,35 c	23.525
0.06	24	25,978 de	23.52 b	21,42 ab	24.061 d
0.06	48	24,273 c	24.33 cd	22,82 bc	24.551 e
0.06	72	22,794 b	24.52 cd	23,33 c	24.134 d
0.08	24	26,584 e	24.88 d	23,12 c	25.011 f
0.08	48	25,454 d	24.64 d	23,61 c	25.153 f
0.08	72	23,697 c	24.26 c	23,27 c	24.610 e
DMRT (α = 5 %)		0,74 –0,84	0.29-0.32	1,12-1,28	2.97-3.39

Note: dextrozyme concentration for arenga starch were 0,08%; 0,12% and 0,16%w/w.

In the case of arenga starch, we found that glucoamylase and pullulanase found the difficulties to hydrolyze its substrate. It was needed 0.16% w/w dextrozyme concentration and 48 hours of incubation time to achieve 25.15% of reducing sugar. This phenomenon might be more related to the form and size of arenga starch granule than to amylose:amylopectin ratio. The amylose:amylopectin ratio of arenga starch was in between arrowroot and gembili/taro starches.

Data in Table 3 showed that dextrose equivalent (DE) value increased in accordance with the increase of dextrozyme concentration. The highest DE obtained in glucose syrup from arrowroot starch, followed by taro, gembili syrups and the lowest DE was found in the glucose syrup of arenga starch.

In 0.04% w/w of enzyme concentration, DE values in glucose syrup of arrowroot, taro and gembili starches were 84.06; 82.57 and 79.61, respectively. Meanwhile DE value of arenga syrup was 75.55 obtained by 0.08% w/w of enzyme concentration. Its mean that alpha amylase, glucoamylase and pullulanase activities on arenga starch hydrolisis was faced on difficulties. Pontoh and Low, 1994, found that the liquefaction time for palm

starch (*Metroxylon* starch) samples was longer than that observed for the other starch samples. They also found, based on the liquefaction time that arenga pinnata was difficult to be processed to glucose syrup.

Table 2. Dextrose Equivalent of glucose syrup obtained from different starches.

Dextrozyme concentration (%)*)	Incubation time (hour)	Dextrose Equivalent			
		Gembili	Arrowroot	Taro	Arenga*)
0.04	24	84,99 d	82.10 a	78,73 a	72.48 a
0.04	48	78,93 b	84.44 b	82,94 c	76.02 b
0.04	72	74,93 a	85.64 c	86,05 e	78.17 b
0.06	24	87,03 e	86.80 d	80,08 b	79.87 b
0.06	48	81,97 c	90.84 e	83,85 cd	81.74 c
0.06	72	80,12 b	91.44 ef	85,42 e	80.39 c
0.08	24	89,43 f	92.14 f	83,12 c	82.05 c
0.08	48	88,11 ef	91.66 ef	87,08 f	82.85 d
0.08	72	84,88 d	87.49 d	84,27 d	81.73 d
DMRT (α = 5 %)		1,34 –1,52	1.06-1.19	0,91 –1,03	2.97-3.39

Note: dextrozyme concentration for arenga starch were 0,08%; 0,12% and 0,16%w/w.

The highest DE value 92.14 was obtained from 0.08% w/w of dextrozyme and 24 hours incubation time followed by DE value 91.66 obtained from 48 hours incubation time. Data showed that 0.06% w/w of dextrozyme and 72 hours of incubation time could produced glucose syrup with 91.44 DE value. DE value of arrowroot glucose syrup declined when incubation time was prolonged up to 72 hours. This mean that after 48 hours incubation, the substrate concentration of arrowroot strarch have been reduced as a consequense of glucoamylase and pullulanase activities.

CONCLUSION

Glucoamylase and pullulanase can synergistically hydrolyze starches into glucose syrup sequentially with alpha amylase activity. The different sources of starch will showed the different in glucose syrup characteristic.

Based on dextrose equivalent values, it was shown that dextrozyme could hydrolyzed arrowroot starch more easily into dextrose than for gembili and taro starch, respectively. It was found that the saccharification of arrowroot starch by 0.06% w/w of dextrozyme concentration during 72 hours incubation and the saccharification in 0.08%w/w of dextrozyme during 24 and 48 hours incubation showed as the best treatment where the dextrose equivalent of glucose syrup of arrowroot were 91.44; 92.14 and 91.66, respectively. For gembili, 24 and 48 hours of incubation time in 0.08%w/w of dextrozyme yield 89.43 and 88.11 of dextrose equivalent, respectively. The saccharification of taro starch (48 hours of incubation time and 0.08% w/w of dextrozyme) gave 87.08 DE. Meanwhile, arenga starch was the most difficult substrate for glucoamylase, where the use of 0.16% w/w of dextrozyme could only yield 82.85 of dextrose equivalent at 48 hours of saccharification process.

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CONSTRUCTION OF pYαF-Af VECTOR FOR SECRETION OF α-L-ARABINOFURANOSIDASE (AbfA) IN *Saccharomyces cerevisiae*

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ABSTRACT

Gene encoding thermostable α-L-arabinofuranosidase of *Geobacillus thermoleovorans* IT-08 (*abfA*) was successfully cloned and expressed into system of *Saccharomyces cerevisiae* BJ1924/pY-Af. Secretion of heterologous protein in yeast is the preferred mode of protein production due to easy of product recovery. In this research, construction of new vector for secretion of α-L-arabinofuranosidase (AbfA) in *S. cerevisiae* was conducted with α-Factor (αF) signal peptide addition. The plasmid pYES2 (Invitrogen, USA) was as parental vector in construction. The αF-FLAG-abfA fusion gene was amplified by PCR, in which the plasmid YEpFLAG1-Af was used as a template. PCR was conducted with a pF*SacI*-Af primer and a pR*Bam*HI-Af primer. After amplification, the amplicon was treated with *Sac*I and *Bam*HI and then subcloned to the pYES2 plasmid, which was previously digested with *Sac*I and *Bam*HI. The recombinant plasmid was designated as pYαF-Af and propagated first in *E. coli* before transformed into yeast for AbfA production.

INTRODUCTION

Hemicellulose is the second most abundant renewable biomass and accounts for 25–35% of lignocellulosic biomass. There are various enzymes responsible for the degradation of hemicellulose. In xylan degradation, for instance, endo-1,4-*β*-xylanase, *β*-xylosidase, α-glucuronidase, α-L-arabinofuranosidase and acetylxylan esterase all act on the diferent heteropolymers available in nature. Like cellulose, hemicellulose is also an important source of fermentable sugars for biorefning applications (Kumar et al. 2008).

L-arabinose is useful in preventing postprandial hyperglycemia in diabetic patients (Saha 2000, Saha 2003)**.** Therefore, effective L-arabinose production is a vital perquisite for its use in this respect as well as for its importance in food industry. To achieve this goal, it is necessary to use arabinose-releasing enzymes α-L-arabinofuranosidases, as well as defined polysaccharides and oligosaccharides from different agricultural raw materials (Numan & Bhosle 2006).

A thermostable α-L-arabinofuranosidase gene (*abfA*) from *Geobacillus thermoleovorans* IT-08 (Genebank Accession No. DQ387046) was successfully cloned and expressed in *Escherichia coli* DH5a/pTP510 (Puspaningsih 2004), then subcloned to the pET101/D-TOPO resulting in pET-abfA and overexpressed in *E. coli* BL21 (Puspaningsih et al. 2008).

E. coli is not as safe commercial host for big scale enzyme production. Yeasts, mainly *Saccharomyces cerevisiae* has been used for centuries in food production and considered as Generally Recognized As Safe (GRAS). The yeast *S. cerevisiae* has been extensively used in industrial processes and it possesses a number of attributes that render it an attractive host for the production of foreign proteins. *S. cerevisiae*, however, cannot degrade polysaccharides, including xylan (Kim et al. 2000). The advantage of *S. cerevisiae* is that it produces practically no hydrolases attacking polymeric

(hemi)cellulosic substrates and that possible background activities against oligosaccharides can be easily checked (Clark et al. 1996).

The α-L-arabinofuranosidase from *G. thermoleovorans* IT-08 has never been expressed and secreted in *S. cerevisiae*. The expression of some α-L-arabinofuranosidases from other microorganisms have ever published. The expression of α-L-arabinofuranosidase from *Aspergillus niger* (*abfB*) in *S. cerevisiae* was reported by Torres et al (1996). Cloning and expression of α-L-arabinofuranosidase from *A. niger* (*ABF2*) in *S. cerevisiae* was also published by Crous et al (1996). The α-L-arabinofuranosidase from *Trichoderma reesei* was cloned and expressed in di *S. cerevisiae* and secreted using signal peptide itself (Clark *et al*., 1996).

Secretion of heterologous protein in yeast is the preferred mode of protein production due to easy of product recovery. The aim of the present study was construction of secretion vector for thermostable α-L-arabinofuranosidase (AbfA) of *G. thermoleovorans* IT-08 in *S. cerevisiae*. In this research, we constructed the secretion vector of AbfA with α-Factor (αF) signal peptide addition.

MATERIALS AND METHODS

Microbial strains, plasmids, and culture conditions

Escherichia coli strain DH5α was used as host for subcloning. The plasmid YEpFLAG1-Af was used as templete, the source of the αF-FLAG-*abfA* fusion gene. For the construction of yeast expression and secretion vector, the plasmid pYES2 (Invitrogen) was used. *E. coli* containing recombinant plasmid was cultured at 37°C in Luria–Bertani medium supplemented with 100 μg/ml ampicillin.

Primers design and subcloning

Design of a sense primer and an antisense primer for PCR amplification of the αF-FLAG-*abfA* fusion gene was performed by using Clone Manager program. The αF-FLAG-*abfA* fusion gene (about 1.8 kb) was amplified by PCR, in which the plasmid YEpFLAG1-Af was used as a template. For construction of *S. cerevisiae* expression vector, PCR was done with the sense primer pF*Sac*IAf , with a *Sac*I restriction cleavage site and the antisense primer pR*Xho*IAf, with a *Xho*I restriction cleavage site. After amplification, the *abfA* gene was treated with *Sac*I and *Bam*HI and then subcloned to the pYES2 plasmid, which was previously digested with *Sac*I and *Bam*HI. The recombinant plasmid was designed as pYαF-Af. This recombinant plasmid was introduced in *E. coli* DH5α using $CaCl_2$ method (Sambrook et al. 1989). Restriction analysis of recombinant plasmid was used *Sac*I and *Bam*HI enzymes, and its results was looked at by agarose gel electrophoresis.

RESULTS AND DISCUSSION

The plasmid pYαF-Af was constructed for expression of an αF signal peptide-FLAG-AbfA fusion protein using the *GAL1* promoter. The *αF-FLAG-abfA* fusion gene was amplified by PCR. PCR was conducted with a pF*Sac*I- αF primer and pR*Bam*HI-Af primer that designed in this research (Table 1)

The condition of PCR was performed with first denaturation at 94°C for 2 min; 30 cycles (denaturation at 94°C for 30 s, annealing at 50°C for 1 min, and elongation at72°C for 2 min); and the final extension at 72°C for 10 min. Amplicon was analyzed by agarose gel electrophoresis (Figure 1). The amplicon, a fragment DNA of the αF-*abfA* and pYES2 vector were digested by *Sac*I dan *Bam*HI. The result of restriction analysis of recombinant plasmid can be looked at with agarose gel electrophoresis (Figure 2).

Tabel 1. The result of primers design, the sense primer pF*Sac*IAf, with a *Sac*I restriction cleavage site (underlined) and the antisense primer pR*Xho*IAf, with a *Xho*I restriction cleavage site (underlined).

Name	The sequence of primer	Length of primer	Length of amplicon
pF*Sac*I- αF	5'-GCGAGCTCATGAGATTTCCTTCAATTTTTAC-3'	31 pb	1782 pb
pR*Bam*HI-Af	5'-GCGGATCCTTATCGTTTTCCTAAACGAATCAC-3'	32 pb	

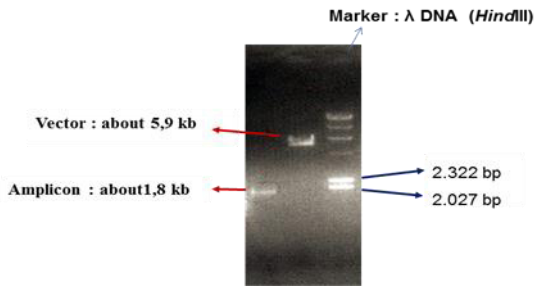

Figure 1. The amplicon and pYES2/*Bam*HI vector were analyzed by agarose gel electrophoresis.

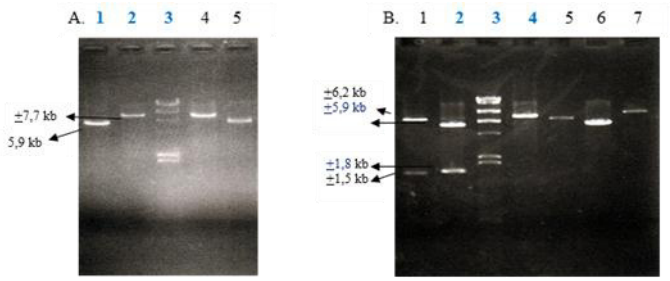

Figure 2. Electrophoregram of restiction analysis of recombinant plasmid.
A. (1) pYES2 : *Bam*HI, (2). P1 : *Bam*HI, (3) marker: λ *Hind*III, (4) P2 ; *Bam*HI, (5) pYES2 : *Bam*HI.
B. (1) YEpFLAG1-Af : *Xho*I and Bam*HI*, (2) P1 : *Sac*I dan *Bam*HI, (3). Marker: λ *Hind*III, (4) P3 : *Bam*HI, (5). YEpFLAG-1 : *Bam*HI (7,2 kb), (6). pYES2 : *Bam*HI (5,9 kb), 7. YEpFLAG-Af : *Xho*I (8,7 kb).

In general, the secretion of heterologous proteins in *S. cerevisiae* is affected by a variety of genetic and environmental parameters such as leader peptide, host, promoter, culture conditions, etc. The key variables determining translocation efficiency are therefore likely to be signal peptide affinity for soluble targeting components, such as signal recognition particle, and duration of ribosome attachment (Arnold et al, 1998). In this study, the secretion vector was constructed to investigate the αF signal sequence on secretion of AbfA in *S. cerevisiae*. It was designed to direct the secretion of AbfA by the aid of one of the signal sequences, the αF leader in the plasmid pYαF-Af (a homologous leader sequence).

Plasmid pYαF-Af was able to construct for expression of an α-factor signal peptide-FLAG-abfA fusion gene in *S. cerevisiae*. In this new plasmid, the expression of the abfA gene is regulated by the inducible *GAL1* promoter *CYC1* terminator, and the its secretion is facilitated by α-factor signal peptide.

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MICROPATTERNED BIOACTIVE LAYER ON NONBIOFOULING SURFACE FOR HIGHLY SENSITIVE IMMUNOASSAY

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ABSTRACT

Micropatterned biointerfaces especially on polymeric substrates are very important for biological studies and applications including biosensors, studies of cell-surface interactions, cell patterning and the like. For applications such as immunoassay-based microarray biosensors, biointerfaces should have regions at which nonspecific bindings of biomolecules eliminated while at the other regions the specific bindings enhanced. To create such surface, a novel method has been performed. That is, the well-known biocompatible cell-membrane-like surface based on 2-methacryl- oyloxyethyl phosphorylcholine (MPC) brush type and micropattered biorecoginition layer (methacryl poly(ethylene glycol)-*N*-succinimidyl carbonate) were constructed by using living radical polymerization technique based on dithiocarbamate chemistry as photo-iniferter. An immunoassay-based microarray biosensor was performed for evaluating sensitivity of these surfaces and we found that this technique could detect rabbit IgG antigen up to 14.7 pM. Due to the less nonspecific adsorption of antigen (background), the response for the sandwich immunoassay performed with micropatterend biorecognition was much higher than that for the corresponding antigen concentration when using standard ELISA techniques. This method can be effectively applied for microchip and microfluidic devices.

Keywords: *living radical polymerization, nonbiofouling surface, micropatterned bioactive layer, photoiniferter, highly sensitive immunoassay*

INTRODUCTION

To create useful biomaterials for many biotechnology applications, interfaces are required that have both enhanced specific binding and reduced nonspecific binding. Thus, in applications such as biosensing, the tailoring of biointerface chemistry and the use of micro or nanofabrication technique becomes an important avenue for the production of surface with specific binding properties and minimal background interference[1]. It has already well known that cell-membrane-like surfaces based on 2-methacryloyloxyethyl phosphorylcholine (MPC) is very effective to inhibit blood coagulation on the medical devices or to prevent nonspecific biological interactions at the interface of biosensors [2, 3]. The aim of this study is to prepare micropatterned biorecognition layer on nonbiofouling surface bearing highly biocompatible poly(MPC) brushes for enhancing high signal/noise (S/N) ratio in biosensing using living radical photopolymerization based on diethyl- dithiocarbamate as photoiniferter (initiator, transfer, and terminator)[4]. The ideal construction of this microsensor chip devices is illustrated in Figure 1. It is expected that the biomolecules conjugated on poly(MPC) brushed surface keep high activity due to the reduction of denaturation of immobilized biomolecules in the presence of the MPC unit and the chemical binding to the polymer via the oxyethylene chain[5,6].

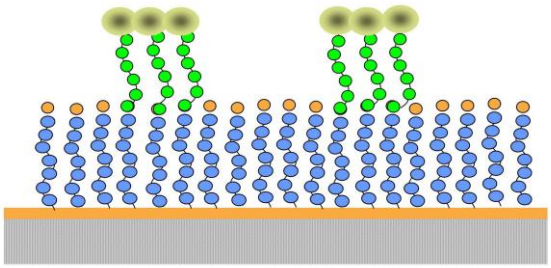

Figure 1. The ideal construction of micropatterned biorecoginiton layers over the nonbiofouling poly(MPC)-brushed type surface.
substrate, macrophotoiniferter layer, MPC,
PEG-NSC, biomolecules (protein/cell)

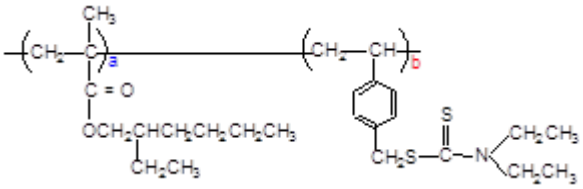

Figure 2. Chemical Structure of macro- photoiniferter; poly(EHMA-co-VBDC) (PEV)

EXPERIMENTAL

The macrophotoiniferters (PEV as shown in Figure 2) comprised of 2-ethylhexyl methacrylate (EHMA) and 4-vinylbenzyl *N,N*-diethyldithiocarbamate (VBDC) were synthesized with various of VBDC content from 10% to 40% (termed as PEV10, 20, 30, 40).

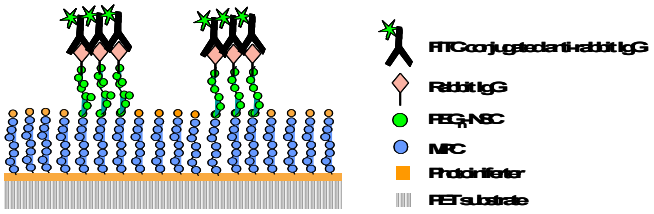

Figure 3. Schematic illustration of constructing micropatterned biorecognition layer (PEG_{200}-NSC) on nonbiofouling surface PEV30-g-MPC_{1h})

Properly cleaned polyethylene terephthalete (PET) substrates were coated by dipping in 0.25% (by weight) solution of macrophotoiniferter dissolved in toluene. In the presence of aqueous solution of 0.3 M MPC monomer, the photoiniferter-coated substrates were irradiated with UV lamp (365 nm) at room temperature. The properties of the poly(MPC)-modified (termed ad PEV_x-g-MPC_y where x and y are VBDC content and photoirradiation time, respectively) surfaces were carefully characterized and evaluated with water contact angle (Kyowa Interface Science, Saitama, Japan), ellipsometry (NL-MIE, Nippon Laser and Electronics Lab., Nagoya, Japan), X-ray photoelectron spectroscopy (XPS, AXIS His 165 and ULTRA, Krotos Analytical, Manchester, UK), and atomic force microscope (AFM, Nanoscope® IIIa, Veeco Instrument, CA, USA). Nonspecific protein adsorption resistance of these modified surfaces was thoroughly investigated by contacting with a mixture of 0.045 g bovine serum albumin (BSA) and 0.030 g human plasma fibrinogen (HPF) in 100 ml phosphate buffer saline (PBS, pH 7.4). The micropatterned biorecognition layer of methacrylated poly(ethylene glycol) conjugated with *N*-succinimidyl carbonate

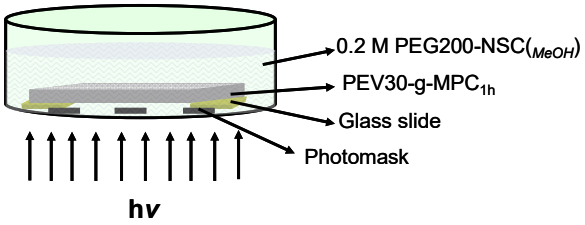

Figure 4. Schematic illustration of immunoassay-based biosensors of IgG-anti-IgG-FITC performed on nonbiofouling surfaces (PEV30-g-MPC_{1h})

(Methacryl-PEG_{200}-NSC) was polymerized by using photomask with 100 μm in size as shown in Figure 3. Finally, for immunoassay-based biosensor application (Figure 4), various concentrations of rabbit IgG ranging from 0 to 220 μg/mL in PBS (pH 7.4) were contacted to the micropatterned biorecog- nition surfaces overnight at room temperature and after washing with fresh PBS they were contacted with anti-rabbit IgG-FITC for 2 hours at 4°C and then washed with fresh PBS before observed with fluorescence microscope equipped with confocal camera.

RESULTS AND DISCUSSION

The nonbiofouling surfaces based on biomimetic poly(MPC)-brushes were prepared on PET. The density and length of the poly(MPC)-brushes which are the very important factors in suppressing the nonspecific protein adsorption were controlled by composition of VBDC on the macrophotoiniferter and irradiation time respectively. Figure 5 shows the thickness and molecular weight of poly(MPC)-brush on PEV40 coated PET as a function of photoirradiation time. The thickness and molecular weight of the grafted layer also increase by grafting time as characteristic for living radical polymerization and reach at 100±14 nm and 32 kDa after 3 hours photoirradiation time respectively.

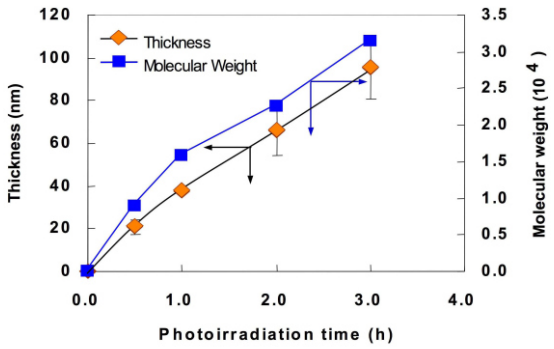

Figure 5. The dry-state thickness and molecular weight of poly(MPC) grafted on PEV40 coated PET (PET/PEV40) as a function of photoirradiation time.

Every modified surface had XPS signals at 285 eV, 288 eV, 133 eV, and 403 eV attributed to C1s in methylene, C1s of carbonyl, P2p, and N1s from grafted poly(MPC) chains, respectively. The value of phosphorus/carbon (P/C) ratio increases by grafting time and composition of VBDC unit on the surface, especially in the lower grafting time (Figure 6). The highest P/C value (0.07) is close to the theoretical one (0.09) indicates the well oriented states of the poly(MPC) layer. Thus, we could control the polymer chain density and length of the poly(MPC) brush by composition of VBDC in the macrophotoiniferter and UV irradiation time, respectively. The static contact angle of

poly(MPC) brush surfaces as a function of photoinifeter composition changed from more than 88° to around 20° after 1 hour irradiation indicating that the poly(MPC) chains are well-oriented on the surface and completely covers the PET surface.

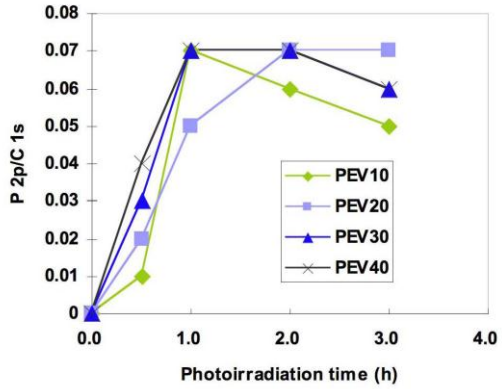

Figure 6. P_{2p}/C_{1s} atomic ratio available on the poly(MPC)-modified surfaces as a function of photoirradiation time at various content of photoiniferter

Table 1. RMS roughness of poly(MPC)-modi- fied surfaces

Surface	Roughness RMS (nm)		
	Photo irradiation time (hr)		
	0.5	1.0	3.0
PEV10-g-MPC	16.0±0.5	17.6 ±0.5	27.3 ±0.5
PEV20-g-MPC	21.7 ±0.4	6.8 ±0.3	9.9 ±0.3
PEV30-g-MPC	18.9 ±0.4	6.4 ±0.3	9.9 ±0.3
PEV40-g-MPC	20.0 ±0.4	6.2 ±0.3	35.8 ±0.7

The morphology of the poly(MPC)-modified surfaces at wet state was studied by an AFM. The root-mean-square (RMS) roughness of PET and macrophotoiniferter coated PET was 4.3± 0.3 nm and 5.7± 0.5 nm, respectively, and the roughness of the poly(MPC)-modified surfaces was summarized in Table 1. The surfaces prepared on lower photoiniferter composition(PET/PEV10) at different photo-irradiation time were very rough, and the RMS roughness of the surfaces increased with increasing the polymer chain length. The rough surfaces were also observed at short chain length (PEV-g-MPC0.5) and the RMS roughness on the surfaces was varied independently from the grafting density. Meanwhile, the smooth surfaces were generally generated from medium chain density and chain length (PEV20-g-MPC1.0, PEV30-g-MPC1.0, and PEV40-g-MPC1.0 with RMS roughness of 6.8± 0.3, 6.4± 0.3, and 6.2± 0.3 nm, respectively). Figure 7 shows the typical AFM height images of the PET and the poly(MPC)-modified surfaces preparing with a different photoirradiation time on PEV30 coated PET. The brushed-like structure was observed at the poly(MPC)-modified surfaces in a wet state. Based on the results shown in Table 1 and AFM height images, it was confirmed that the topology of the surface is greatly depending on the chain density and chain length of poly(MPC). The similar phenomenon was also reported by Feng et al.[7] where at the same chain density but different MPC unit generated different topology and surface roughness of poly(MPC)-modified surfaces using ATRP process although they took the images in dry condition.

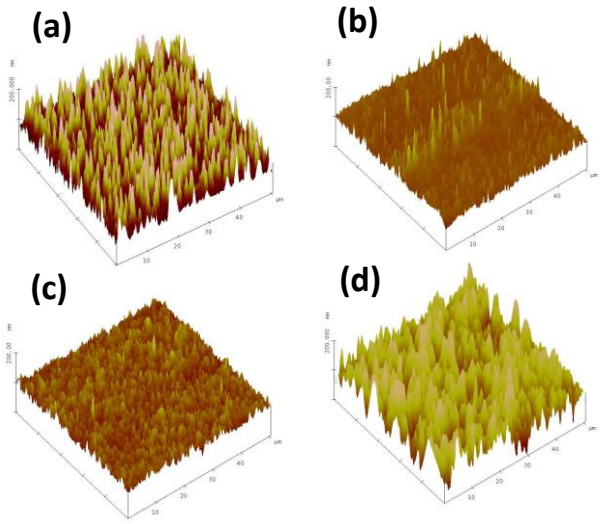

Figure 7. 3D topography profile of poly(MPC) brush surfaces on different chain density for 3 hours photoirradiation time. PEV10-g-MPC_{3h} (a), PEV20-g-MPC_{3h} (b), PEV30-g-MPC_{3h} (c), and PEV40-g-MPC_{3h} with rms roughness of 27.31 nm, 9.86 nm, 9.83 nm, and 35.79 for a, b c, and d respectively.

Protein adsorption repellence of these poly(MPC)-modified surfaces was evaluated and the results of PEV30-g-MPC, and PEV40-g-MPC are seen in Figure 8. Protein adsorption significantly reduces on all poly(MPC)-modified surfaces compared to control one (PET/PEV substrate) except for the result of PEV40-g-MPC3.0. All the smooth surfaces effectively reduced nonspecific protein adsorption up to 86%. The reduction of protein adsorption on poly(MPC)-modified surfaces is due to highly surface hydrophilicity showing a phosphorylcholine group of MPC unit, and protein adsorbs on PEV/PET through hydrophobic interaction, as expected. While, all the rough poly(MPC)-modified surfaces remain severe for inevitable nonspecific protein adsorption even at the very highly poly(MPC) chain density and on highly hydrophilic surfaces like PEV40-g-MPC3.0. These phenomenon pop up us to the question about the mechanism of nonspecific protein adsorption especially on poly(MPC)-modified surfaces. So it could be concluded that surface morphology or surface roughness plays a very important factor to be considered in generating nonbiofouling surfaces of poly(MPC)-modified surfaces.

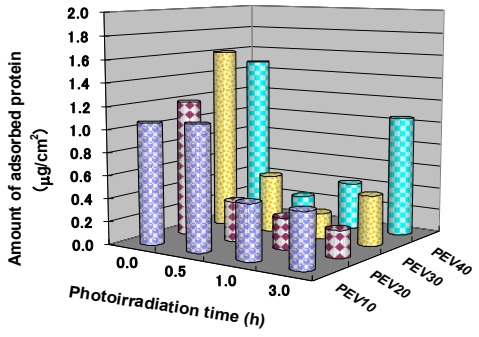

Figure 8. Total amount of adsorbed protein on photoiniferter-coated surfaces (control surfaces) and on poly(MPC) brush surfaces on various chain density and irradiation time.

Micropatterned polymer brushes are of crucial importance to the development of biochips, microarrays, and microdevices for cell growth, regulation of protein adsorption, and drug delivery. Thus, the micropatterning of poly(MPC)-brush and biorecognition

layer were tried to conduct by the process described in the Figure 3. Micropatterning biorecognition layer (PEG200-NSC) was successfully conducted on nonbiofouling surface (PEV30-g-MPC_{1h}) with high fidelity. For evaluation of the micropatterned surface, an immunoassay-based biosensor was conducted using rabbit IgG – anti rabbit IgG-FITC specific interaction. The profile of the fluorescence intensity was shown in the Figure 9.

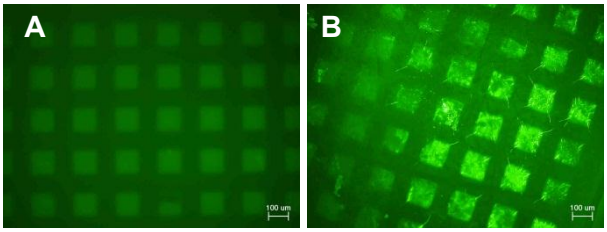

Figure 9. Fluorescence profile images of IgG-anti-IgG-FITC at different concentration of rabbit IgG of 14.7 pM and 1.47 nM for A and B, respectively.

The `sigmoidal binding behaviour` was observed as intensity of fluorescence increased by increasing the concentration of the rabbit-IgG but somehow the intensity became stagnant since the saturation of immobilized rabbit IgG (Figure 10). Detection limit of this method was 0.0022 µg/mL which is equal to 14.7 pM which proves the highly sensitivity of this method although somehow the S/N for this concentration was still remained low. The S/N significantly increased as the concentration of rabbit IgG increased before reaching the saturated condition. Due the less nonspecific adsorption (background), the response for the immunoassay performed with micropatterend biorecognition layer (PEG_{200}-NSC) was much higher than that for the corresponding antigen concentration when using standard ELISA techniques.

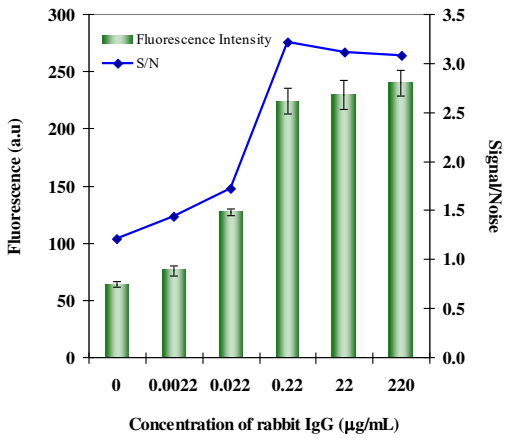

Figure 10. Fluorescence intensity and S/N of the IgG-anti-IgG-FITC interaction as a function of IgG concentration.

CONCLUSION

A novel method for preparing nonbiofouling surfaces on polymeric substrates using living radical polymerization based on dithiocarmabate chemistry has been conducted. Further the construction of micropatterned biorecognition layer (PEG200-NSC) on the nonbiofouling surface has been performed. Immunoassay-based biosensor on microchip was used to evaluate the S/N. The sensitivity of the surface can be enhanced to the level of pM of rabbit IgG antigen.

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THE DEVELOPMENT APPLICATION OF ULTRAFILTRATION TECHNOLOGY ON AQUACULTURE: HARVESTING AND CONCENTRATING MICROALGAE FOR LARVICULTURE PURPOSES

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ABSTRACT

One of the critical factors determines the success of aquaculture system is the availability of feed continuously. Feed for fish/shrimp farming can be obtained in form of live/natural feeds or artificial diet. Live feed such as microalgae is particularly used for hatchery system. Advantages of natural feed over artificial diet are relatively small size that fit with the opening mouth of fish/shrimp larvae, high nutritional content, easy to culture, and do not contaminating culture medium. Live feeds will continue to be the primary feedstock for larviculture purposes in shrimp/fish hatcheries. Culture of microalgae includes production step, harvesting, and post-harvesting. Harvesting usually done when the concentration of microalgae reach up to 10^6 cell/l. Conventional process to harvest microalgae, such as chemical flocculation, coagulation and centrifugation technique are time- and chemical consuming. Ultrafiltration (UF) as alternative of conventional process offers several advantages for harvesting and concentration of microalgae. The suspension was continuously recycled from and to the vessel through the UF membrane module by a feed pump with constant speed. Permeate was removed from the culture while microalgae cell as retentate was returned to the feed tank to increase microalgae concentration. Preliminary study showed the capability of UF membrane to concentrate microalgae culture up to 10^{10} cell/l. Performances of membrane depend on hydrodynamics condition, initial concentration and properties of culture (shape, age, AND DEBRIS). In the meantime, it is also feasible for hatchery unit to simultaneously run the microalgae culture to minimize the preservation and distribution problem. Using UF membrane technology for harvesting of microalgae is alternative solution to solve microalgae/culture medium separation problem in term of time- and chemical consuming efficiency.

Keyword: Aquaculture, microalgae, ultrafiltration

INTRODUCTION

There is a growing interest in microalgae, and the development of biotechnology has allowed their commercial exploitation. In addition to aquaculture and the health food market, the uses of microalgae currently focus on the production of various high value metabolites (Rossignol et al., 1999). Approximately 90% of aquaculture-produced animals were reared using phytoplankton (microalgae) as feed source during one or more stages. Worldwide aquaculture production is still growing with trends toward intensification and greater control over total nutritional input.

Microalgae production for feeds is divided into intensive monoculture for larval stages of bivalves, shrimp, and certain fish species, and extensive culture for grow-out of bivalves, carp, and shrimp. Favoured genera of microalgae for larval feeds include *Chaetoceros, Thalassiosira, Tetraselmis, Isochrysis* and *Nanochloropsis*. These organisms are fed directly and/or indirectly to the cultured larval organism. Indirect means of providing the algae are trough Artemia, Rotifers, and Daphnia, which are, in turn, fed to target larval organisms.

Apart of the development of separation technology, the application of some filtration technology for harvesting microalgae cell has been investigated, such as filter press technology (Mohn, 1980), rotary drum vacuum and rotaru drum precoat technology (Gudin et al., 1980), sand filter (Bent-Amotz et al., 1991), and membrane technology

(Petrusevski et al., 1995; Borowitzka, 1997; Rossignol et al., 1999). Application of membrane technology, especially ultrafiltration technology seems most potential and rational. Cheryan (1986) stated that ultrafiltration membrane could harvested 100% algae cell from their culture medium. One of the ultrafiltration problems for harvesting microalgae is decreasing of flux caused by membrane fouling.

This study aims to investigate the effect of pressure and concentrate factor on flux characteristic and their rejection in case of harvesting *Nannochloropsis* sp prior use as live feed to shrimp larvae.

MATERIALS AND METHODS

Setting Up of Ultrafiltration System

This study use a cross-flow hollow fiber ultrafiltartion membrane. The internal membrane consisted of polyacrylonitrile (PAN) ultrafiltration membrane material with molecular wight cut-ofF (MWCO) 100000 Dalton and 98.125 cm^2 of total area. The experimental set-up is shown in Fig. 2.

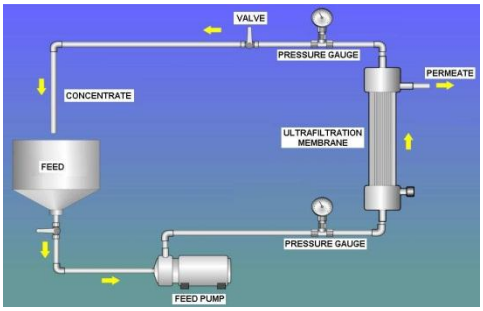

Figure 2. Experiment setup for Microalga harvesting by UF membrane

Microalgal strain

The study was performed using a strain of *Nannochloropsis* sp. which isolates obtained from Laboratory of Aquatic Ecosystem, School of Life Science and Technology, Insitut Teknologi Bandung. Cultures were maintained in the mix media described by Priyambodo et al. (2003).

Procedures

In this study, the effect of transmembrane pressure (TMP) variations and feed concentration on flux profile and membrane rejection were tested. On the other hand, the influence of flux and membrane rejection on volume concentration ratio was also tested. The optimum operation pressure for harvesting and concentratiing microalge was obtained from the experiment data.

The initial microalgae density of 10^7 cell/l was performed and concentrating until 10^8 cell/L. The microalge concentration both of initial and after harvesting were determined by measuring cell by using Haemositometer *Improved Neubeuer*.

RESULTS AND DISCUSSION

Transmembrane Pressure (TMP) Influence on Membrane Flux

Theoretically, flux will increase proportionally to transmembrane pressure. Fig. 3 showed permeation flux during filtration process at TMP 0.75 – 2.5 bar and constant temperature of 25°C. At Figure 3, flux decreasing significantly at first 60 minute caused by polarization concentration and membrane fouling (Cheryan, 1986)

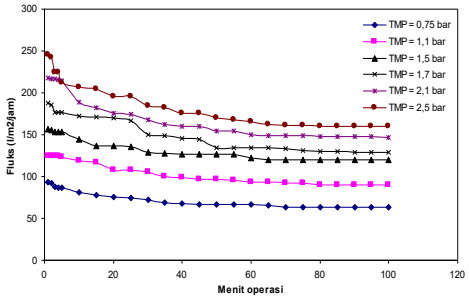

Figure 3. Influence of various TMP on membrane flux

Influence of TMP on steady state flux depicted on Figure 4. The permeation flux increase to TMP and it is interesting at TMP 1 bar still result on a high average flux of 80 $l.m^{-2}.h^{-1}$

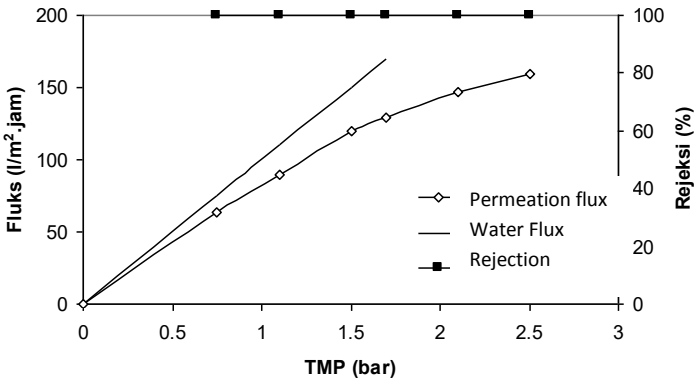

Figure 4. Flux profile and rejection to various TMP

The experiment results also showed that microalgae totally rejected by membrane. It could understand that caused by microalgae size (2-10 µm) larger than membrane pore size (100000 Da equivalents with 0.1 µm). This is indicate that microalgae could be harvesting as concentrate, but still need a consideration about cell condition after harvesting, because we found broken cell increased to TMP. Based on the results, the next experiment conducted at maximum TMP 1 bar.

Influence of Volume Concentration Ratio (VCR) on Membrane Flux Characteristic

VCR is the ratio of initial feed volume (Vo) to feed volume at time t (Vt) that have significant influence on membrane flux and rejection profile. This study was conducted on various VCR 1, 1.25, 2.5, 5 and 10. The experiment showed that lower flux obtained on high VCR, although we get constant rejection (100%) to increasing VCR, as depicted on Figure 5.

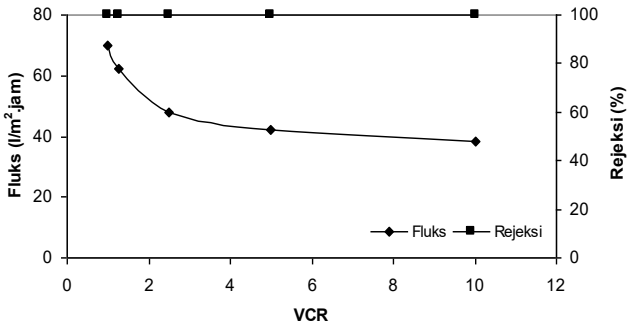

Figure 5. Flux and rejection profile to VCR

Membrane flux obtained on 38.34 $l.m^{-2}.h^{-1}$ at VCR 10 (concentration of microalgae 10^8 cell/l), this is indicated that increasing of concentration will decreasing flux caused by membrane fouling. Fouling caused by accumulation of microalgae cell layer on membrane surface. On the other hand, increases the concentration will also increasing osmotic membrane pressure that could be decrease of solute driving force passing the membrane (Mulder, 1996). Having considering of membrane flux, rejection and microalgae cell condition, ultrafiltration membrane could be harvesting and concentrating microalgae up to 10^{10} cell/liter, with still high flux and 100% rejection.

CONCLUSION

Harvesting and concentrating microalgae with Ultrafiltration membrane is a suitable and promising technique for supporting live feed availability on larviculture purposes. It is also have economically and environmentally advantages in term of time and chemical consuming, but still need to be consider about how to manage the membrane fouling on harvesting microalgae process.

ACKNOWLEDGEMENT

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TAMARIND LEAF EXTRACTION (*Tamarindus indica* L.) ETHANOL-DEXTRIN ENCAPSULATION: STUDY OF ANTIRADICAL AND ANTIOXIDANT

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ABSTRACT

Tamarind leaf has a flavonoid as an antiradical with antioxidant activity. Tamarind leaf is extracted through ethanol concentration (10%, 30%, 50%, 70% dan 90%) with a ratio of 1 : 2. It is filtrated and added dextrin concentration (5%, 7.5%, and 10%) and then dried. Tamarind leaf extract is tested by total phenolic content and diphenylpicrilhydrazyl (DPPH). Antioxidant activity on soy bean oil is tested with peroxide number (ferric thiocyanate) and malonaldehyde (TBA). BHT synthetic antioxidant was used as the control. This research is designed using a multiple randomized design. The results showed the following optimal levels: extraction 70% ethanol dextrin 5%, diphenylpicrilhydrazyl and total phenolic content: 0.18% with 3.24% gallic acid equivalent. Tamarind leaf extract in concentration 200 ppm, is not able to inhibit peroxide. However, it does inhibit malonaldehyde on oxidized soy bean oil. Tamarind leaf extract and BHT have a similar capability as an antioxidant in oxidized soy bean oil.

Keywords: *Tamarindus indica* L., dextrin, antioxidant, antiradical and soy bean oil

INTRODUCTION

Antioxidant compounds called phytochemicals (Pratt, 1992) that come from natural materials are safer for humans. The derivative flavonoids are as important a phytochemical as an antioxidant (Johnson, 2001). Phenolic compounds in plants have been widely studied and found to show antioxidant activity (Pokorny, 2001). Natural antioxidant compounds are expected to change synthetic antioxidants such as butylated hidroksianisol (BHA) and butylated hidroksitoluene (BHT).

Research has been conducted showing Tamarind *(Tamarindus indica* L.*)* to have antioxidant activity (Suwariani *and* Suhendra, 2008; Chanwitheesuk *et al.,* 2005; Maisuthisakul *et al.,* 2008; Siddhuraju, 2007). Tamarind has shown potential as an antidiabetic and anti-hiperlipidemik (Maiti *et al.,* 2005; Maiti *et al.,* 2004), by inhibiting nitric oxide production in cells (Choi *and* Hwang, 2005). The antioxidant compound in tamarind is 2-hydroxy acid-30, 40-dihydroxy aseto fenon, 3.4-dihydroxy methyl benzoate, 3.4-dihydroxy phenyl acetic acid, epicatechin and oligomerik proanthocianidin (Tsuda *et al.,* 1994).

Tamarind *(Tamarindus indica* L.*)* contains xyloglucan. The xyloglucans (XGs) are a group of storage or structural heteropolysaccharides from plants, and their structure is composed of a 1,4-linked β-D-glucan main chain that is partially substituted with α-D-Xyl side-chains at the O-6 atoms. Depending on the source, the side-chains can be β-D-Gal-1,2-α-D-Xyl or α-L-l-Fuc-1,2- β-D-Gal-1,2- α-D-Xyl (Carpita & Gibeaut, 1993; Fry, 1989; Hayashi, 1989; McNeil, Darvill, & Fry, 1984; Varner & Lin, 1989) or more complex chains (Hantus, Pauly, Darvill, Albersheim, & York, 1997; Freitas et al., 2005; Jia, Qin, Darvill, & York, 2003; York, Kumar-Kolli, Orlando, Albersheim, & Darvill, 1996). The XGs are water-soluble, but the individual macromolecules typically do not fully hydrate, and consequently, aggregated species remain present even in very dilute solutions. For encapsulation, dextrin is one of the compounds required to make tamarind leaf extract.

Cyclodextrins (CDs) are cyclic oligomers of a-D-glucopyranose that can be produced due to the transformation of starch by certain bacteria such as *Bacillus*

macerans (Jeang, Lin, & Hsieh, 2005; Qi, Mokhtar, & Zimmermann, 2007; Qi & Zimmermann, 2005; Rimphanitchayakit, Tonuzuka, & Sakano, 2005). The torus-like cyclodextrin molecules have an outer polar surface and an inner non-polar surface. The small hydrophobic cavities within the cyclodextrins have diameters of 0.50, 0.62, and 0.79 nm respectively, for the α, β, and γ dextrins. Cavities are potential binding sites for a wide variety of both organic and inorganic molecules. Cyclodextrins can be used to "encapsulate" food additives, and their complexes may be useful in the separation of enantiomers, of drugs.

In recent years, several published reviews have described the use of CDs in food and flavour applications (see Cravotto et al., 2006; Hedges & McBride, 1999; Hedges, Shieh, & Sikorski, 1995; Qi & Hedges, 1995; Samant & Pai, 1991; Szente & Szejtli, 2004). CDs have been recommended for applications in food processing as well as for food additives with a variety of reason. One reason is to protect lipophilic food components that are sensitive to oxygen and due to light- or heat-induced degradation.

The aim of the research is to find out whether tamarind leaf extract (*Tamarindus indica* L) with an ethanol-dextrin encapsulation method can act as anantiradical and antioxidant.

MATERIALS AND METHODS

Materials and Equipment

The research is focused on local varieties the tamarind leaf *(Tamarindus indica* L.) collected from Kertalangu Kesiman Village, Denpasar - Bali. The chemical were obtained from Merck and included : Soybean oil (linoleic acid), tiobarbituric acid, phosphate buffer, sodium carbonate, *ferry* and *tiobarbituric thiosianat acid*, and *Folin Ciocalteu phenol*, ; The chemical were obtained from Brathaco Chemical included : ethanol, BHT while Sigma provided : gallat acid, DPPH radical (-2.2-1-picryldhydrazyl dhiphenil radical). The equipment used in this research included : Vacuum Rotary Evaporator, spectrophotometer (Turner SP-870), Centrifuge (EC HN-S II 0000-9000 rpm), Vortex (Thermolyne), Oven (Blue M), and an incubator (Memmert, Model 500).

Experimental Design

Experiments were conducted using a completely randomized factorial design (CRD)Factor 1 focused on : the concentration of ethanol as a solvent : K1: 10%, K2: 30%, K3: 50%, Q4: 70% and K5: 90%. The second factor looked at : the concentration of dextrin adding solution, (w/w), which consisteds of three levels : R1: 5%, R2: 7.5%, and R3: 10%. The experiment was repeated twice.

Research and Analysis

Fresh tamarind leaves were washed, drained, and then ground to make powder. The mixture was than added to the ethanol ratio (2:1) and extracted after one hour then filtered and leaf extracts added with dextrin as above. The compound was then dried in an oven at a temperature of 50°C. The dried tamarind leaf extract was then ground to make a powder and seived through a 60 mesh.

Analysis: Stage 1 involved determining DPPH and the components of phenolic compounds. Stage 2 looked at the antioxidant activity using TBA (Kikuzaki and Nakatami, 1993) modified. Stage 3 focused on antioxidant activity *Ferry thiosianat* (Kikuzaki and Nakatami, 1993) modified.

RESULTS AND DISCUSSION

Total Phenol Tamarind Leaf Extract

The total phenolic quantity in the tamarind leaf extract had a high solubility in 90% ethanol. The addition of 7.5% dextrin caused a higher polyphenol binding ability than alternative levels (Table 1). Polyphenol solubiliti differs depending upon the structure of the compounds. Dextrin as an encapsulant has the ability to bind the polyphenol compounds.

The binding ability of polyphenol compounds on dextrins is due to cyclodextrin molecules that are hydrophobic. Binding capacity of polyphenol compounds depends on the type of cyclodextrin (α, β or γ). The hydrophobic ability of cyclodextrin has a similar tendency to polyphenol in that when sufficiently polarized it becomes soluble in an ethanol. Optimum concentration of dextrin for binding to polyphenols was found to be 7.5%.

Table 1. The average of total phenolic of tamarind leaf extracts in ethanol concentration and dextrin

Dextrin (%)	Ethanol Solvent (%)				
	10	30	50	70	90
5	1.183^{j}	1.835^{i}	2.599^{h}	3.235^{fg}	3.797^{d}
7,5	3.410^{ef}	6.451^{a}	2.952^{g}	4.593^{c}	5.690^{b}
10	3.295^{f}	2.624^{h}	3.620^{de}	3.472^{ef}	5.410^{b}

Antiradical Activities of Tamarind Leaf Extract (DPPH test)

Extraction of ethanol with concentration 70% displayed the highest antiradical activity (Table 2). Tamarind antioxidant compound is 2-hydroxy-30, 40-dihydroxy aseto fenon, 3.4-dihydroxy methyl benzoate, 3.4-dihydroxy phenyl acetic acid, epicatechin and oligomeric proanthocianidin (Tsuda *et al.,* 1994), an has tendency to become soluble in ethanol 70% concentration. Research using ethanol concentration of 70% for extraction tamarind fruit also done by Martinello et al. (2006) and Ramos et al. (2003).

Addition to dextrin concentration did not influence radical activities (Table 2). Dextrin with a concentration of 5% showed the ability to bind phenolic compounds and protect from heat damage. Dextrin has the ability to separate molecules XGs, so that the tamarind leaf extract forms an anhydride compound.

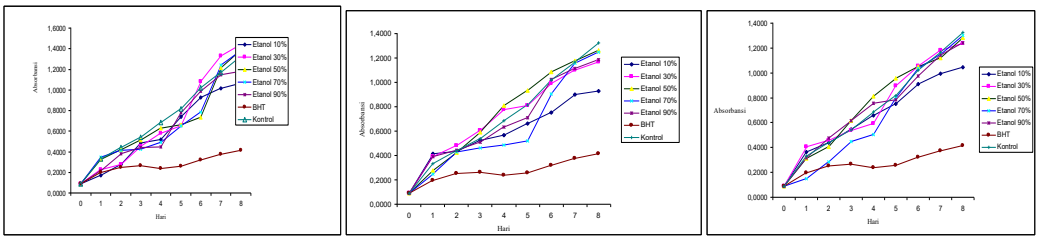
Table 2. Average antioxidant activity (DPPH) of tamarind leaf extract at various concentrations of ethanol and dextrin

Dextrin (%)	Ethanol Solvent (%)				
	10	30	50	70	90
5	0.0597^{i}	0.0946^{f}	0.1066^{d}	0.1177^{b}	0.0857^{gh}
7,5	0.0430^{j}	0.0440^{j}	0.0980^{e}	0.1143^{c}	0.1042^{d}
10	0.0425^{j}	0.0372^{k}	0.0849^{h}	0.1232^{a}	0.1064^{d}

Antioxidant Activity of tamarind leaf extract using the FTC method.

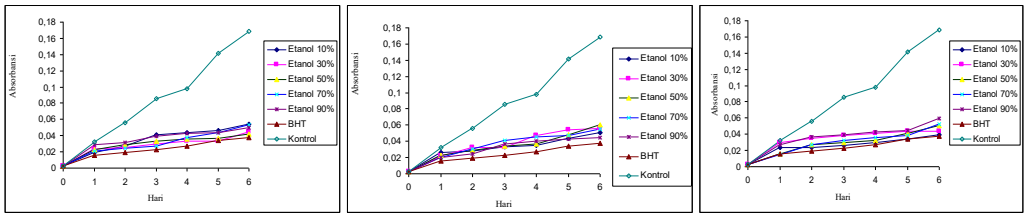
Measurement of antioxidant activity conducted by the FTC method based on the formation of peroxide which is oxidation of linoleic acid from soybean oil. Peroxide will oxidize ferrous ions to ferri, and then ferrithiosianat form when measured at λ = 500 nm.

Figure 1a, 1b, 1c: The antioxidant activity of tamarind leaf extract at addition of 5%, 7.5% & 10% dextrin with FTC test



The FTC test showed that polyphenol compounds of tamarind leaf extract haves no ability to inhibit peroxides.
Peroxide is the primary products of oxidation which could not be inhibited / prevented by the polyphenol compound of tamarind leaf extract (Fig. 1a, 1b.1c).

Antioxidant Activity of Tamarind Leaf Extract with TBA Method
To determine the ability of antioxidants to inhibit the formation of the malonaldehid reaction rate oxidation process on lipids using the thiobarburic acid test (TBA).
ind leaf extract at addition of 5%, 7.5% and 10% dextrin with test TBA



MDA is a secondary product from the oxidation of soybean oil. Tamarind leaf extract varied for ethanol and dextrin concentration showed a high ability to prevent / inhibit the formation of MDA (Figure 2a, 2b. 2c). Martinello et al. (2006) examined the tamarind fruit showed the ability to prevent / inhibit the formation of MDA.

CONCLUSION

1. Dextrin has a high ability to isolate xyloglucans (XGs) on the encapsulation of tamarind leaf extract
2. Tamarind leaf extract has low affect on antiradicals and has no ability to prevent formation of peroxides.
3. Tamarind leaf extract has the ability to inhibit or even / prevent the formation of MDA, similar to BHT.

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USING OF *PEDIOCOCCUS ACIDILACTICI* U318 POWDER AS STARTER CULTURE IN PRODUCTION OF *URUTAN*: STUDY ON CONDITIONING PERIOD AND CASING USED IN *URUTAN* PRODUCTION

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ABSTRACT

Using of inoculum in powder form is more convenience than the pure starter culture, especially for commercial and industrial scale production of fermented food. Starter culture has an important role to improve the quality of the products. The application of pure starter culture of *Pediococcus acidilactici* U318 have been experimented in production of *urutan* and showed an improvement of product quality. In order to make more convenient using starter culture, experiment was done to produce starter culture in powder form. In present study, using of this inoculum powder was experimented in *urutan* production. The aim of the experiment was to find out the effects of conditioning period and casing on microbiological and sensory characteristics of *urutan*. The experiment results showed that the interaction of conditioning period and kinds of casing affected to the total microorganism, and total *Enterobacteriaceae* of the product. On the other hand, water content of *urutan* was only affected by the treatment of conditioning period, and total of lactic acid bacteria was only affected by casing used in processing of *urutan.* Sensory evaluation showed that 192 hours conditioning period and synthetic casing gave the highest preference with the characteristics as follow: brown color, normal-relatives texture, normal-specific *urutan* aroma, and normal flavor.

Keywords: *Pediococcus acidilactici* U318, *urutan*, production, conditioning, casing

INTRODUCTION

LAB play important roles in food fermentation which provide advantages such as modification and improvement of flavor characteristics and contribute in preservation of food stuffs. LAB starter cultures have already been widely applied in numerous fermentation such as milk (Parente *et al.*, 1997; Fitzsimons *et al.*, 1999), vegetables (Sanchez *et al.*, 2000; Kalac *et al.*, 2000), alcoholic beverages (Patarata *et al.*, 1994; Pattison *et al.*, 1998) and meat (Hammes and Hertel, 1998). It has also been demonstrated that a better quality of sausage is achieved by using starter cultures. Since it has been discovered that *urutan* has a different characteristics from other fermented sausages, and the fact that spices are always incorporated in *urutan* fermentation unlike the European and American sausages, the development of specific starter cultures for *urutan* is urgently required (Antara *et al.*, 2002).

Un-consistence of quality and risk of fermentation failure are the common problems on traditional fermented products which are produced under uncontrolled condition. The failure of *urutan* fermentation that produced using natural casing and dried under sun shine was signed by off-odor product. The application of pure starter culture have been experimented in production of *urutan* and showed an improvement of product quality (Antara *et al.*, 2002). In order to make more convenient using starter culture, experiment was done to produce starter culture in powder form and applied them in fermentation of *urutan*. The objective of the present research was to determine the effects of conditioning process and the kinds of casing used on the microbiological and sensory characteristics of *urutan*.

MATERIALS AND METHODS

Starter cultures

LAB strain, *Pediococcus acidilactici* U318, originally isolated from *urutan* was used as starter culture in this study. The strains were grown in MRS broth (Difco, Detroit, MI) for 16-18 h and the liquid inoculum was then powdered by vacuum dryer after addition of skim and maizena as filling agents. The inoculum powder was then used as starter culture in production of *urutan***.**

Urutan production

Two fermentation lots inoculated with powder of starter culture of *P. acidilactici* U318. The *urutan* batter was prepared as follows: 2 kg of lean pork and fat (meat and fat ration was 3:1) was mixed with 100 g garlic (*Allium sativum*), 20 g aromatic ginger (*Kaempferia galangal*), 30 g "laos" (*Galanga pinata*), 10 g pepper (*Piper nigrum*), 20 g chilli (*Capsicum frutescens*), 20 g turmeric (*Curcuma longa*), 60 g table salt, and 20 g sugar. The mixture was divided into four equal lots and then inoculated as described above to give a final cells count of about 10^7 cells/g. Each lot was thoroughly mixed and stuffed into collagen casing (inner diameter of about 2.5 cm and length 12 cm). The *urutan* were incubated for 120 h at two different temperatures; 30°C and 45°C, which was changed every 12 h.

Destructive sampling was periodically done at 0 h (soon after the mixture was stuffed into casing), 24, 48, 72, 96, and 120 h. Microbiological, colour, and texture analysis were conducted immediately after sampling and samples for chemical analysis were kept at -80°C until the time of analysis. The experiments were conducted in two independent replications.

Microbiological analysis

Ten gram of samples were blended and homogenized with 90 ml saline solution (0.85% NaCl). One ml of homogenate of each sample was serially ten fold diluted in saline solution. Diluents (0.1 ml) were plated in appropriate agar medium for microbiological analysis. LAB population were enumerated on MRS agar after 24 h incubation at 30°C, aerobic plate count (APC) were determined on Plate Count Agar (Difco, Detroit, MI) after incubating at 30°C for 48 h, *Enterobacteriaceae* counts were determined on Violet Red Bile Dextrose agar (Difto, Detroit, MI) after 24 h incubation at 37°C.

Analysis of pH

Ten grams of sample were blended and homogenized with 40 ml destilized water, and the pH was measured by digital pH meter H-10B (TOA Electronic Ltd., Japan).

Sensory evaluation

Sensory evaluation was done using score test for sensories criteria of color, texture, aroma, and taste, and overall hedonic test was done to evaluate the prefference of the products.

RESULTS AND DISCUSSION

Microbiological Characteristics

Total of LAB and APC were not significantly changes during process of conditioning, but using of natural casing was significantly different in total of LAB and APC compared with using of synthetic casing (Fig. 1 and Fig 2). Low temperature (10°C) during conditioning inhibited the growth of the bacteria which this condition was needed to avoid increasing of product acidity, but flavor components were still produced during conditioning. The implication of inhibition of the bacterial growth was the acidity of the products was stabile during conditioning (Fig. 3). On the other hand, total of *Enterobacteriaceae* decreased during conditioning (Fig 4). Exposure of these bacteria in

low pH for days of conditioning caused the bacteria retarded to grow, even killed by the organic acid present in *urutan*. From the experiment we found that total of *Enterobacteriaceae* contained in *urutan* at final conditioning was 4.25 x 10^2 cfu/g and 1.05 x 10^3 cfu/g for *urutan* made of synthetic and natural casing, respectively.

The conditioning process is needed to enhance the flavor formation and to solidify in order producing compact and sliceable product. In this experiment, the water content of the product decreased during conditioning (Fig. 5) that affected its compactness (Fig. 6). The water content of the final product was in the range of 26.7% – 32.6%, which was in the criteria of dried sausage.

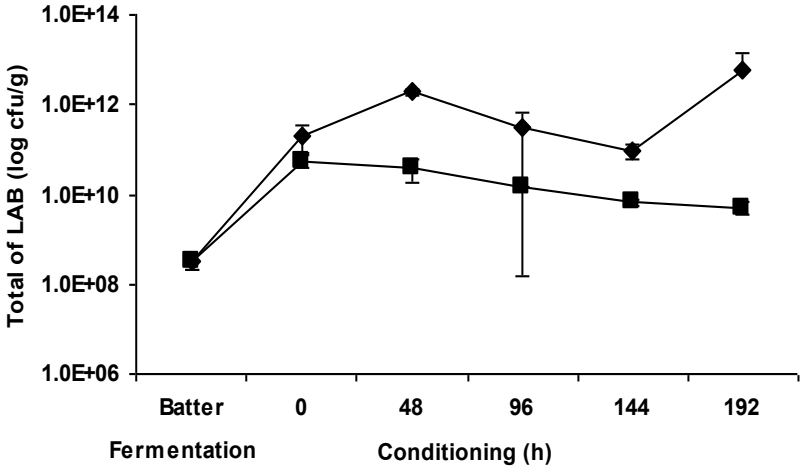

Fig. 1. Change of LAB in *urutan* during process of fermentation and conditioning. ♦: natural casing and ■:synthetic casing. Bar indicate standard deviation data.

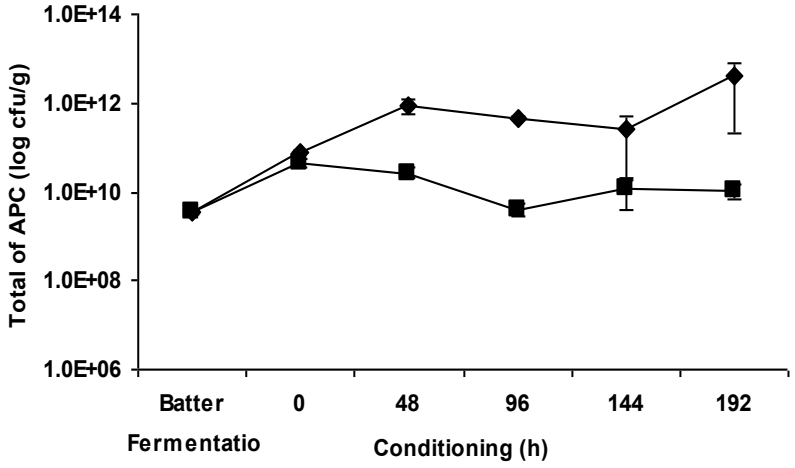

Fig. 2. Change of APC in *urutan* during process of fermentation and conditioning. ♦: natural casing and ■:synthetic casing. Bar indicate standard deviation data.

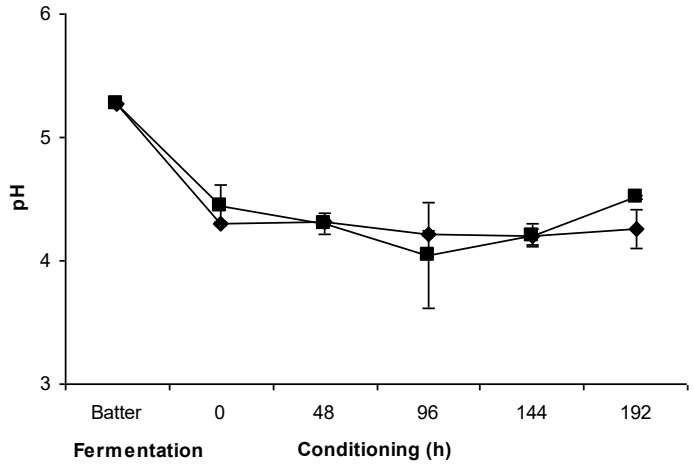

Fig. 3. Change of pH *urutan* during process of fermentation and conditioning. ♦: natural casing and ■:synthetic casing. Bar indicate standard deviation of data.

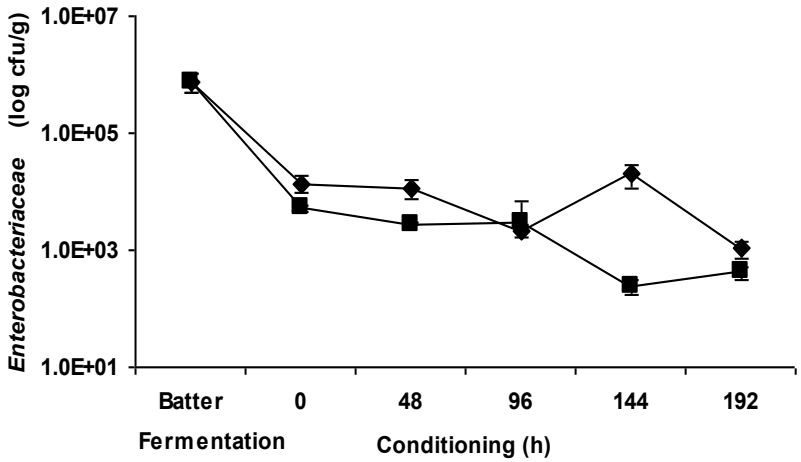

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Fig. 4. Change of *Enterobacteriaceae* in *urutan* during process of fermentation and conditioning. ♦: natural casing and ■:synthetic casing. Bar indicate standard deviation of data.

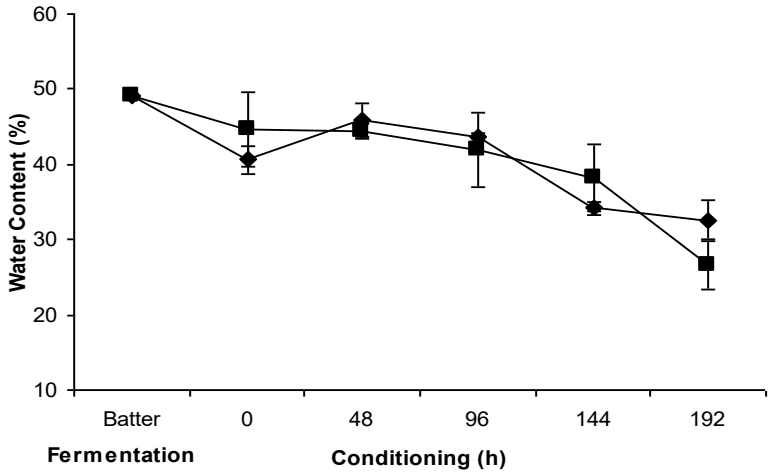

Fig. 5. Change of water content of *urutan* during process of fermentation and conditioning. ♦: natural casing and ■:synthetic casing. Bar indicate standard deviation of data.

Sensory Characteristics

Sensory characteristics of *urutan* was evaluated by organoleptic test with a standard methods. Texture, color, aroma, and taste were evaluated by using scoring test, which the panelists were selected their ability to differentiate the sensory characteristics of the samples by using of duo-trio test (Soekarto, 1985). The selected panelists were then ordered to evaluate the *urutan* samples.

From the evaluation that were done by 16 selected persons showed that the interaction between conditioning period and the casing used did not has any effects to the sensory characteristics of *urutan*. However, there were changes of *urutan* color, texture, and taste during conditioning period, but aroma was not significantly change (Fig. 6). From overall hedonic test, the panelists preferred *urutan* with the longer conditioning period, and using of synthetic (collagen) casing was more accepted than natural casing.

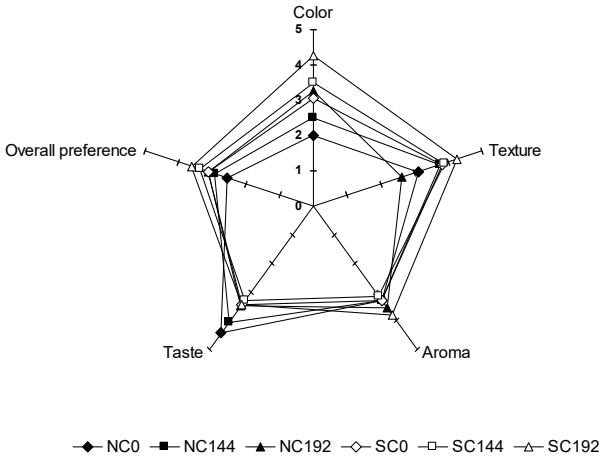

Fig. 5. Change of sensory characteristics of *urutan* during process of conditioning. NC: natural casing; SC: synthetic (collagen) casing.

CONCLUSION

Conditioning process was needed to improve the quality and safety of *urutan*. Lactic acid bacteria applied as starter culture were stabile during conditioning and the acidity as well. On the other hand, *Enterobacteriacea* decreased during process of conditioning. Process of conditioning also affected the panelist preference due to improvement of sensory characteristics of *urutan*, especially color, texture and taste of *urutan*. Using of casing also influence panelist preference to the product, which preferred *urutan* using collagen casing than natural casing.

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STUDY OF ANTIOXIDANT ACTIVITY OF GRAPE SKIN AND GRAPE SEED FROM THE SOLID WASTE OF A WINE INDUSTRY

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ABSTRACT

Grape is one of the sources of natural antioxidant. Grape skin contains several *phenolic* compounds, and grape seeds have much flavonoid compound, in which they can act as antioxidant (Kammerer, *et al*., 2005; Khomsan, 2006). Grape skin and grape seeds can be found in the solid waste of the production of grape juice in the wine industry. In this research, the grape used for the wine industry was the species of *Alphonso lavalle*. The grape skin and grape seed were extracted by using a various concentration of ethanol, namely 0%, 20%, 40%, 60%, and 80%.The antioxidant activity of a compound can be measured by its ability to bind the free radicals. The ability to bind the free radicals can be examined by DPPH method. In the DPPH method, a higher DPPH's value means a higher antioxidant activity. Research results showed that the levels of antioxidant activity of the grape skin extract were varying between the DPPH's value of 38.5% to 60.2%. However, the levels of antioxidant activity of the grape seed extract were varying between the DPPH's value of 69.9% to 87.4%. The highest antioxidant activity was found on the grape skin and grape seed which were extracted by using ethanol 80%. The DPPH's value of grape seed extract was significantly higher than the grape skin extract. The DPPH's value disparities between grape skin and grape seed extract were about 27.2% to 41.6%. In this research, it was also undertaken a comparison of the levels of antioxidant activity between the grape seed extract and one brand of commercial antioxidant capsule. The research results showed that the DPPH's value of the antioxidant capsule was 93.6%. The DPPH's value of grape seed extract was only 6.2% lower than the antioxidant capsule. It can be said that the antioxidant activity of the grape seed was almost similar to the commercial antioxidant capsule, therefore there is a possibility to produce commercial antioxidant product from the grape seeds from the solid waste of wine industry.

Keywords: grape skin, grape seed, antioxidant, and wine industry

INTRODUCTION

Free radical is a molecule that has one unpaired electron, and it becomes unstable therefore it always takes the electron from other molecules. Free radical is can be produced in our body from a metabolic process and from external sources such as ultraviolet light, gas emission from vehicle and factory, chemical substances contained in foods, and many other contaminants. The presence of free radicals in our body which are higher than normal can cause health problems, such as coronary heart disease (CHD), diabetes mellitus, cancer and early aging (Auroma, *et al*., 1997). So, that´s why we need a substance that can reduce the concentration of free radicals in our body. Antioxidant has the ability to reduce the concentration of free radicals in our body by neutralizing those free radicals, therefore it becomes harmless to our body (Hall, 2001).

Grape (*Vitis vinifera*) contains a high concentration of antioxidant. Grape fruit can be processed into many products such as wine and grape juice. During the production process, there are some kinds of waste, such as grape seed and grape skin (Sirait, 2004). About 20-25% of grape becomes production waste, and only 75-80% can be processed into wine and grape juice (Kresna, pers. comm., 2008). Grape fruit contains *ellagic acid* and *quercetin* that can act as an antioxidant (Scwharz and Berkoff, 2004). Grape skin contains many phenolic compounds, such as *cathecins, flavonol glycosides* and *antochyanins* that can act as an antioxidant (Kammerer *et al*., 2005). *Antochyanins* is very useful for health in reducing the risk of cancer, heart attack, and can decrease the level of cholesterol in human body.

Grape seed contains flavonoid, that is one third of the flavonoid content in the grape fruit (Khomsan, 2006). Grape seeds contain *poliphenol* on *flavonoid* form such as *gallic acid*, *monomeric flavon-3-ols catechin*, *epicatechin*, *gallocatechin*, *epigallocatechin*, *epicatechin 3-O-gallate*, *procyanidin dimers*, *trimers* and polimer *procyanidins.* Grape seeds extract contains many *Oligomeric Proanthocyanidins* (OPC's) that consider as a super antioxidant that can prevent human from certain diseases. Research showed that the antioxidant from grape seed is 20 times better than vitamin E, and 50 times greater than vitamin C (Shi, *et al*., 2003).

This research was aimed to give an information regarding the activity of antioxidant contained in grape skin and grape seeds in order to neutralize the free radicals. In order to achieve the aims of this research, it was undertaken the extraction of antioxidant in the grape skin and seed from the waste of wine industry by using several concentrations of ethanol. The results of the extraction then was analyzed to know the activity of the antioxidant content. It was also undertaken the analysis of an antioxidant capsule sold commercially in order to compare the antioxidant activity of the grape skin and grape seed to the antioxidant capsule.

METHODS

The grape skins and grape seeds were taken from the wastes of a wine industry (PT. Arpan Bali Utama, Sanur – Bali) as the samples of this research. The grape fruits that are used in this wine industry are the species of *Alphonso lavalle*. The samples then were analyzed at the Laboratory of Food Technology, Faculty of Agriculture Technology, Gadjah Mada University, Yogyakarta. The extraction process was undertaken by using ethanol with concentration of 0%, 20%, 40%, 60%, dan 80%. The examinantion of antioxidant activity was undertaken by using DPPH (α, α diphenil β-pikrilhidrazil) Method (Pyzybylski *et al*., 1998 *in* Mahardika, 2007). The similar examinantion of antioxidant activity was undertaken for the antioxidant capsules (one brand of antioxidant capsule that are produced and sold commercially in Australia).

RESULTS

A. Antioxidant Activity of the Extracted Grape Skin

Based on the laboratory analysis on the samples of grape skin which were extracted by using a various concentration of ethanol, the results of the antioxidant activity (DPPH's Value) are shown on the Table 1 below. The largest average of the DPPH's value was derived from ethanol concentration of 80%, i.e. the DPPH's value of 60.20%, and the lowest average of the DPPH value was derived from ethanol concentration of 40%, i.e. the DPPH's value of 38.50%.

Table 1. Antioxidant activity of the extracted grape skin.

Ethanol Concentration (%)	DPPH's Value (%)			
	Repetition 1	Repetition 2	Repetition 3	Average
0	38.57	38.92	38.05	38.51
20	39.62	39.27	39.80	39.56
40	38.60	38.90	38.00	38.50
60	45.38	45.50	45.72	45.53
80	60.20	60.00	60.40	60.20

B. Antioxidant Activity of the Extracted Grape Seeds

Based on the laboratory analysis on the samples of grape seeds which were extracted by using a various concentration of ethanol, the results of the antioxidant activity (DPPH's Value) are shown on the Table 2 below. The largest average of the DPPH's value was derived from ethanol concentration of 80%, i.e. the DPPH's value of 87.42%, and the lowest average of the DPPH value was derived from ethanol concentration of 0%, i.e. the DPPH's value of 69.87%.

Table 2. Antioxidant activity of the extracted grape seeds.

Ethanol Concentration (%)	DPPH's Value (%)			
	Repetition 1	Repetition 2	Repetition 3	Average
0	69.98	70.16	69.46	69.87
20	71.03	71.20	70.86	71.03
40	77.75	77.31	77.32	77.46
60	87.27	86.91	87.27	87.15
80	87.78	87.27	87.21	87.42

C. Comparison of Antioxidant Activity of the Extracted Grape Skin and Grape Seeds

Based upon of the both tables above, it can be said that the antioxidant activity from the grape seeds are bigger than those from the grape skins. The differences of the average DPPH's value between the grape seeds and the grape skins varied between 27.22 % up to 41.62 %. Graphically, the comparison of the average DPPH's value of grape seeds and the grape skins can be shown in the Graph 1.

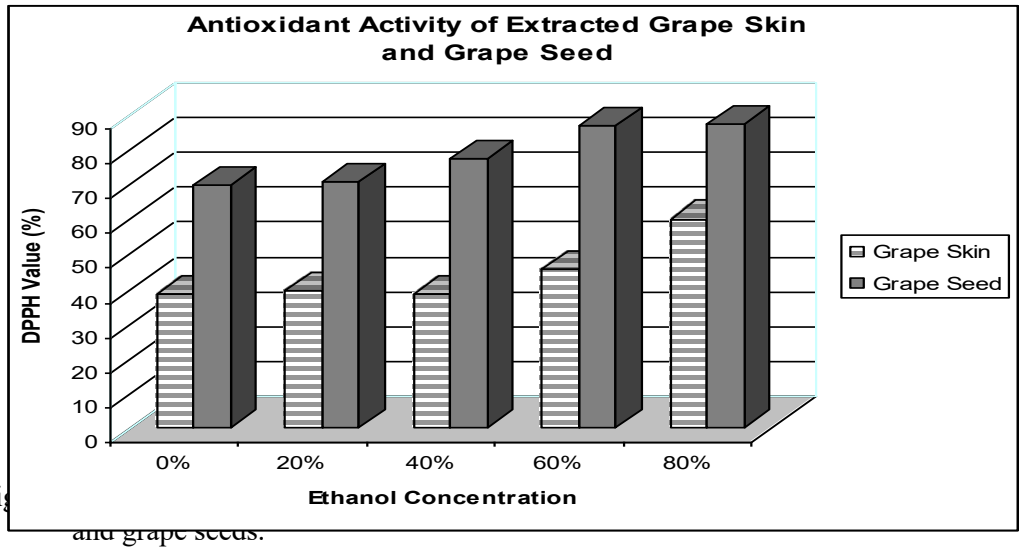

Fig
and grape seeds.

D. Comparison of Antioxidant Activity of the Extracted Grape Skin, Grape Seeds and the Commercial Antioxidant Capsules

According to the results of laboratory analysis by using DPPH method, the comparison of antioxidant activity of the grape skin, grape seeds, and the commercial antioxidant capsules are shown in Table 3. The average value of DPPH at antioxidant capsule is 93,58 %.

Table 3. Antioxidant activity of the extracted grape skin (80% ethanol), grape seeds (80% ethanol), and antioxidant capsule

Samples	DPPH's Value (%)			
	Repetition 1	Repetition 2	Repetition 3	Average
Extracts of grape skin (ethanol 80 %)	60,20	60,00	60,40	60,20
Extracts of grape seed (ethanol 80 %)	87,78	87,27	87,21	87,42
Commercial antioxidant capsule	94,26	93,30	93,18	93,58

The antioxidant activity of the extracted grape skin was much lower than the antioxidant activity of the commercial antioxidant capsules, in which the difference between these two antioxidant activities was 33,38% (measured in the DPPH's value). On the other hand, the antioxidant activity of the extracted grape seed was relatively similar to the antioxidant activity of the commercial antioxidant capsule, in which the difference of these two antioxidant activities was only 6.16% (measured in the DPPH's value). It means that the extracted grape seed has a similar antioxidant activity to the commercial antioxidant capsule.

CONCLUSIONS

1. The extracted grape skin has antioxidant activities varying from the DPPH's value of 38.50% up to 60.20%. On the other hand, the extracted grape seeds has antioxidant activities varying from the DPPH's value of 69.87% to 87.42%. The variation of the antioxidant activity was dependent on the concentration of ethanol used for extraction.
2. The antioxidant activity of the extracted grape seeds was not much different to the antioxidant activity of the commercial antioxidant capsules. The difference of the DPPH's value was only 6.16%. It means that the extracted grape seed has a similar antioxidant activity to the commercial antioxidant capsules. This shows that the extracted grape seeds can be used as an antioxidant supplement.

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FORMULATION AND EVALUATION OF COMPACT POWDER WITH ETHYL VITAMIN C IN ALLYL METHACRYLATE CROSSPOLYMER (AMP) AS A DRUG DELIVERY SYSTEM

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ABSTRACT

A research on the formulation of a compact powder with ethyl ether preparation Ascorbyl with Various mixtures of ethyl ether in allyl methacrylate Ascorbyl crosspolymer (0.5% and 1%) and compact powder with Ascorbyl ethyl ether (0.5% and 1%) has been carried out. Based on the evaluation data, it was found stable That the preparations were the resource persons in consistency, color, smell, and homogenity after 28 days. Scanning Electron Microscope based on (SEM), it shown the formula with ethyl ether in allyl methacrylate Ascorbyl crosspolymer 1% Had particle size 5-10μm. Based on smoothness, quality spreads, and adhesiveness in 20 females in allyl ethyl ether methacrylate Ascorbyl crosspolymer 1% Had a highest score. Based on the physical evaluation of compact powder preparation, it was found That the preparation Had good stability. The safety investigation of compact powder with ethyl ether in allyl methacrylate Ascorbyl crosspolymer 1% give thenon-skin irritation on consumers.

INTRODUCTION

Preparation of cosmetics that aims to make skin tones look more homogeneous, and cover the stains on the skin. Powder for facial skin usually consist of solid powder and powder. Excess solid powder that is easier to use and carry.

The purpose of this study was to present the composition as a cosmetic compact cover between the phase composition of powder and binder phase and can provide favorable characteristics such as regulation of sebum and improve the smoothness of texture without causing a heavy feeling on the skin surface. Examples of adsorbent used is kaolin, silica spherical shape , acrylates co-polymer such as allyl methacrylate Polytrap and Crosspolymer like Polypore E200. Poly-Pore E200 is a trade name of AMP.

The use of vitamin C in the field of cosmetic, dermatological, and pharmaceutical are limited, although very useful. Due to the chemical instability of vitamin C. Another way to overcome its limitations is with derivatisasi vitamin C from a salt such as magnesium Ascorbyl Palmitate or ascorbylphosphate. Ethyl vitamin C or is one of the implications of vitamin C, which can be dissolved in water or oil. They are also more easily penetrate the tissue to reach the dermis. Then the enzyme in the network will process ethyl vitamin C and release of vitamin C.

MATERIALS AND METHODS

The materials used in the formulation of solid dosage allyl methacrylate powder Crosspolymer (AMP), Aquadestilata, Ethyl Vitamin C, Kaolin, magnesium stearate, Methyl Paraben, Mica uncoated, Paraffin Liquidum, Perfume Khatia, Red Iron Oxide, Talc, TitaniumDioxide, and Yellow Iron Oxide. Entrapment and Characterization of Vitamin C in ally methacrylate crosspolymer.

That is the solid powder samples, performed the sifting and then pasted on the specimen holder. In the specimen holder attached double-sided tape, then powder sprinkled on double-sided tape. After it is cleaned with a hand blower to remove dust impurities. Then the sample was given a thin layer (coating) by gold-palladium (Au: 80% and Pd: 20%) with ion engines Sputter JFC - 1100. After the coating process, then the

sample is inserted into the specimen chamber of SEM machine to do the shooting. Smoothness and Testing Coverage, and Power Sticking Powder Solid

To evaluate smoothness, dispersive power, and the adhesiveness powder on the skin sensory testing. Selected 20 women at the age of 19-23 years to use the formula of each of the compact, including the control formula. Evaluation scale figures include five scale (5: excellent, 4: good, 3: on average, 2: sufficient, 1: not good), and the results obtained with an average of 20 women, and then the results from these scales were aggregated averaged . Security Testing of Solid Powder with ethyl Vitamin C in allyl methacrylate Crosspolymer . Compact irritation test was conducted to observe the reaction of the skin after use of the solid powder of vitamin C with allyl ethyl methacrylate crosspolymer the most stable during the test containing the stability of vitamin C in the allyl ethyl methacrylate crosspolymer. As a comparison made also to the solid powder which does not contain ethyl vitamin C.

RESULTS AND DISCUSSION

The result of entrapment and Characterization of Vitamin C with allyl ethyl methacrylate Crosspolymer with Various Comparisons . The result of adsorption and characterization of vitamin C with allyl ethyl methacrylate crosspolymer with a ratio 6: 1, 8: 1, 10: 1

Physical observations indicate that the trapping with allyl methacrylate crosspolymer comparison, the ratio of allyl methacrylate and ethyl crosspolymer vitamin C 1: 6 in the form of powder, but still moist. Therefore, the necessary process of drying powder. After that the dry powder is obtained, which is ready to be formulated in solid powder. When you enter is the ratio of the mixture with 1: 8 or 1: 10, both physically more wet formula, so that will happen because the attachment of dust clumping in the mixture with talc powder, when printed on the godet, will get the results of the uneven surface of the powder.In the dry powder in the ratio of vitamin C in the allyl ethyl methacrylate 6:1 crosspolymer evaluated microscopically using the SEM (Scanning Electron Microscope), respectively for the ethyl vitamin C, allyl methacrylate and the entrapment of crosspolymer allyl ethyl methacrylate crosspolymer of vitamin C. Results from SEM photographs of ethyl vitamin C can be seen in Figure 1.

Ethyl vitamin C crystals shaped like needles and larger than 200μm, and physically is a white powder. Thus ethyl vitamin C must be dissolved first in distilled water before snared by allyl methacrylate crosspolymer. Because if in powder form, will not be possible to ensnared by allyl methacrylate as allyl methacrylate crosspolymer crosspolymer physically well shaped white powder. But according to the literature, with SEM, allyl methacrylate crosspolymer round and there are hollow.

From the results of SEM images for these crosspolymer allyl methacrylate, allyl methacrylate proved that crosspolymer used particle shape and size according to the literature, that is round or spherical and hollow, and measuring about 10-20 μm. Hydrophilic substance will be snared by crosspolymer allyl methacrylate and allyl methacrylate crosspolymer sticking out. Examples such as entrapment AMP with a solution of ethyl vitamin C.

Results Formulation of Solid Dosage powder with entrapment Ethyl Various Concentrations of Vitamin C with allyl methacrylate Crosspolymer Results formulation Solid Powder with Vitamin C Ethyl mixture with allyl methacrylate crosspolymer and various concentrations. From the observation data in table 3, found that the solid powder in the formula A and B are colored brown, while the FC and FD formula younger brown. Brown color is due to the addition of a special dye to solid powder, which is a mixture of red iron oxide and yellow iron oxide. While the formula C and D added ethyl vitamin C, which was bound by the allyl methacrylate crosspolymer. However, all formulas have the form of a solid. The smell of powder solid in all formulas

khatia smelling perfume. The smell is weak because the perfume khatia who added just 0.1%.

Physical Examination Results / organoleptic . can be seen that the four solid powder formula has a pretty good organoleptic stability. This is proven by the absence of changes in consistency, color, odor, and homogeneity during the 28-day storage period. That is because the dosage form of a dense and compact powder. Hardness Testing Results Solid Powder Compact Powder with Vitamin C in allyl ethyl methacrylate Crossspolymer (AMP.It was revealed that the solid powder mixtures with various concentrations of vitamin C with allyl ethyl methacrylate crosspolymer have different hardness significantly. However, there is an average difference between real violence powder Formula another. In the control powder FCD, it is known that the null hypothesis (H0) was rejected, with the assumption that there are differences in Ho rejected. With a level of 95% ($\alpha = 0.05$) revealed that the solid powder mixtures with various concentrations of ethyl vitamin C with allyl l methacrylate crosspolymer have different hardness significantly. Fragility Testing Results Solid Powder (Droptest) . From the data table showed that the FD with a scale of 1 is not destroyed. This was caused by the presence of AMP with ethyl vitamin C in the levels of 1%. AMP in addition to the delivery systems for active substances, but in a solid powder, also serves as forming a dense mass of powder and compact, so the powder is not fragile and easily broken. The observation above formula D by SEM on the FD, the particle size between 5-10μm, with a rounded shape. It is obtained from the bondage allyl ethyl methacrylate crosspolymer with vitamin C 1%. So to penetration of the active substance into the skin can be used FD. Because of the spherical particle shape, and size is much smaller than the other three formulas. A spherical particle shape, which is due to a combination of base powder with a mixture of vitamins C and allyl ethyl methacrylate crosspolymer. Smoothness Test Results, Coverage, and Power Sticking Powder Solid FD crosspolymer more, to absorb sebum and sweat with a high capacity class, so as to increase the adhesiveness powder, and last longer on the skin.

Safety Testing of Solid Powder with ethyl Vitamin C in allyl methacrylate Crosspolymer It is known that the preparation of solid powder with allyl methacrylate and ethyl crosspolymer vitamin C 1% does not irritate the skin, characterized by the absence of reaction heat, redness and irritation, or itching of the skin of volunteers. The result of entrapment of vitamin C with allyl ethyl methacrylate crosspolymer in various comparisons (6:1, 8:1, 10:1) showed that the most optimal entrapment occurs in a mixture with a ratio of 6:1. Solid powder with vitamin C in the allyl ethyl methacrylate 1.0% crosspolymer have consistency, color, odor, homogeneity, which is good and has a size of 5μm, so the formula has the softness and the best dispersive power than others.

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PROBIOTIC PROPERTIES AND GENETIC IDENTIFICATION OF *LACTOBACILLUS* SP. SKG34

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ABSTRACT

Lactobacillus sp SKG34 isolated from sumbawa mare milk had good probiotic properties as shown by its capabilities to survive on modified gastro intestinal tract conditions. Further studies revealed that the *Lactobacillus* sp SKG34 deconjugated glycodeoxycholic acid and hence this bacterium is possible to be applied as cholesterol lowering probiotic. *Lactobacillus* sp SKG34 shared 98% similarity with *Lactobacillus rhamnosus* ATCC 53103 based on the 16S rDNA sequence analysis.

Keywords: Lactobacillus, probiotic, genetic identification

INTRODUCTION

Lactic acid bacteria are among the most common bacteria associated in milk and food stuff, human and animal intestines. These bacteria have been widely used in the production of fermented foods. The utilization of this bacterium especially in fermented milk in Europe and in production of fermented foods in the orient provide a new in sight of this bacterium not only in production of a safe and palatable food product, but also in more functionality especially in maintaining the human health (Steinkarus, 1990). A long history in the consumption of food products containing living lactic acid bacteria such yoghurt in Europe and plant based fermented product such as kimchi in Korea, sauerkraut in Japan, tape in Indonesia, bring deep understanding that this group of bacteria has been considered as *generally recognized safe* or being considered equally like a food component.

The Metchnikoff finding about the importance of the intestinal microbiota in maintaining the human health (Tannok, 1999), stimulates studies on application of LAB for human health rather than for production for fermented foods. Following this, Mitsuka (a pioneer in application of direct consumption of fermented milk containing high number of viable *Lactobacillus casei* strain Shirota in Japan) has proved that consumption of living cell of LAB can maintain human health. Recently this term is well known as probiotic, and this has been defined as a consumption living cell in adequate amount to stimulate the health of their hosts (WHO, 2001).

Perception of Indonesian consumers on maintaining heath through diet management gradually increased in the recent years. Some probiotic products and fermented foods containing probiotic can easily be found in the supermarkets or event has been prescribed by medical doctors. This circumstance elevates the research on the exploration of the Indonesian biodiversities in developing a so called indigenous probiotics or a strain of probiotics which are originated from the Indonesian biodiversities such fermented food products and human intestine. We recently isolated *Lactobacillus* sp. SKG34 from Sumbawa mare milk, famous fermented milk with several health claims (Rijatmiko, 2003: Harmawatio *et al*., 2004), although this is still a weak claim since inappropriately supported by scientific evident. The strain has been characterized for its ability to survive in the gastrointestinal tract as shown by its capability to survive in the

solution containing 0,2-0,4 mM deoxycholic acid and its ability to inhibit some pathogenic bacteria (Sujaya *et al.* 2008). The activities of the Lactobacillus sp, SKG34 to metabolize some sugars has been determined and this lead to the conclusion that this strain was found to be quite distinct when compared to reference strain of *Lactobacillus rhamnosus*. Thus, the objectives of this study were to determine the probiotic characteristics and to molecularly identify of the *Lactobacillus* sp SKG34 by sequencing its 16S rDNA.

MATERIALS AND METHODS

Strain and cultivation methods

Lactobacillus sp SKG34 was obtained from the Udayana University Culture Collection (UNUDCC), Integrated Laboratory for Bioscience and Biotechnology, Udayana University. The strain was stored in glycerol stock at -20^{o}C. The Lactobacillus was refreshed and cultured in MRS broth (Pronadisa) as instructed by the manufacturer, incubated for 24h at 37^{o}C in anaerobic condition using Oxoid gas generating kit.

Bile salt Hydrolase activity

Activity of bile salt hydolase was performed on MRS broth containing 0,3% glycodeoxycholic acid (GDCA) and and L-cystein (Begly *et al*., 2006; Tanaka *et al*., 1999). The SKG34 was refreshed for 24h at 37^{o}C in anaerobic condition. One loop full of active culture was streaked onto MRS agar containing GDCA, and then incubated aerobically at 37^{o}C for 4 days. The presence of hallow around colonies indicates the presence of BSH. At the same time, negative control was performed by streaking the same strain onto MRS agar without GDCA amendment.

Genetic identification of Lactobacillus sp SKG34.

Genomic DNA was isolated from 1 ml culture broth following incubation at 37^{o}C for 24 h. The cells were harvested by centrifugation at 5000 rpm for 5 minute at 5^{o}C and washed twice using saline-TEE buffer pH 8. The product of cell mass was then added with 100 ul lyzozym solution (10 mg/ml) and incubated at 37^{o}C for 60 min. The lyzed cell was added with 150 ul SDS 10% following incubation at 37^{o}C for 60 min, added with 225 ul phenol saturated solution and 225 ul cloroform-isoamyl alcohol (24:1), briefly vortexed, and then centrifuged at 15,000 rpm for 30 min at 5^{o}C. Subsequently, the upper portion was carefully taken, transfered into a new sterile eppendorf tube, added with 2,5X ice cold etanol 99,9%, kept ice for 15 minutes, and centrifuged for 30 minutes at 15,000 rpm, 5^{o}C. The DNA pooled in the botoom of the eppendorft tubes was then washed twice with etanol 70%, dried, and diluted using 50 ul TE Buffer before being electrophorized.

Amplification and sequencing of the 16S rDNA

The DNA was amplified using bacterial 16S rDNA as describe in Mori *et al*. 1997 and Sujaya *et al*., 2001. Briefly the PCR was performed in 50ul PCR reaction containing 1 ul DNA, 50 pmol of forward and reverse primers, 1X PCR buffer II, 75 mM $MgCl_2$, 0,5 U amplitaq. The volume was adjusted using deionized water up to 50ul. PCR was performed at 94^{o}C for 5 min, following 30 cycles at 94^{o}C for 20 sec, annealing at 55^{o}C for 20 sec and 72oC for 30 sec. The final step was added with 72^{o}C for 5 min. About 5 ul of amplicon was electrophoresed in 1,5% agarose, stained with 50ng EtBr and the presence of band was visualized using UV illuminator.
The PCR product was then subjected to DNA sequencing, which was performed at the DNA Sequnces Service, Singapore (1st BASE Pte Ltd**.).**

RESULTS AND DISCUSSION

The recent trend in the development of novel probiotics is based upon its functionality to the host. Each probiotic strain has it own characteristics and functional effect. *Lactobacillus acidophilus* LC1 has been reported, based on clinical studies, to be effective for balancing intestinal microbiota (Bernet, *et al*. 1984); *Lactobacillus rhamnosus* GG is good for prevention of antibiotic associated diarrhea, treatment as well as prevention from rotavirus infection causing Chron' disease (Salminan *et al*., 1993): *Lactobacillus casei* strain Shirota has been proven to be effective for balancing intestinal microbiota, lowering fecal enzyme, inhibiting the superficial bladder cancer (Aso and Akazan, 1992); *Lactobacillus gasseri* was effective for fecal enzyme reduction (Pedrosa *et al*. 1995).

Our result showed that *Lactobacillus* sp SKG34 deconjugated bile salt as shown in Fig 1. The presence of the BSH activity in probiotic strains is preferred since this activity is linked to the bile metabolisms in the human body and significantly lowering the blood cholesterol content. Bile is excreted from the liver in the form of glycol or tauro cholic and then deconjugated by bile salt hydrolase to form cholic, taurine, and glycine. Cholic acid (primary bile acid) has high pKa 6.4 while deoxycholic acid has pKa of 6.53 (Kurdi *et al*., 2003). Thus, when probiotic grow in the gastrointestinal tract (GIT) and lowering the pH of GIT, the CA and DCA will precipitate and excreted together with the feces. More cholic (bile) precipitated concomitants with increase cholesterol utilized by the body to produce fresh bile.

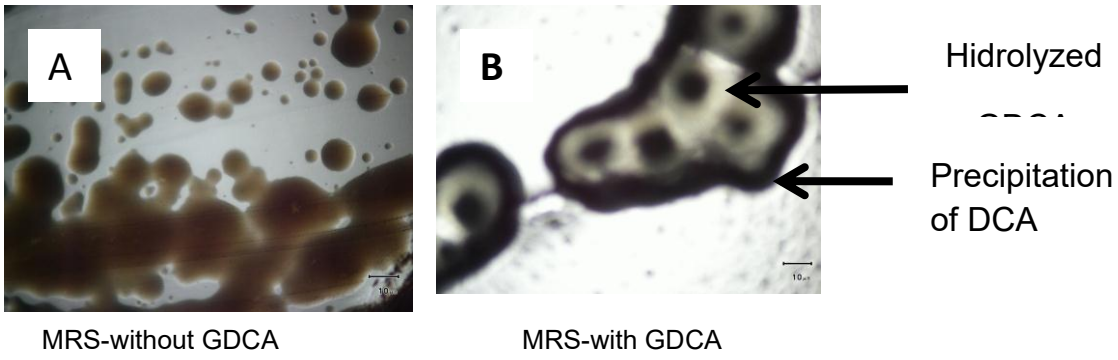

Figure 1. Colonies of *Lactobacillus* sp SKG34 grown on MRS without (A) and with GDCA (B). Hallow and clear zone around the colonies indicates the activity of BSH.

Table 1. Homology results of 16S rDNA sequence of Lactobacillus sp SKG34

Sequences producing significant alignments:

Accession	Description	Max ident
AP011548.1	Lactobacillus rhamnosus ATCC 53103 DNA, complete genome	98%
FM179323.1	Lactobacillus rhamnosus Lc 705 whole genome sequence, strain Lc 705	98%
FM179322.1	Lactobacillus rhamnosus GG whole genome sequence, strain GG (ATCC 53103)	98%
EF533991.1	Lactobacillus rhamnosus strain IDCC 3201 16S ribosomal RNA gene, complete sequence	98%
HM125050.1	Lactobacillus rhamnosus strain LP1 16S ribosomal RNA gene, partial seq.	98%
AB008211.1	Lactobacillus rhamnosus gene for 16S rRNA, partial sequence, strain: YIT 0105 (= ATCC 7469)	98%

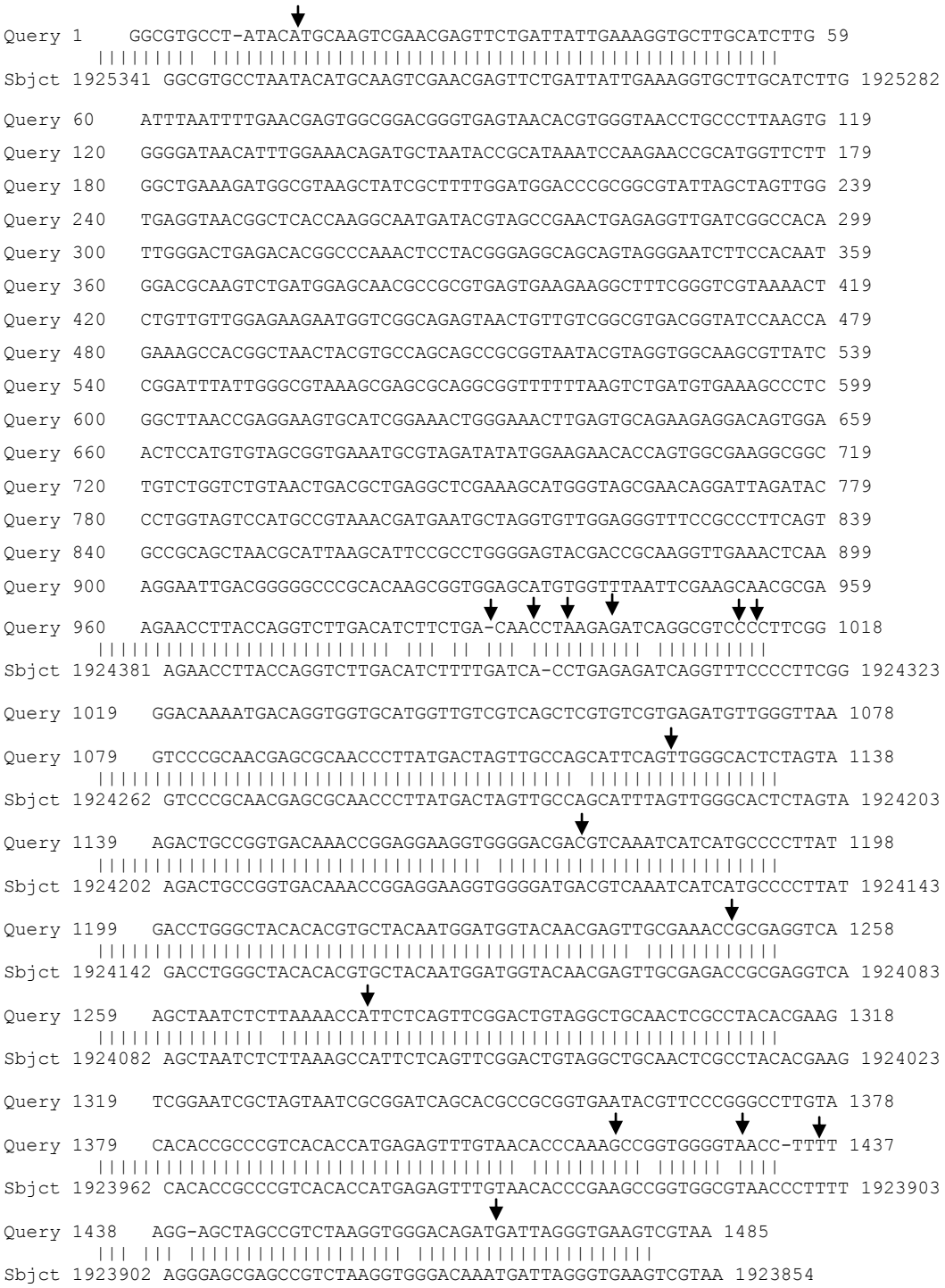

Figure 2. Sequences of 16S rDNA of *Lactobacillus* sp SKG34 compares with *Lactobacillus rhamnosus* ATCC 53103 in GenBank (using blast system). Only sequences which have different nucleotide with reference data are shown in double strand. Nucleotide differences are indicating by arrow head. Query: SKG34; Subject : *L rhamnousus* ATCC 53103.

Lactobacillus sp SKG 34 was closely related to *Lactobacillus rhamnosus* ATCC53103 with 98% DNA homology (Table 1). The differences in nucleotides are shown in Figure 2. Although the high value of homology shared with *L rhamnousus*, the *Lactobacillus* sp SKG34 is though to be a different strain compared to ATCC53103. The SKG34 is very

important strain as shown by its probiotic properties and might be different from ATCC53103, and it has a possibility to be developed as a novel probiotic from the Indonesian biodiversity.

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EFFECT OF MATURITY STAGE OF *Carica papaya*-THAILAND VARIETY ON LIPIDS SERUM PROFILE OF SPRAGUE DAWLEY RATS

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ABSTRACT

The effects of *Carica papaya* maturity stage on lipids serum profile of Sprague Dawley Rats were investigated. Forty rats randomly assigned to one of four treatments: a) Standard Diet, b) Green Ripe Stage of Papaya Diet, c) Ripe Stage of Papaya Diet, or d) Mannitol Diet. Each diet of the four treatments was consists of 5% cellulose; 5% dietary fiber; 5% dietary fiber + 6% mannitol; and 5% cellulose + 6% mannitol, respectively. The diet fed to male Sprague Dawley Rats (255-330 g) *ad libitum* for two weeks. The result showed the decreasing of total cholesterol, HDL and triglyceride after feed intervention during two weeks period. The highest decreasing of mentioned lipids profile affected by mannitol diet, and followed by ripe stage papaya diet, green stage papaya diet and the lowest decreasing was showed by standard diet. It was related to the composition change of soluble and insoluble dietary fiber during ripening of *Carica papaya*.

Keywords: *Carica papaya*-Thailand variety, maturity stage, mannitol, dietary fiber, lipids profile

INTRODUCTION

It is well established that consumption of dietary fiber can prevent many degenerative diseases, such as heart disease, atherosclerosis, diabetic, obesity, or colon cancer. Heart and blood artery diseases are the first caused of death in Indonesia (DepKes RI, 1992).

Heart and blood artery diseases are related to metabolism of lipid components. The effect of dietary fiber consumption on preventing of diseases that mentioned can be detected from the lipid profile of blood serum. Chylomicron is a lipoprotein that has a low density because 98% is composed of lipids. The function of chylomicron is to transport dietary fats, especially in the form of triglycerides and ester cholesterol, into the body. Fatty acids are changed to be triglyceride by almost of all body tissue. Triglycerides synthesis by heart was used to produce blood lipoprotein. The cholesterol also needs as structural components of plasma lipoprotein. Deddy *et al*. (1993) said that in the human body there are 65% (1.5 mg/dl) of plasma cholesterol existed in form of low density lipoprotein (LDL). LDL is the main of blood lipoprotein which is has the function of cholesterol transfer from heart to the tissue, whereas high density lipoprotein (HDL) works as a catalyst to facilitate chylomicron catabolism.

The role of dietary fiber in lipid metabolism maybe related to its ability to binding the organic compound, such as sterol (Spiller and Freeman, 1983). There are any evidences that showed the specific role of dietary fiber in decreasing of cholesterol content in blood. The differences of maturity stage of papaya-Thailand can be the difference sources of dietary fiber. Total fiber content and proportion of soluble fiber and insoluble fiber derived from previous research were 16.27% (soluble fiber : insoluble fiber = 2.3: 13.98) in green ripe stage, and changed to 8.13% (soluble fiber : insoluble fiber = 3.62 : 4.51) in ripe stage. This study is an initial study that aims to examine the effect of papaya maturity stage on serum lipid profile of Sprague Dawley rats. This research was conducted in conjunction with assessment of laxative effects of components (fiber and mannitol) in the *Carica papaya*-Thailand variety with a different maturity stage.

MATERIALS AND METHODS

Animals and diets. Forty male Sprague Dawley rats weighing 255-330 g were randomly assigned to one of four treatments (10 rats/treatment). The animals were allowed free access to water and feed *ad libitum* for a total of 21days. During the period of adaptation (7 d), the rats were individually housed at room temperature and fed with AIN-93 (American Institute of Nutrition 1993) standard diet. Following the adaptation phase, rats were feed intervention for two weeks (14 d). Treatment included Standard Diet (containing 5% cellulose), Green Mature Stage of Papaya Powder Diet (containing 5% dietary fiber), Ripe Stage of Papaya Powder Diet (containing 5% dietary fiber + 6% mannitol), or Mannitol Diet (containing 5% cellulose + 6% mannitol), as shown in Table 1. Daily feed consumption were recorded and body weight were recorded every 3 days during the experiment.

Tabel 1. Daily Feed Consumption.

Compound	Diet			
	Standard AIN'93 (g)	Green Ripe Papaya (g)	Ripe Papaya (g)	Mannitol (g)
Corn starch	618.3	614.5	617.3	558.3
Casein	142.3	133.9	136.9	142.3
Sucrose	100.0	-	70.4	100.0
Soybean oil	40.0	39.3	39.6	40.0
Cellulose	50.0	25.4	40.5	50.0
Mineral mixture*)	35.0	27.8	30.3	35.0
Vitamin mixture*)	10.0	10.0	10.0	10.0
L. cystine	1.8	1.8	1.8	1.8
Choline bitartrat	2.5	2.5	2.5	2.5
Papaya powder	-	151.5	117.4	-
Mannitol	-	-	-	60.0
Total	1000.0	1006.7	1066.7	1000.0
Total Dietary Fiber	50.0	50.0	50.0	50.0
Total Mannitol	-		60.0	60.0

*) Reeves *et al*., 1993

Sampling procedures. Blood samples were obtained from the rats after the adaptation phase and after the treatment. Before blood sampling, the rats fasted for overnight (12 hours). Retoorbital plexus used as the method of blood sampling. Before analysis of serum lipid profile, the serum separated from blood plasma by centrifugation at 4,500 x g for 15 min.

Analytical procedures. Total cholesterol was measured by CHOD-PAP enzymatic method (Richmond, 1973). Cholesterol LDL and HDL were determined by CHOP-PAP enzymatic method (Wielan and Seidal, 1983) and CHOD-PAP enzymatic method (Eckel *et al.*, 1977), respectively. Finally, the triglyceride was determined by GPO-PAP enzymatic method (Mc Gowan *et al*., 1983).

Statistical analysis. Values are given as the means and, where appropriate, significance of differences between mean values were determined by ANOVA and multiple range comparison by Fisher's least significant difference procedures. Values of $p < 0,05$ were considered significant.

RESULTS AND DISCUSSION

Provision of treatment diets tend to lower triglyceride concentrations (Figure 1). Decrease in triglycerides are 6.09%, 17.91%, 34.77% and 37.85% respectively due to a standard diet, green ripe papaya diet, ripe papaya diet and mannitol diet. The decrease of

triglyceride levels in group of ripe papaya diet greater than green ripe papaya diet. This is because the proportion of soluble fiber is higher at ripe papaya diet. Some researchers argue that soluble dietary fiber can reduce triglycerides by inhibiting the absorption of triglycerides (Haskell *et al*.,1992 in Sembor, 1998; and Chandalia, 2000). Mayes (1997) claimed that it resulted in the formation of chylomicron and chylomicron remnan more slowly, thus giving a chance on lipoprotein lipase to hydrolyze chylomicron to triglycerides more before reach the heart and deliver triglycerides to the network. Therefore triglycerides in the blood decreases.

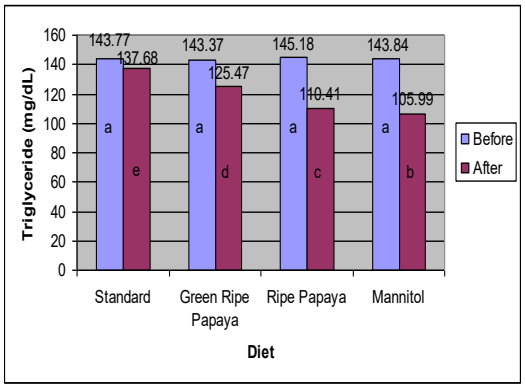

Figure 1. Effect of Diet on Triglyceride Serum Serum

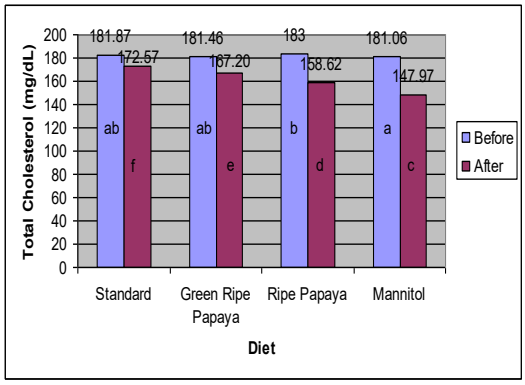

Figure 2. Effect of Diet on Total Cholesterol

All rats that received dietary treatment decreased total cholesterol (Figure 2). The decrease amounted to 9.3% for the standard diet group; 14.26% for the group of green ripe papaya diet; 24.38% for the group of ripe papaya diet and 33.09% for the group of mannitol diet. These data indicate that soluble fiber with a larger proportion of the ripe papaya diet potentially greater role in lowering cholesterol than a diet of green ripe papaya. Soluble fiber that is viscous may decrease the rate of glucose and sterol absorption in the intestine (Kahlon *et al*., 2000). Soluble fiber has the ability to bind and hold water because the polysaccharides have a residual sugar with polar groups. Hydration ability of soluble fibers can cause the formation of the gel matrix that increase the viscosity of intestinal contents and reduce the speed of nutrient absorption (Schneeman, 1986). In addition, soluble fiber is more capable of binding bile acids and carried out with feces. Decreased bile acid will cause the liver to synthesize bile from cholesterol as base ingredients. Therefore, the amount of cholesterol in plasma and in tissues will be reduced.

It was proved by Anderson *et al*. (1990) in the Mc Intosh M (2001), namely that the administration of psyllium (rich of soluble fiber) as much as 5.1 g in patients with hypercholesterolemic male patients during 8 weeks resulted in a decrease by 8.9% cholesterol compared to placebo. Chandalia *et al*.(2000) also mentioned that the decrease in the rate of absorption of insulin will suppress excretion thereby reducing the stimulation of HMG Co A reductase by insulin which causes a decrease of serum cholesterol.

Rats that received a standard diet treatment decreased triglyceride and total cholesterol levels in the lowest since the fiber components in the form of insoluble fiber. Conversely, groups of rats with mannitol diet decreased triglyceride and total cholesterol levels are highest. This can be caused by low feed intake (Table 2) and calories, so that has stimulated the use of fat reserves to be broken as a source of energy. Mannitol is a sugar alcohol that can not be used as a source of calories that cause the biggest loss of rats weight (Figure 3).

Decrease in feed intake and body weight during the study were also result in increased LDL (cholesterol carrier to the network) and a decrease in HDL (catalyst of chylomicron catabolism). The increase in LDL due to the range of 7.7% - 14.27%, while the decrease in HDL ranged 26.13% - 36.7%. This leads to the use of fat reserves for the needs of all body tissues. In other words metabolism leads to fat catabolism process because the calories lack of the body.

Table 2. Feed Intake (g/d/rat)*)

Diet	Observation Time		
	First Week**)	Second Week	Third Week
Standard	17.6 a	11.9 ab	5.1 a
Green Ripe Papaya	17.7 a	12.8 b	8.0 a
Ripe Papaya	17.5 a	12.8 b	7.1 b
Mannitol	18.0 a	10.9 a	6.4 ab

*) The same notation in the same column indicate no significant difference

**) All groups of rats fed with standard (adaptation)

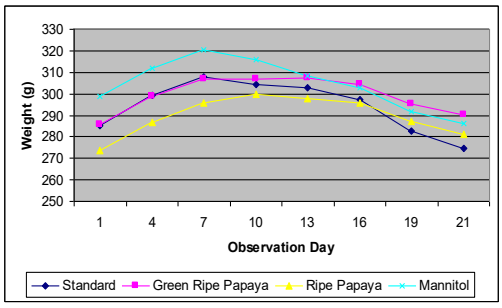

Figure 3. The Change of Rats Weight During The Study

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PRODUCTION AND PURIFICATION OF LIPASE FROM *Aspergillus niger* AND IT'S POSSIBILITY FOR α-LINOLENIC ACID PRODUCTION

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ABSTRACT

Lipase from *Aspergillus niger* which produced under solid state fermentation using soybean pulp as a medium for carbon and nitrogen source has been purified and characterized. Through 3 steps purification, this enzyme has molecular weight 51 KDa with 11.6% yield and 1,303 folds purification. The enzyme had stability at pH 3 to 6 and at temperature range 25°C to 60°C while optimum for its activity at pH 5 and at temperature 50°C, respectively. For the comparison purpose, commercial lipases from *Mucor miehei*, *Rhizomucor miehei*, porcine pancreas, D-Amano and AY30 (Amano) were used. To measure activities of lipases linseed oil (LO) substrate was used. To quantify of free fatty acid released (FFA) as α-linolenic acid (ALA), the enzymatically method using non esterified fatty acids methods was employed. Hydrolization LO for 18 hours by using purified lipase produced 0.65g/L FFA. No significant difference when lipase from *R. miehei* was used where resulting in 0.63g/L FFA after assaying at the same incubation time. Hydrolysis at optimum pH and temperature showed that the purified lipase from *A. niger* more effective hydrolyzed LO and release 89.8 g/L FFA as ALA while lipase from *R. miehei* only gave the result 85.8 g/L. Suggested, this purified lipase can be used as a cheap source of lipase for ALA production from LO.

Keywords: lipase, *Aspergillus niger*, linseed oil

INTRODUCTION

The omega 3 (ω-3) fatty acids, composed from eighteen carbon polyunsaturated fatty acid with three double bonds ALA is one of essential lipid to human health but it cannot be manufactured by human body [1] therefore must be present in the diet. Evidence suggests that ALA intake can decrease the risk of cardiovascular diseases [2]. ALA, as well as the fatty acids eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), belongs to a group of fatty acids called omega-3 fatty acids. EPA and DHA are found primarily in fish [3, 4, 5, 6, 7] and some fungi *Phytium, Phytophtora* and the Zygomycetes genus *Mortierella* [8]. It was also reported that ALA is highly concentrated in certain plant oils such as flaxseed oil and to a lesser extent, canola, soy, perilla, and walnut oils [9]. However, this oil cannot absorb efficiently by human body so that in this work production of ALA as *free fatty acids* from LO through enzymatically hydrolysis was investigated.

MATERIALS AND METHODS

Lipases collection; Crude lipase from *A. niger* was obtained by solid state fermentation according to previous papers [10, 11] was used as a lipase source. For the comparison, commercial lipases of *R. miehei* (L4277-SIGMA), *porcine pancreas* (L3126-SIGMA), *porcine pancreas* (20552-02 Nacalai), *M. miehei* (L9031-SIGMA), Lipase AY30 (Amano) and Lipase D-Amano (Amano) were obtained from chemical supplier.
Lipase sterilization and storing; Membrane filter 0.45 micron Low Protein Biding Durapore (PVDF) SLHV 013-Millipore was used for lipase sterilization, and the sterilized lipase solution stored at 4°C.
Purification and molecular weight analysis; All purification steps were carried out at room temperature, using 20 mM acetate buffer, pH 5. Details of the purification are presented in the section of result and discussion. SDS-PAGE with various marker protein

standard were used to estimate the molecular weight of purified lipase [11].

Analysis of lipase activities; The activity of lipases was evaluated in SD medium containing 1g/L LO, 2.5 g/L tergitol-NP40 as described in previous research [10]. This examination was done in series of time incubation at 30°C and shaking at 120 rpm using rotary shaker. Total FFA released was examined by Non-Esterified Fatty Acids (NEFA)-C kit (Wako) and oleic acid was used as standard. Component of FFA produced was also identified and quantified by Gas Chromatograph (GC) after transmethylation [10]. The percentage of ALA produced during LO hydrolysis was calculated using formulation below.

$$DH = \left(\frac{TFP}{TL}\right) x100\%$$

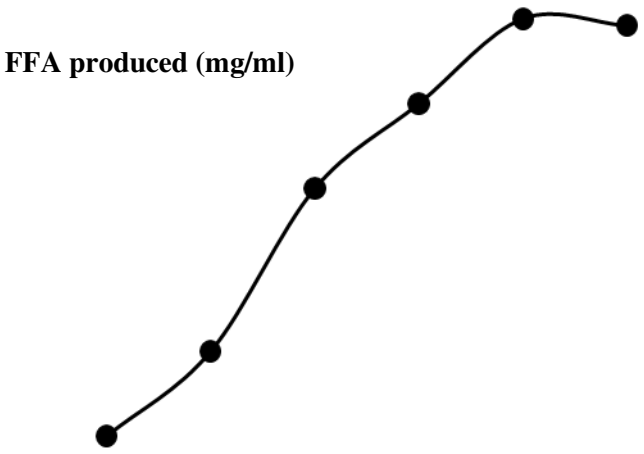
where: DH = The percentage ALA produced (%)

TFP = Total FFA produced as ALA

RESULTS AND DISCUSSION

Acording to the previous research [10, 11], the crude lipase was produced through solid state fermentation from 500 g sample of soybean pulp. After sterilization by autoclaving, the medium was inoculated with pre-culture *A. niger* and incubated at 30°C. During fermentation the lipase activity was analyzed using NEFA for measuring the FFA released. The optimum lipase activity was achieved after 4 days cultivation (Figure 1) and the crude lipase harvested by adding 1% NaCl and 0.1% toluene, followed by shaking 120 rpm at room temperature for 9 hours and was then filtered. The rest of debris in crude lipase solution was removed by centrifugation 4000 rpm for 10 minutes followed by re-filtration, stored at 4°C and used for the next lipase purification source.

Figure 1. Optimum cultivation of lipase production was optimized by monitoring of FFA



produced (mg/ml) during solid state fermentation.

For the first step of purification, the crude lipase was precipitated. The crude lipase was made in 65% saturated in ammonium sulfate followed by centrifugation at 12,000 rpm for 20 minutes. And then remaining ammonium sulfate in precipitates was removed by overnight dialysis at 4°C against buffer. This dialyzed enzyme was loaded into DEAE Toyopearl 650M ion exchange column pre-equilibrated with buffer. The column was then eluted by 0–0.5M NaCl linear gradient. Active fractions were pooled, re-dialyzed against buffer to remove NaCl. This peak was identified as lipase,

respectively. Finally, the purified lipase was obtained through Mono-Q anion exchanger column, resulting in 11.6% yield and 1,303 folds (Table 1).

Table 1. Purification steps of lipase from *A. niger*

Purification step	Total ABS-280	Total Activity (unit)	Specific Activity	Yield (%)	Fold
Ammonium sulfate Precipitation	56,400	1,800	0.03	100.0	1.0
DEAE Toyopearl	11,300	760	0.07	42.2	2.1
Mono-Q	5	208	41.60	11.6	1,303

The molecular weight of the enzyme was estimated by SDS-PAGE, this lipase was 51 KDa approximately (Figure 2).

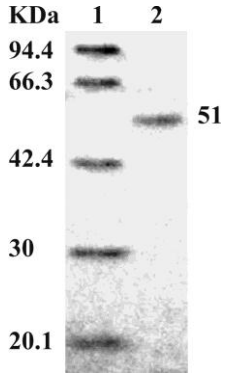


Figure 2. SDS-PAGE of protein standards (lane 1) and purified lipase (lane 2)

It was shown in Table 2, purified lipase from *A. niger* have highest activity and it seem hydrolyzed LO efficienly comparing with others commercial lipases. This lipase produced 0.65g/L after 18 hours incubation. With the same time of incubation, lipase from *R. miehei* (L4277-SIGMA) released FFA at similar amount (0.63g/L). Lipase AY30, *M. miehei* and D Amano only produced 0.38g/L, 0.27g/L and 0.28g/L, while other lipases produced FFA at very low concentration.

Tabel 2. Hydrolysis of LO by using various lipases

Lipase	Unit/mL applied in medium*	FFA produced (g/L) in a series time (hour) hydrolysis			
		0	6	12	18
Purified Lipase from *A. niger*	100	0.01	0.26	0.54	0.65
Porc. Panc. (20552-02 Nacalai)	100	0.01	0.04	0.11	0.13
R. miehei (L4277-SIGMA)	100	0.01	0.04	0.31	0.63
Porc. Panc. (L3126-SIGMA)	100	0.01	0.04	0.04	0.04
Lipase AY30 (Amano)	100	0.01	0.13	0.30	0.38
M. miehei (L9031-SIGMA)	100	0.01	0.03	0.20	0.27
D "Amano" (Amano)	100	0.01	0.10	0.20	0.28
Without lipase	-	0.01	0.01	0.01	0.02

*: 5 mL SD medium contain 1 g/L Linseed oil and 2.5 g/L tergitol-NP40

To improve the FFA production, optimizing of LO hydrolysis using purified lipase was conducted at a series of pH using 50mM acetate and phosphate buffer at a range pH 3 to 8 and temperature at 25 to 65^{o}C. The reaction mixtures of lipase and substrate were done in 5 mL SD medium contain 1 g/L LO and 2.5 g/L tergitol-NP40 and incubated in 12 hours at 30^{o}C 120 rpm. We found that the enzyme had stability at pH 3 to 6 and at temperature range 25^{o}C to 60^{o}C while optimum for its activity at pH 5 and at the temperature 50^{o}C respectively. Further steps, the components of FFA were also analyzed

using GC, and it will be compared with other FFA which hydrolyzed by using lipase from *R. miehei* (L4277-SIGMA).

Table 3. Fatty Acids composition of hydrolyzates analyzed by GC

Fatty acids composition of hydrolyzates	Fatty acids (g/L)		
	Without Lipase	*A. niger*	*R. miehei*
Palmitic acid (16:0)	0.059	0.02	0.09
Stearic acid (18:0)	0.031	0	0.08
Oleic acid (18:1)	0.18	0.06	0.05
Linoleic acid (18:2)	0.139	0.14	0.13
α-Linolenic acid (18:3(n-3))	0.57	0.512	0.489
Total FFA released	0.979	0.732	0.839

It was shown in Table 3, although total production during hydrolysis by these two lipases (from *A. niger* and *R. miehei*) are relatively different where *R. miehei* produced much more higher than purified lipase from *A. niger*, it was proved that hydrolysis using purified lipase from *A. niger* had capability to release 0.512 g/L (or 89.8%) ALA from LO, while lipase from *R. miehei* only released 0.489 g/L (or 85.8%). Suggested that crude lipase from *A. niger* more effective to produce ALA from LO than lipase from *R. miehei*.

CONCLUSION

An enzyme as lipase from *A. niger* hydrolyzed and produced ALA from LO effectively comparing with six selected commercial lipases has been proved. It was suggested that the crude lipase *A. niger* can be used as a cheap source of lipase for ALA production from LO and may be implemented for industrial scale.

ACKNOWLEDGEMENT

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OPTIMALIZE GENISTEIN OF REJECTED EDAMAME SOYBEAN FLOUR USING β-GLUCOSIDASE PRODUCED BY BACTERIA

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The development in many sectors brings the social status change and creates human lifestyle which causes the degenerative disease increase. One way to overcome this condition is by explore genistein. In developed/industrialized countries, barley's genistein is already being extracted, commercialized and used as an alternative therapy for cancer sufferer (Coral, 2008; Caderroth and Serge, 2009). Indonesia is an edamame soybean exporter for Japan and USA. The biggest edamame soybean produced by PT. Mitratani Dua Tujuh Jember, exported in each year reaches 6,152 – 8,000 tons on average, but out of the total production, roughly 12.8 tons of fresh edamame per month is rejected. The rejected edamame soybean contains only 0.122 mg/g genistein on average (Wibisono and Warsito, 2009), but can be optimally used by β-glucosidase enzyme from microbe. The current research examines the optimal condition of enzyme and the application of such enzyme to get the rejected edamame soybean flour with optimal genistein. The result shows that B. adolencentis produces the most optimal β-glucosidase enzyme, along with B. animalis, L. casei and B. bifidum. Bifidobacterium and L. casei grow optimally at 35oC, but the enzyme itself has an optimal temperature of 45oC. The optimal pH of both enzyme and bacteria is pH 6. The fermentation index (proposed to be known as Wibisono index) of B. adolencentis has the highest index of 6 hour incubation in 1.153 and followed by B. animalis which has fermentation index 1.012. Extracted β-glucosidase enzyme produces the highest activity in ratio flour : water = 1:10 and the optimal incubation time to hidrolysis 80.5% genistin reached in 12 hours. Another finding indicates that the increase of genistein's content in rejected Edamame soybean flour becomes 0.487 – 0.513 mg/g. Our food product from rejected edamame which enrichment of genistein have been patented with certificate number: 050.0226A (28 January 2010).

Keywords: Genistein, β- glucosidase, Edamame

BIOETHANOL FERMENTATION FROM SAGO (Metroxylon sagu Rottb.) PITH POWDER USING COCULTURES Pichia stipitis CBS 5773, Saccharomyces cerevisiae D1/P3GI AND Zymomonas mobilis FNCC 0056

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The research of sago (*Metroxylon sagu* Rottb.) pith powder hydrolysis using sulfuric acid and enzyme also ethanol fermentation from its hydrolysates by cocultures *Pichia stipitis* CBS 5773, *Saccharomyces cerevisiae* D1/P3GI and *Zymomonas mobilis* FNCC 0056 has been conducted. The research method used was Complete Randomized Design with factorial in three factors, which were cocultures type, sugar concentration and incubation periods. The type of cocultures was comprised of (1) *Pichia stipitis* and *Saccharomyces cerevisiae* and (2) *Pichia stipitis* and *Zymomonas mobilis*. The sugar concentration used were 5% and 10%. The fermentation was performed 120 hour. The research methods used were descriptive and experimental method. The descriptive method was used for first step, which were sago pith powder hydrolysis be a reduction sugar. The experimental method was used for second step, which were sago hydrolysate sugar fermentation process be an ethanol. The data were statistically analyzed using Duncan's Multiple Range Test. The research showed that hydrolysis using 6 M sulfuric acid with temperature for hydrolysis was 120ºC results the reduction sugar about 22,26% (w/w) with DE value about 28,63%, hydrolysis using α-amylase enzyme (0,17µl/g), hemicellulase enzyme (1/3 x 0,001g/g), cellulase enzyme (0,55 µl/g), and amyloglucosidase enzyme (0,37 µl/g) results reduction sugar about 53,28% (w/w) with DE value about 68,52%. Fermentation process with cocultures *Pichia stipitis* and *Saccharomyces cerevisiae*, sugar concentration of 10% and 72 hours of fermentation, produced the highest ethanol concentration about 4,15% with fermentation efficiency about 50,12%.

Keyword: hydrolysis, sago pith powder, sulfuric acid, enzyme, reduction sugar, fermentation, cocultures, *Pichia stipitis* CBS 5773, *Saccharomyces cerevisiae* D1/P3GI, *Zymomonas mobilis* FNCC 0056, ethanol.

ORAL PRESENTATIONS:

BIODIVERSITY

AND

ENVIRONMENT

ALLELIC DIVERSITY OF SAMPOERNA AGRO'S EKONA PISIFERA OIL PALM BASED ON MICROSATELLITE MARKERS

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ABSTRACT

Germplasm collection, knowledge on genetic distances and relationships among breeding materials data are important in plant breeding activities and has significant impact on crop improvement. Sampoerna Agro (SA) has established oil palm germplasm collections consisted of 3 ekona pisifera populations. In this experiment the allelic diversity among SA's pisifera collection were investigated using SSR marker. Microsatellite markers are highly reliable, inherited in codominant fashion whereby heterozygotes and homozygotes are distinguishable, easy to score and can rapidly produced using PCR. A total of 12 palms from the populations were genotyped using 20 SSR loci. Mean number of alleles per locus was 3.85 while mean of *Polymorphic Information Content* (PIC) of the SSR marker analyzed was 56.2%. Observed mean of heterozygosity was 0.604 while expected mean of heterozygosity was 0.645. Results of genetic dissimilarity coefficient calculation and dendogram construction using DARwin 5.05 indicated that 12 SA's Ekona pisifera populations was clustered into three groups. Accession 22 of Ekona pisifera and part of accession 1 belonged into group I; that of accession 7 belonged into group II; while number of individuals of accession 1 belonged into Group III. Implication of allelic diversity of SA's pisifera germ plasm on SA's breeding program will be discussed in detail.

Keywords: allelic diversity, microsatellite marker, oil palm, pisifera

INTRODUCTION

Oil palm (*Elaeis guineensis* Jacq.) is currently one of the strategic plantation commodities in Indonesia. Therefore, activities to improve oil palm characters are needed to develop better planting materials. Such objectives may be achieved through oil palm breeding program. Effective oil palm breeding activities depend on the availability of oil palm germplasm collections and their genetic variability.

A number of microsatellite markers analysis has been done for oil palm germplasm collections of Sampoerna Agro (Putri, 2010). The main objectives of this study were to utilize microsatellite markers for analyzing Sampoerna Agro's (Sumatra, Indonesia) 12 Ekona pisifera populations and to characterize their genetic diversity. The generated data will be used to draw preliminary conclusion on this germplasm for breeding purposes.

MATERIALS AND METHODS

Plant material, DNA extraction and genotyping.

Total genomic DNA was extracted from fresh leaf 12 samples of each individual palm using a conventional CTAB method. The genomic DNA concentration was

estimated with a fluorimeter (Fluoroskan Ascent®, Thermo Fisher Scientific®, USA) and the DNA quality was checked using agarose minigel electrophoresis.

Twenty independent microsatellite loci were chosen from the oil palm reference map published by Billotte *et al.* (2005). The amplified SSR allelic patterns were analysed with the SAGAGT® software (LI-COR, Lincoln, USA) and alleles were identified according to their base pair size. The genetic diversity was estimated under PowerMarker v3.0 (Liu and Muse, 2005) and two types of descriptive analysis on genetic diversity were performed under DARwin5 (Perrier and Jacquemoud-Collet, 2009).

RESULTS AND DISCUSSIONS

The pisifera originated from Ekona showed the highest value for PIC and mean number of alleles per locus (56.2% and 3.85, respectively). High diversity of pisifera of Ekona origin that has been used as parent for production of comercial seeds (Bina Sawit Makmur, 2004), showed high level of heterozygosities. Such data may indicate that this pisifera origins may have previously been exposed to only little selection pressure. Germplasm collection that had high polymorphism level could be recommended for breeding purposes and further germplasm exploitation.

This slightly lower numbers of alleles, with a higher major allele frequency and a lower H_o mean value (0.604) of the SA pisifera accessions, were in accordance to the effects of past hybridization and selections previously conducted. The past hybridization and selections tended to decrease the average number of alleles per locus and the proportion of heterozygous loci. Expected heterozygosities (H_e) in the SA's pisifera samples indicated a high average polymorphism rate (0.645) for the loci.

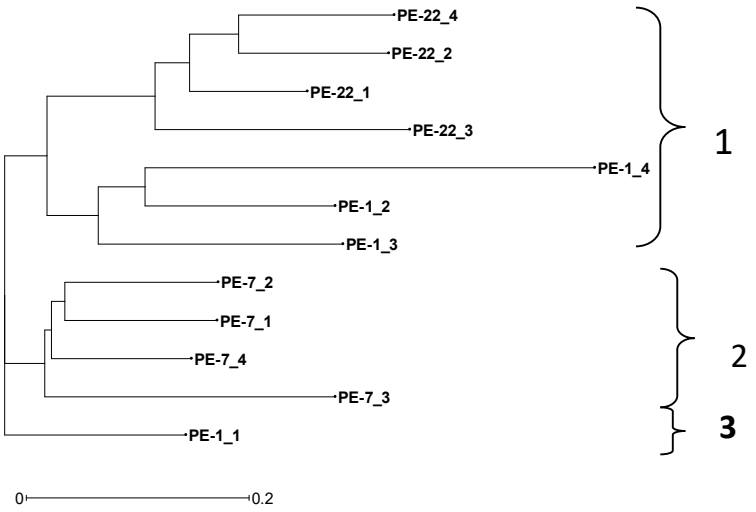

Figure 1. Neighbor-Joining profile from 12 Ekona's individu based on *Matrix Dissimilarity Simple Matching* analysis

Genetic relationship among Ekona

Analysis using factorial methods was conducted since the purpose of analysis was mainly to give an overall representation of diversity and not the individual effects. The Principal Coordinates Analysis (PCoA) identified several independent axes or eigenvectors that are linear combinations of the characters studied (SSRs in this case), which account for the largest part of the variation (SSR length polymorphism). The PCoA was performed on the 77 alleles, revealed by the 20 SSR loci over the 12 SA Ekona pisifera. Distinct groups were discriminated, with axes 1 and 2 explaining 60.47% of the

total molecular variation (*Figure 2*). One group was made of Ekona-22 along with three individuals of Ekona-1 Nigeria accession. One individual E-1_1 was separated from their main group. These materials may have been mislabelled materials. Such materials should be discarded and should not be used as pollen sources and in breeding program.

As described by Perrier and Jacquemoud-Collet (2009), tree method was another approach for presenting diversity structure. This method indicated individual relationship that might be less accurate than factorial analyses on the overall structure. Our results of unrooted Neighbour-Joining tree gave a quite nice picture about the relations among SA Ekona pisifera (*Figure 1*).

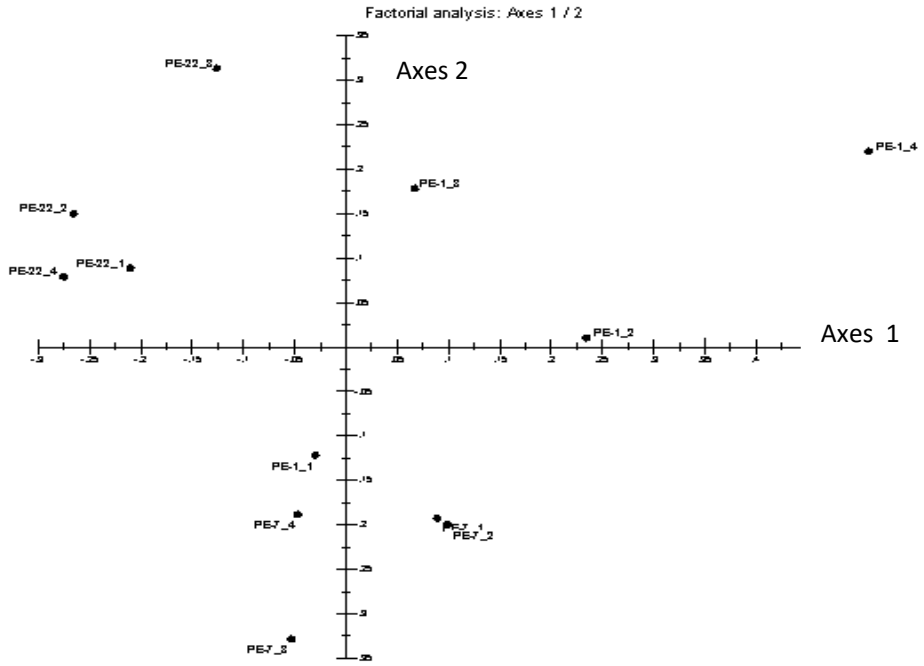

Figure 2. Multivariate Factorial Analysis on axis 1 and axis 2 based on 20 microsatellite locus

All of these demonstrated that results of SSR analysis were usefull for genetic diversity of pisifera oil palms germplasm. Moreover, SSR-genotyping should allow more efficient selection of pisifera parents for oil palm breeding programs. Selection of more genetically divergent parents would theoretically maximize heterosis and thus increase hybrid vigour of oil palm hybrids.

The SSR polymorphism provides a valuable tool for the analysis of pisifera oil palms germplasm. These results have significant implications for pisifera conservation purposes, since they could be used to identify a core genepool for ex situ pisifera conservation and for future SA's oil palm breeding programs. Based on generated SSR markers, mean number of allele per locus, expected heterozygosities and PIC value, the selected pisifera originated from Ekona had high level of allelic diversity. The geographic and genetic structures of the pisifera oil palm diversity suggested the possibility of new breeding approaches.

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PLANT COMMUNITY STUDY IN LAKE BUYAN-TAMBLINGAN FOREST AREAS BALI

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ABSTRACT

Plant community study in Buyan-Tamblingan Nature Recreation Area aimed to identify whether there were differences between the two communities in terms of vegetation structure and composition and whether habitat factors such as land slope and altitude have some effects on species distribution. NMDS ordination revealed that the two communities were different ($R_{ANOSIM=0.7}$ $p<0,001$). Species diversity was also different between these communities. Buyan area had higher diversity index (Shannon 2,00) compare to Tamblingan (1,60). CCA ordination showed that both slope and altitude have significant effect on the distribution of some species in axes 1 and 2 (sum of all canonical eigenvalues 0.619). The invasive grass *Panicum repens* had the higher contribution to the differences between the plant communities in the two lake areas as detectedusing SIMPER analysis. This invasive species exist in Buyan and absence in Tamblingan. Differences in tourism activities and impacts were proposed to be part of the reasons behind the results.

Keywords: Plant community, NMDS, CCA, Buyan-Tamblingan Nature Recreation Area, Bali

INTRODUCTION

Mountain forest has become the last sanctuary for most of Bali's biodiversity as is the case in Java. Mountain forest ecosystem is important when we consider that most of the lowland forest in Indonesia has been disturbed and degraded (Lavigne and Gunnell 2006; Smiet 1992; Whitten *et al.* 1996). Forest areas near the Buyan and Tamblingan Lake are one of the remaining tropical mountain rain forests in Bali and they play significant role in maintaining the ecosystem and as buffer zone for the surrounding areas and for the lower areas of Bali. However, nowadays mountains rain forests are becoming more and more threaten due to the increase in human activity (Horn *et al.* 2001; Lavigne and Gunnell 2006), including forest near Buyan and Tamblingan Lakes. As is the case for Beratan Lake, the Buyan Lake is also slowly more exposed to tourism activities. The clearing of forested areas near the lake in 2001 was conducted to establish camping ground. Hence, ecological research on the plant communities in the forests surrounding these lakes is needed to be conducted. This study will provide baseline data to the next stage of plant biodiversity monitoring that can be use to generate a more comprehensive conservation and restoration efforts in these areas.

MATERIALS AND METHODS

This study was conducted in the Nature Recreation Area - Batukaru Nature Reserve, the Buyan and Tamblingan Lake. This area is located at 8° 14' 8" S dan 115° 05' 15" E, ± 60 km North of Denpasar. Buyan and Tamblingan Lake is a mountain rain forest located at an altitude of 1.210 – 1.350 m. Vegetation was sampled by making transects through the forest and using circular plots of 10 m radial to measure the tree species (number of individual, height, girth). Within the 10 m circular plot, a nested circular plot with 2 m radial was also made to measure the groundcover species (species, number of individual) (Kent and Coker 1992). The distance between plots is 50 m and there are a total of 30 plots, in Buyan and Tamblingan. Data were assessed using multivariate analysis with PRIMER and CANOCO. Shannon-Wiener species diversity on each site was calculated using the DIVERSE routine in PRIMER. The differences in

community composition among the two forested lake areas were tested using Non Metric Multidimensional Analysis in PRIMER V.6. (Clarke and Gorley 2005). Variation in community composition among deposits was subsequently tested for its significance level with an ANOSIM (Clarke 1993). The SIMPER routine in PRIMER V.6 was then used to explore the relative contribution of individual species to dissimilarity among sites. A Canonical Correspondence Analyses was used to identify the distribution of species along the environmental gradients (Ter Braak 1986). The CCA axes were evaluated statistically using a Monte Carlo permutation test. The CCA analysis was done using CANOCO program V.4.5 (ter Braak and Smilauer 2002).

RESULTS AND DISCUSSION

The forest areas in these two lakes although located near to each other, have different species diversity rate and community composition. The Buyan Forest area had higher species diversity rate based on Shannon diversity index compare to Tamblingan (Figure 1). According to Barbour *et al.* (1980), Shannon index of 0-2 is categorized as low diversity, and so the diversity index in these two lake forest areas is low. The difference in Shannon Diversity index in these two lakes is probably due to the difference in the type of tourism activities. At the Buyan Lake Forest area, a wide gap of forest was cleared for camping ground area. The establishment of pathways from the entrance to the camping ground through the forest also contribute to the increase in the number of species especially exotic and weed species. The opening of the forest creates a gap where direct sunlight reaches the floor and catalyses the germination of species that were dormant as a soil seed bank, especially grasses (Poaceae) (Aubert *et al.* 2003; Austin and Pausas 2001; Pena 2003).

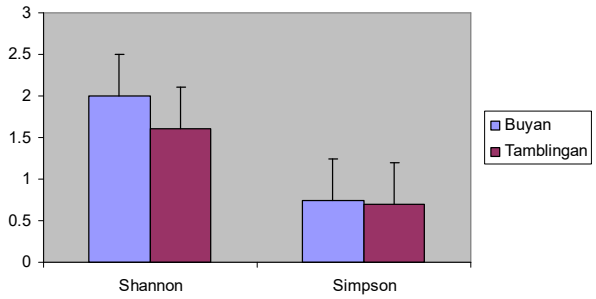

Figure 1. Species diversity in Lake Buyan and amblingan Forest using Shannon and Simpso Diversity Index

Vegetation analysis using NMDS ordination model showed that there were significant differences in community composition between the two forest areas (Figure 2, R_{ANOSIM} of 0.7 ($p<0.001$)). As seen in Table 1, plant community at Buyan, is characterized by the domination of grass species such as *Panicum reptans,* shrubs such as *Solanum* sp and *Coffea* sp and tree species such as *Leucaena leucocephala,* and *Altingia excelsa.* These species were not found at Tamblingan. Whereas at Tamblingan, plant community was mainly composed and dominated by tree species such as *Laportea* sp, *Mescereh midtia*, *Lucuma luzoniensis*, *Rauvolfia javanica*, *Ficus* sp, and *Erythrina variegata* , species of from Zingiberaceae family and a fern species *Gleichinia* sp. These species except for *Lucuma luzoniensis* were also found at Buyan although with lower average abundance. This phenomenon is also probably one of the reasons of the higher diversity index at Buyan. The grass species, *Panicum* reptans was the species that was the mainly responsible for the difference between Buyan and Tamblingan with total percentage of contribution of 9.33%. Whereas the tree species, *Laportea* sp was the main

species that chaacterized the community vegetation at Tamblingan with total percentage of contribution of 7,36%.

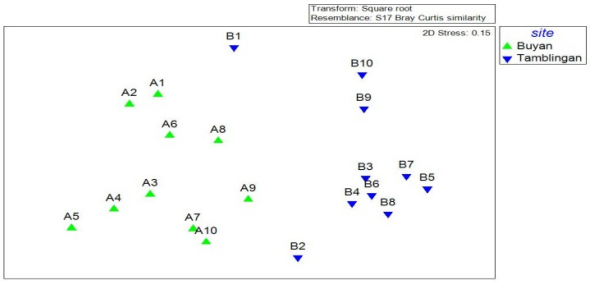

Figure 2. NMDS plots ordination (2D stress = 0.15) of the two plant communities in Buyan-Tamblingan Lake Forest Areas

Table 1. Similarity percentages (SIMPER) of top four differentiating species among habitat similarity comparison. Av Diss abbreviation refers to the average dissimilarity which is the average contribution for each species to the overall dissimilarity between groups. Diss/SD is the ratio of average dissimilarity with the standard deviation which indicates how consistent a species contributes to dissimilarity among study sites/groups. Cumulative contribution is the contribution of each species to the dissimilarity between the groups. Average abundance data are the abundance data after transformation. SIMPER was done for the species so that species contributing to 90% dissimilarity were analysed, only eight most important species contributing to the dissimilarity between the groups have been listed here.

Species	Group Buyan Av. abundance	Group Tamblingan Av. abundance	Diss/SD	Contrib%
Panicum reptans	3.53	0.00	0.70	9.33
Laportea sp	0.10	2.87	0.89	7.36
Leucaena leucocephala	1.68	0.00	0.83	4.83
Zingiber sp	1.49	0.57	0.82	4.52
Mescereh midtia	1.48	0.37	1.36	3.98
Lucuma luzoniensis	0.00	2.13	0.33	3.98
Solanum sp	1.37	0.00	0.72	3.87
Altingia excelsa	1.42	0.00	0.94	3.55
Rauvolfia javanica	0.65	1.08	1.18	2.56
Ficus sp	0.47	0.63	0.86	2.45
Erythrina variegata	0.80	0.17	1.08	2.29
Coffea sp	0.81	0.00	0.61	2.23
Gleichinia sp	0.22	0.71	0.53	2.22

Results from this study are important as baseline data for the conservation areas managers in order to develop a management and site rehabilitation plan. For rehabilitation purposes, species selection is an important part of the process. Native species or species that are less aggressive are preferred than exotic and aggressive species and these species should also positively correlated with elevation and slope gradients. This can be drawn from the CCA diagram (Figure 3). *Eucalyptus*, *Homalanthus*, *Rauvolfia*, *Leucaena*, and *Erythrina* are the tree species that are correlated with the two gradients and are potential to be used as species for rehabilitation purposes.

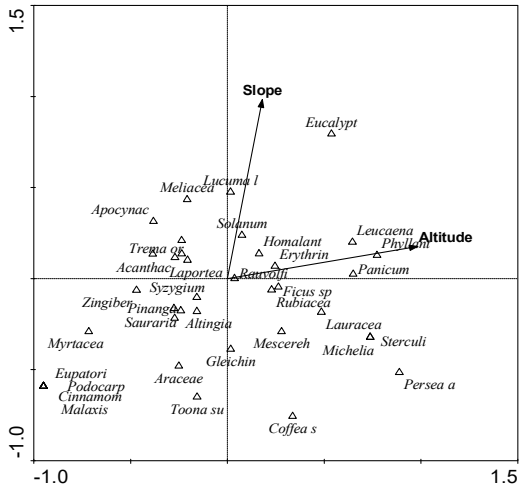

Figure 3. Ordination diagram derived from Canonical Correspondence Analysis showing distribution of the species on the gradients of slope and altitude.

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THE EFFECT OF LAND-USE TYPE ON BIRD COMMUNITY IN NORTH BANDUNG AREA, WEST JAVA

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ABSTRACT

Changes in vegetation structure and physical environmental factor can have effects on the variety of bird species in the established landscape. North Bandung is undergoing a significant change of landscape in the last several years. The objective of this research was to study the relationship of land use type and habitat factors on bird community in North Bandung area. Bird data were collected from May 2006 to April 2007 using Point Count method. Vegetation data such as strata complexity and percentage of canopy cover were collected using direct observation method, while altitude, percentage of built-up area and nearest distance of land use type to built-up area were collected by extracting Landsat TM image (year 2004). The effect of habitat factors on bird distribution was analyzed using Canonical Correspondence Analysis (CCA). Based on Landsat TM image analysis, landscape of the research area was classified into four land-use types: forest, agricultural area, cropland and residential area. During observation, 59 species of birds were found. The highest richness was in forest (51 species, H' = 2.428), and the lowest was in cropland (31 species, H' = 2.329). Canopy cover and strata complexity were landscape factors with the highest effect on bird abundance in residential area (r = 0.933 and 0.808, $p < 0.05$). Bird abundance in cropland was affected by canopy cover and altitude (r = 0.854 and 0.747, $p < 0.05$), while in agricultural area it was affected by distance to nearest residential area and strata complexity (r = 0.958 and 0.815, $p < 0.05$). Forest bird abundance was affected by altitude and canopy cover (r = 0.960 and 0.774, $p < 0.05$). In general, bird abundance in North Bandung area was affected by two factors, canopy cover and altitude (r = 0.898 and 0.896, $p < 0.05$). This result highlighted the importance of vegetation as a critical habitat factor affecting the existence of bird community in all land-use types in North Bandung area. It was also implied that mountain forest is important as the habitat of the most bird species in North Bandung area.

Keywords: land-use type, bird community, North Bandung area, CCA ordination

INTRODUCTION

The presence of organisms in a landscape is associated with landscape environmental factors. Changes that occur in a landscape will affect environmental factors that regulate the landscape, and eventually will have impact on the organisms that occupy the landscape, such as affecting species abundance and diversity (Forman, 1996). One group of organisms that is vulnerable to changes in its habitat is bird (Hansen and Urban, 1992). Husodo (2006) has shown the influence of landscape changes caused by human activities on diversity and abundance of bird species in West Bandung area.

Badan Pengelolaan Lingkungan Hidup Daerah (BPLHD) Jawa Barat (2004) noted that during the last 10 years there have been significant changes in the landscape of North Bandung area. Approximately 1,545 ha of natural forest area in North Bandung were converted into other land-use types, while there was an additional area for agriculture, settlements and cropland. Landscape changes in North Bandung are predicted to continue in the subsequent years. This continued changes will threatens bird communities in North Bandung area. In this study, an analysis on several landscape environmental factors, and their impact on bird communities on different land-use types in North Bandung area were conducted. Based on the analysis I determined the level of influence of each factor to the abundance and distribution of bird species found on various land-use types which are formed from prior landscape changes were determined. This study will provide baseline

data that can be use to determine the appropriate policies for land-use management and biodiversity conservation in North Bandung area.

METHODS

This study was conducted in North Bandung area from May 2006 to April 2007. Area of study was limited between UTM coordinates 787000-793000 mE and 9235000-9252000 mN and divided into observation plots forming grids of 1 × 1 km. Data collection was conducted in selected grids. Three grids were positioned horizontally from west to east and 16 grids were positioned vertically from south to north. Using point count method (Bibby et al., 1992), four subplots of observation (point count, ∅ 80 - 100 m) were randomly selected in each grid for bird data collection. Bird observation was conducted in the morning from 05.30 to 10.00 am with a 10-minute recording period. For the entire study sites, total point count obtained was 2304 of 10 minutes sample points. Identification of bird was based on MacKinnon et al. (1992).

Land-use type was determined for each grid by extracting Landsat TM year 2004 using remote sensing techniques. Vegetation index of radiometric and geometric corrected image was calculated using NDVI (Normalized Difference Vegetation Index) formula. NDVI image was then classified using supervised classification method.

Landscape environmental factors were measured using two methods. Altitude, nearest distance of point count to built-up area and percentage of built-up area were obtained from satellite imagery data. Vegetation data such as strata complexity and percentage of canopy cover were obtained from direct measurement in the field.

Parameters measured for bird communities were relative abundance, diversity index and evenness index. Relationships of bird abundance and landscape environmental factors were analyzed using CCA (Canonical Correspondence Analysis) ordination in R program (Venables and Smith, 2010).

RESULTS AND DISCUSSION

Area of study was classified into four land-use types, namely residential area, cropland, agriculture area and forest. Total area of each land-use type was 18.68% for residential area, 40.21% for cropland, 31.85% for agriculture area and 5.26% for forest. In southern part of the study area, landscape mosaic was dominated by residential area, adjacent to cropland. Moving to north, landscape mosaic was shifted into a matrix of natural ecosystems. Northern part of the study area was dominated by agriculture matrix with few number of small forest patches. One large forest patch was part of the Tangkuban Perahu Natural Reserve.

During the study 59 bird species from 28 families and 5261 individuals were recorded. A total of 51 species were recorded in forest, 45 species in agriculture area, 33 species in residential area and 31 species in cropland. Predominant species, with relative abundance > 5%, were *Collocalia esculenta*, *Passer montanus*, *Zosterops palpebrosus*, *Pericrocotus miniatus*, *Pycnonotus bimaculatus*, *Pycnonotus aurigaster*, *Aethopyga mysticalis*, *Dicaeum sanguinolentum* and *Orthotomus cuculatus*. Dominance of a certain species was not the same for each land-use type. Based on our observation, there were some bird species with large number of individuals but had limited distribution to certain type of land-use.

In general, bird communities in all land-use types had the same evenness index values, ranging from 0.813 to 0.873, which means that each bird species was evenly distributed and that individual distribution was not different for each land-use type. Agriculture area and forest had the highest bird diversity (H' = 2.487 and 2.428), while as the lowest was in cropland and residential area (H' = 2.329 and 2.127). Based on these

index values, characteristics and environmental condition of each land-use type would affect the differences in bird occurrence and number of individuals. Each species of birds had different ability to adapt to landscape environmental factors, and this would affect its presence or absence in a particular landscape (Mason et al., 2006). For example, forest bird community were generally consist of species that highly depend on vegetation and not tolerant to disturbances, while as almost all bird species found in residential area and cropland were those that tolerant to disturbances and changes on its habitat.

The CCA showed an ordination that was constrained by landscape environmental variables. The CCA for forest showed 98.2 from the total 133.2 variances, or 73.7% of variance was shown. For agriculture area, cropland and residential area, variance showed by CCA were 44.1%, 63.2% and 68.5%. This means that not all differences in chi-square distance among sites were shown, but only those differences that could be related to differences in environmental variables (Kindt and Coe, 2005).

The CCA also gave eigenvalues which basically show how much variance is expressed on each axis (Kindt and Coe, 2005). Eigenvalues are listed from highest to lowest, thus the first axes of the graphs will result in most variance being shown. CCA of all land-use types showed the highest eigenvalues on axis1 and axis2, thus interpretation of the graph would be based on the two axes.

Table 1. Biplot scores of CCA ordination showing correlation of bird community composition and landscape environmental variables in all land-use types ($p<0.05$).

Landscape env. factors	Forest		Agriculture area		Cropland		Residential area	
	CCA_1	CCA_2	CCA_1	CCA_2	CCA_1	CCA_2	CCA_1	CCA_2
Altitude	**0.96**	0.03	**0.81**	0.08	**0.52**	0.01	0.46	-0.04
Nearest distance to built area	-0.06	**-0.65**	-0.02	**-0.96**	**-0.53**	-0.24	0.02	-0.47
% of built-up area	**-0.67**	-0.08	0.22	-0.01	-0.47	**0.75**	**-0.64**	-0.49
% of canopy cover	0.52	**0.77**	-0.05	0.48	0.02	**-0.85**	**0.93**	0.31
Strata complexity	0.28	**0.63**	**-0.81**	-0.05	0.33	0.27	-0.26	**0.81**

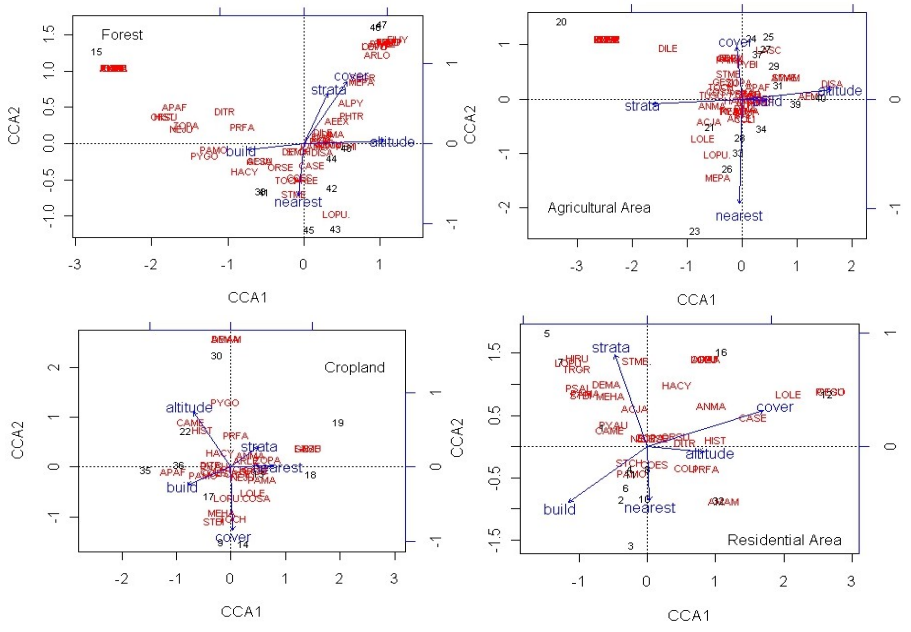

Figure 1. CCA ordination for the bird abundance dataset using all the landscape environmental variables in all land-use types.

Figure 1 shows ordination graphs for the first two axes for the bird abundance dataset in all land-use types using all the environmental variables as constraining variables. Coefficient of correlation for each environmental variable in all land-use types are shown in Table 1. In forest it can be seen that the first axis is very well correlated with altitude (r = 0.96) and percentage of built-up area (r = -0.67), and the second axis is correlated with percentage of canopy cover (r = 0.77), nearest distance to built-up area (r = -0.65) and strata complexity (r = 0.63). Therefore bird community in forest was controlled by two gradients, and all environmental variables have a significant effect on the distribution of bird in forest land-use type. This suggested the vulnerability of forest bird species to changes in its habitat (Hansen and Urban, 1992). Since its presence was controlled by many environmental variables, slightly changes in forest landscape are expected to shift forest bird community composition, and thus prefer more common species than interior species. From the result we can also see that forest bird composition was mostly affected by altitudinal factor. This highlighted the importance of mountain forest as habitat for most forest bird species in North Bandung area.

In agriculture area, bird distribution was affected mainly by nearest distance to built-up area (r = -0.96), altitude and strata complexity (r = 0.81). Bird distribution in a more disturbed ecosystem was strongly affected by canopy cover, as seen in cropland (r = -0.85) and residential area (r = 0.93). Bird community in residential area was also significantly affected by strata complexity (r = 0.81). This result highlighted the importance of vegetation, especially trees, in an urban landscape as refuge for birds (Fernandez-Juricic and Jokimaki, 2001).

Landscape changes caused by anthropogenic activities in North Bandung were expected to change relationship form between bird species and its habitat. Bird is highly depends on vegetation (Thiollay, 1994). Land transformation to meet human needs will simplify vegetation structure and composition. This is predicted to shift bird community composition, in which bird species that highly depend on the presence of vegetation will be replaced with a more common birds that are well adapted to landscape changes.

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IS IT POSSIBLE TO TRACK DOWN WHO'S POLLUTING THE RIVER?

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ABSTRACT

This paper reports the use of faecal steroids, namely coprostanol, cholesterol, ethyl coprostanol, epicoprostanol, cholestanol, stigmastanol, □-sitosterol, and others, to determine the sources of faecal pollution in water environments. The fact that each animal excretes unique steroid composition has been acknowledged world wide and thus the faecal steroid profiles have been used as finger print for the animal. In this paper the compositions of steroid compounds in faeces of various animals including human are confronted against those of water samples collected from various locations. Principal Component Analysis (PCA) is applied as a technique to extract the data and highlight the similarities and differences between animals and locations samples. This technique is proven to be reliable for the determination of the pollution sources.

Keywords: *coprostanol, ethyl coprostanol, faecal pollution, steroids, PCA*

INTRODUCTION

Mitigation of faecal pollution in aquatic environments requires the identification of the sources, as well as the determination and monitoring of the contamination levels. The conventional procedure in determining faecal contamination, namely enumeration of faecal indicator organisms such as thermotolerant coliforms, *E.coli*, streptococci, and others, are not source specific. These microorganisms are found in most animal faeces indiscriminately.

Levels of faecal steroids have been widely used as chemical biomarkers of faecal pollution in aquatic environments, although there has been no standardised method adopted (Chan, K. H., *et.al*., 1998; Düreth, S., *et.al*., 1986; Gonzalez-Oreja, J. A. and Saiz-Salinas, J. I., 1998; Grimalt, J. O., *et.al*., 1990; Murtaugh, J.J., and Bunch, R.L , 1967; Quéméneur, M. and Marty, Y., 1994; Tabak, H. H., *et.al*.,1972, Leeming, R., 1996).

In this paper, the use of faecal steroids to predict the sources of faecal contamination in catchment water is evaluated. In doing so, there are a number of aspects that need to be considered. If the steroid composition of animal faeces is to be used as a 'finger print' of the faecal pollutant, firstly, the steroid compositions of faeces of the animal that inhabit the area of interest have to be determined. Secondly, the interaction of the steroids with environmental conditions including their stability, interconversion, and physical properties should be understood in order to track their fate in the environment. For these reasons, studies on animal species steroid profiles, steroid degradation, and steroid profiles in sewage treatment effluents were carried out along with the determination of the steroid profiles in the catchment waters. However, due to space limitation, this paper only reports the most directly connected ones namely the animal faecal steroid profiles and the analysis of using the profiles to determine the origins of faecal pollution in the catchment waters. Hence the steroid profiles of water samples are compared with those of the animals known to inhabit the areas of sampling locations.

The source discrimination using ratios indices is difficult in environments contaminated with mixed pollution sources, because the contribution of individual sources to the whole steroid profiles is controlled by various aspects including the numbers of the species and different amount of excretion per day per species, which are

complicated to determine. By using PCA, on the other hand, it is easy to underline important aspects and put aside those having no impact on data discrimination.

The use of PCA as a technique to highlight the differences and similarities of the steroid profiles between animals has been proven to be potentially effective. The applicability of the technique have been tested by applying the analysis to combined data of steroid contents of the animal faeces and sewage effluents collected from a domestic sewage treatment plant at Newhaven, South Australia (Suprihatin, 2004). The analysis was conducted by extracting the steroid concentrations and using the ones with highest impact on discriminating the data into groups. The chosen parameters were then used as factor categories to cluster the data thus samples with similar steroid contents are located in one clustered. By plotting this in a Cartesian diagram one can see clearly which samples have similar profiles. This paper reports the use of the technique to extract the steroid contents of animal faeces as the pollutant sources and the steroid concentrations in water samples collected from several locations in South Australia's water catchments.

MATERIALS AND METHODS

Materials:

- Faecal steroid profiles of animals (human, horse, cow, sheep, pig, dog, cat, duck, seagull, pelican, kangaroo, wallaby, possum.
- Steroid contents of water samples collected from Torrens and Patawalonga cathment in South Australia: near Airport, Mitcham, Waterfall Gully, Gorge, Castambul, Gumeracha, Merchant.

Methods

- Steroid contents (coprostanol, cholesterol, ethyl coprostanol, epicoprostanol, cholestanol, stigmastanol, □-sitosterol, ethyl cholesterol) of the animal faeces and water samples were analysed using PCA (SPSS®).

Description of samping sites

- Mitcham: near the hills, recreation park surrounded by residential area
- Airport: near the beach. close to industrial and urban residential area.
- Gumeracha and Merchant: the upper rural residential communities and forest
- Gorge and Castambul: rural areas with activities such as agricultural and pastoral
- Waterfall Gully: semi rural residential close to recreational and wildlife parklands.

RESULTS AND DISCUSSION

Three parameters were extracted into two factors: coprostanol (C_{27} □ stanol) and in the first factor and ethyl coprostanol (C_{29} □ stanol) cholesterol (C_{27} sterol) in the second. The results were transformed in a Cartesian diagram using the factors as the axes (Figure 1). Positive X axis represents more percentage of cholesterol and negative X-axis represents more percentage of ethyl coprostanol. The ordinate Y represents percentage of coprostanol. This is why pelicans and dogs are located in more positive X-axis than human and other species, since cholesterol comprises about 97% of the total pelican faecal steroid and 88 % of the total dog faecal steroid. All herbivores are clustered in one group with higher percentage of plant steroid like ethyl coprostanol and or ethyl epicoprostanol (C_{29} □ stanol) than cholesterol. This description precisely matches with the steroid profiles of those animals. The position of human on high positive ordinate

shows that it contains extremely high percentage of coprostanol compared to duck, cat, and other animals, which is in accordance with the steroid profiles. This feature further demonstrates the reliability of the technique to distinguish the species on the basis of their faecal steroid profiles.

Comparing the profiles of species with water samples, it is easy to interpret the diagrams in terms of the similarities and differences of species and sampling locations all together. The position of the sampling sites in the diagram show their profile similarities to those of the species clustered close to the site. For example, the Airport site is clustered with duck profile and close to the profiles of seagull, while Mitcham site is located close to the duck, cat, and dog profiles. This suggests that the steroid found in the Airport site is associated with faecal contamination originated from ducks and seagulls, while in Mitcham the contamination is brought about by ducks, cats, and dogs, since it is unlikely to find seagulls at the hills site. The steroid profiles in Gumeracha and Merchant sites indicate faecal pollution from native herbivores, which is very likely, considering that the sites are surrounded by parklands and close to forestry. Gorge, according to the diagram, is contaminated by non-native herbivores faecal steroids. Again, this is supported by the fact that the area are mostly used for agricultural activity. At the same time, the diagram does not suggest that any site is contaminated by steroids that might include human faecal pollution, which is very likely.

The result shows the consistency of the technique to analyse and determine the sources of the pollution. The analyses of other monthly samples (not shown), although not identical, are consistent with the likely sources in accordance with the landuse management covered by the catchment area. This emphasises the strength of using the combination of steroid profiles and PCA in determining the sources of faecal pollution in aquatic environments.

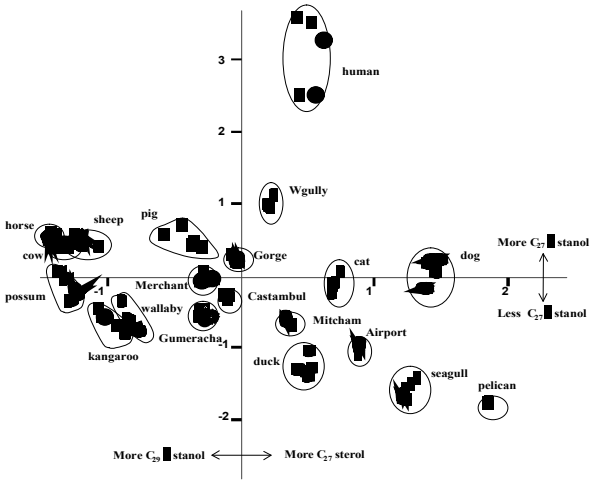

Figure 1. PCA of the water samples and the species faecal steroid. Relative position of samples indicates their common profiles

This novel technique is simpler yet more accurate compared to the ratios index for the determination. More importantly, from a management point of view, this technique provides an opportunity to link the faecal pollution to its responsible species to aid in development of control mechanisms.

It can be clearly concluded that the analysis of faecal steroid using PCA is a powerful technique for identifying sources of faecal pollution. The technique used in determining sources of faecal pollution in catchments waters is demonstrated to be reliable.

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POPULATION DYNAMICS AND IDENTIFICATION OF PHOSPHATE SOLUBILIZING BACTERIA IN COMPOST OF AGRICULTURAL LITTERS

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ABSTRACT

Effort to find out simple, easy and inexpensive technology as an alternative agricultural practices of slash and burn need to be done. Slash and burn in agricultural practices has caused many problems in West Kalimantan. The aim of slash and burn practices is to eliminate weeds. This principle can be done by composting agricultural litters and depend upon microorganisms that work fast in composting the litters. Phosphate solubilizing bacteria (PSB) is one of the bacteria involved in composting. The availability of phosphate in compost indicates the presence of PSB. By having population dynamic of PSB bacteria, processing time of composting can be estimated. The compost using the litters of four dominant plants namely *Imperata cylindrica, Cyperus sp, Ludwigia hyssofolia* and one species from *Pteridophyta* coming from agricultural area was studied to find out population dynamics of PSB and to indentify genus of PSB which involved in the process of compost. The highest temperature during this study was 56 ^{0}C on the third day of composting and reduced to 37^{o}C while pH initially tended to be acid (6.66) and gradually increased to basic neutral pH (7.22) at the end of composting. Based on Gaussian estimation, the duration of PSB availability was 7 days from the ninth to the15th day of composting with the number of 4,28.10^7 CFU.g^{-1} of compost at the peak on 12th day. Based on biochemical test and isolate characterization, two genus of PSB involved in composting of agricultural litters were *Flavobacterium*and *Pseudomonas*.

Keywords: Population dynamics, Phosphate Solubilizing Bacteria, Gaussian, Compost, Pteridophyta, *Imperata cylindrica, Cyperus* sp., *Ludwigia hyssofolia,* Flavobacterium and Pseudomonas.

INTRODUCTION

Slash and burn is often used to clear the land for agriculture in West Kalimantan. For the farmers, slash and burn is an easy, fast, and inexpensive way to give benefit such as providing nutrient, reducing disease, and eliminate the weeds (Nurida, 2001) but it often causes environmental problems. Land clearing by fire in West Kalimantan has caused many problems in health, economy, transport and international relationship due to heavy smoke.

It is urgent to find alternative ways to reduce smoke caused by slash and burn for opening agricultural land. Compost can have similar purpose as it can degrade the plant litters as well as provide nutrient. Compost is the product of organic litters from plants or animals or both (Sutejo, 2002). In the process, compost involves bacteria, fungi, and protozoa to degrade materials becoming other substances. Phosphate Solubilizing Bacteria (PSB), Cellulose Bacteria, and Amonnification Bacteria are involved in the process of compost. Phosphate solubilizing microorganism can be *Pseudomonas*, *Bacillus*, *Escheria*, and *Actinomycetes* (Nurmayulis, 2005).

During the process of composting, the population number of cellulose bacteria, ammonification bacteria, and PSB fluctuates as the C:N ratio, nitrogen, and phosphate changes. Population dynamics of cellulose bacteria, ammonification bacteria, and PSB will indicate time duration for composting. From the previous research (Sunandar, 2008), there were two PSB found in the compost process of three dominant plant litters but was

not identified yet. The aims of this research was to find out population dynamics of PSB and to indentify genus of PSB which involved in the process of compost using four dominant plants from agricultural land. By knowing number and time duration of its bacteria, time duration of compost can be estimated. Isolates from the compost using the plant litters from agricultural land can also be potentially developed into compost starter.

MATERIALS AND METHODS

Compost and estimation of population dynamics of PSB

Compost was made from agricultural litters of four dominant plants (*Imperata cylindrica*, *Cyperus* sp, *Ludwigia hyssofolia,* and one species from *Pteridophyta*) with EM_4 as activator. It was processed for 30 days and its height was 1 m. Sampling was taken daily for the first two weeks and thereafter every other days to count the number of PBS using plate count method with pikovskaya media. Compost temperature and pH were measured for 30 days. The number of PSB was plotted against time to estimate time duration of PSB existence and the time of PSB maximum number using Gaussian Model of ORIGIN Version 5.

Colony and cell morphology

The colony and cell of PSB were observed for shape, color, elevation and the edge shape. Gram staining was done to determine positive or negative reaction.

Biochemical test

Isolate was inoculated on Nutrient Agar (NA) media for motility test and H_2S formation. The capacity to solubilize the phosphate indicated by halo formation on pikovskaya media was tested for phosphate enzyme. Gelatinase was indicated from the ability to solubilize gelatine at 4°C after incubated for 24 hours (Hadioetomo, 1990 in Pratiwi, 2009). Isolate was stricken on NA medium containing starch and incubated for 48 hours at 37°C. The ability to hydrolyze starch was shown as a clear zone around the colony after dropping *lugol's iodine* on the media surface. Bubble formation in bacteria colony after dropped by 3% H_2O_2 was indication of catalase (Pradhika, 2009). Aerobic characteristic was indicated by the presence of bacteria on the surface of pikovskaya liquid (Hadioetomo, 1990 in Pratiwi, 2009). Changes of color into maroon blue after dropped by the reagent showed the presence of oxidase. Tests for glucose, lactose, and sucrose fermentation were done using TSIA media incubated for 24 – 48 hours at 37°C. No color change on slant and butt indicated there was no carbohydrate fermentation. However, if the slant color changed into red partially or whole indicated peptone degradation. Red slant and yellow butt without gas formation showed glucose fermentation only. Both slant and butt color turned into yellow without gas formation indicated glucose, lactose, and sucrose fermentation. Black color on butt indicated the presence of H_2S (Pradhika, 2009). Indole test was done by growing the bacteria on media containing Triptofan Broth (TB). Positive reaction was shown by the color of red after added by 3-5 drops of kovac's reagent (Fardiaz, 1989). Identification referred to *Bergey's Manual of Determinative Bacteriology* (Holt *et al.*, 1994)

RESULTS AND DISCUSSION

Compost and estimation of population dynamics of PSB

The highest temperature of compost was 56^0C, occurred on the third days and reduced into 37^0C on the 30th days of compost (Fig.1). There was direct relationship between an increase of temperature and oxygen consumption by bacteria (Dardjat, 2007). Aerobic compost produces high temperature. The higher temperature, the faster compost

processes. Heat was resulted from bacteria activity to degrade organic matter (Isroi, 2007).

pH was 6.88 at the beginning and increased into 7.22 at the end of compost (Fig 1). According to McKinley and Vestal (1985) and Madigan (2003), pH between 6 – 8 would optimize bacteria growth. Degradation of organic matter can increase soil pH (Hue, 1992 in Nurmayulis, 2005). Hue (1992) stated ligand formation between organic acid such as tartrate with hydroxyl form from Fe^{2+} and H^{+} in soil would bind Fe^{2+} and Al^{3+} and released OH^{-}. Subsequently, pH increased. An increase of pH also accelerated organic matter degradation and produced NH_4^{+} which forms NH_4OH. In turn, NH_4OH will dissociate into NH_4^{+} dan OH^{-}. pH of ready compost is usually closed to neutral (Isroi, 2007).

Based on Gaussian estimation, the duration of PSB availability was 7 days from the ninth to the15th day of composting with the number of 4,28.10^{7} CFU.g^{-1} of compost at the peak on 12th day. PSB was not detected on the first day (Fig. 2). Microorganisms active at the beginning of compost were usually those degrade cellulose either fungi (Sutanto, 2002) or cellulose bacteria. Population of PSB appeared with the number of 3,9.10^{6} CFU.g^{-1}. This appearance was the initial growth of PSB. According to Fardiaz (1998), cell division occurred slowly at the initial growth and increased highly at logarithmic phase, shown at the peak number. The peak number of PSB from the compost used the litters of three dominant plant from agricultural land (Sunandar, 2008) appeared at the 15th days (75.10^{1} CFU.g^{-1}) whilst at this experiment appeared at the 12th (4,28.10^{7} CFU.g^{-1}) of compost using the litters of four dominant plant species. Growth acceleration is influenced by the environment of growth media including pH, nutrient content, temperature and relative humidity (Fardiaz, 1989). Adding one more plant species (*L. hyssofolia*), higher temperature, pH, and smaller size of the plant litters accelerated PSB appearance as well as number of PSB. Reducing number of PSB after the peak number (Fig. 2) indicated phase of death.

Colony and cell morphology of PSB isolate

There were two isolates found in the compost from the plant litters of agricultural land. Both isolates formed halo on pikovskaya media. Both isolates have similar rounded shape, convex with smooth edge of colony but different color (yellow and white). The cell of white isolate was rod whilst yellow isolate was coccus. Both isolate were negative gram (Table 1). Only few colonies of yellow isolate were found in the compost compared to white isolate. Subsequently, only white isolate was used for estimation of PSB population dynamics.

Biochemical test

Identification of two PSB isolates based on the tests of motility, phosphatase, starch hydrolysis, catalase, oxidase, gelatinase, indole, oxygen need, and TSIA resulted in two different genus (Table 2). Yellow isolate fermented glucose, sucrose, and lactose whilst white isolate fermented glucose only. Referring to 13 genus of PSB in the book of *Bergey's Manual Determinative of Bacteriology*, yellow and white isolates were genus of Flavobacterium and Pseudomonas, respectively. Both Flavobacterium and Pseudomonas can be potentially used for starter in composting the plant litters from agricultural land.

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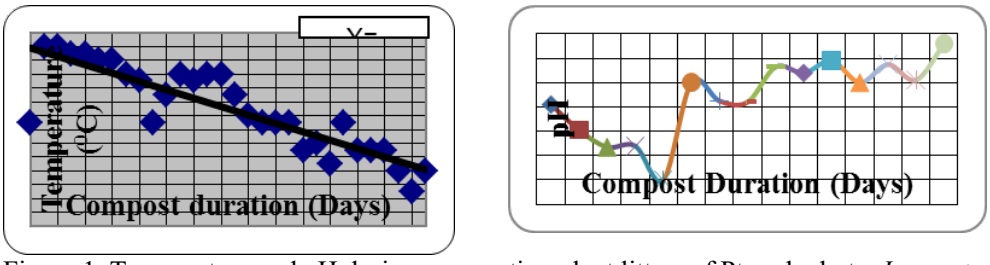

Figure 1. Temperature and pH during composting plant litters of Pterydophyta, *Imperata cylindrica*, *Cyperus* sp, *Ludwigia hyssofolia* from agricultural land

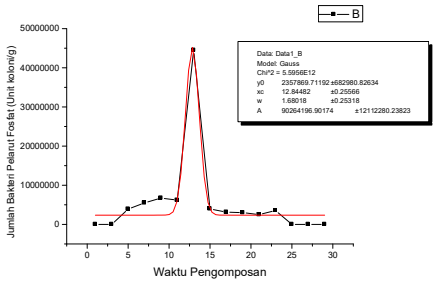

Figure 2. Population dynamics of phosphate solubilizing bacteria during composting plant litters of Pterydophyta, *Imperatacylindrica*, *Cyperus* sp, *Ludwigia hyssofolia* from agricultural land

Table 1. Colony and cell morphology of phosphate solubilizing bacteria in the compost

Morphology		**Species 1**	**Species 2**
Colony	Shape	Round	Round
	Surface	Convex	Convex
	Edge	Smooth	Smooth
	Color	White	Yellow
Cell	Gram reaction	Negative	Negative
	Shape	Rod	Coccus
	Motility	Negative	Negative

Table 2. Biochemical test to identify phosphate solubilizing bacteria in the compost

Biochemical Test	Isolate 1	Isolate 2
Phosphate Enzime	+	+
Gelatine	+	+
Starch Hydrolysis	-	-
Catalase	+	+
Oxigen	Aerobic	Aerobic
Oxidase	+	+
TSIA	Glukoce Fermentation and existing H2S	Glucose, sucrose and lactose Ferementation and Existing H2S
Indole	-	-

+ Reacted positively - Reacted negatively

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ISOLATION, IDENTIFICATION AND DEGRADATION CAPACITY TEST OF PETROLEUM DEGRADATION MICROBE FROM SEA WATER IN CELUKAN BAWANG HARBOUR, BULELENG

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ABSTRACT

Identification of microbes that have ability to degrade petroleum from sea water in Celukan Bawang Harbour, Buleleng was conducted. There were five genus of bacteria found, i.e: (1) *Bacillus* (E and G2 isolates), (2) *Pseudomonas* (D and G1 isolate), (3) *Acetobacter* (H isolate), (4) *Halomonas* (F isolate), and (5) *Neisseria* (A, B, and C2 isolate). The results show that bacteria diversity for degradation of oil in Celukan Bawang harbour is classified as middle grade. Based on data analysis it was found that $F_{calculated}$ = 84,867 > F table = 2.62. It means that there are significant effects of sterilization temperature significantly toward number of bacteria colony and n-octanoic acid concentration. The difference is due to denaturation of protein at high temperature. Based on analysis, it was found that the calculated statistic value was (21.29) > table value (11.07), therefore, H_o is rejected. This means that there is significant difference of urea addition on isolation media towards bacteria capacity to degrade petroleum.

Keywords: isolation, bacteria, degradation, diesel fuel

INTRODUCTION

Human and environment have interaction to each others. Human sometimes ignore conservation environment aspect. Human activities result in pollution which also affected the sustainability of living organisms. The highest pollution sources of aquatic environment in sea is petroleum spill especially diesel fuel. The recovery effort of the pollution can be done by physical, chemical, and biological methods. For biological method, the use of bacteria is the most efficient, effective and environmental friendly method for biodegradation of oil.

The aim of this research are to: (1) identify the genus of bacteria that are able to degrade the diesel fuel in the water sea of Celukan Bawang Harbour, (2) evaluate the effect of temperature differences of sterilization towards the number of oil degradation bacteria colony, and (3) evaluate the effect of C/N addition to degradation media towards bacteria capacity in diesel fuel degradation.

MATERIALS AND METHODS

This research is explorative and experimental research. The data collection in this research was done by two ways (1) field research, and (2) sample analysis in laboratory.
Analysis procedure sample in laboratory:
I. A. Bacteria isolation:
1) 1 ml sample sea water was pour into a petri dish in Bushnell-Haas mineral salts agar media, it was then added with diesel fuel then incubated for 1 x 24 hour
2) From growing colonies, every colony with different type was placed in petri dish until one pure culture was obtained
3) Pure culture was removed in slant agar
B. Bacteria identification: the pure culture was further identify by macroscopic, microscopic, and biochemistry methods. Macroscopic identification includes: shape and type of colony of bacteria. Microscopic identification was done by staining method including gram staining, spore staining, capsule staining, and acid fast. The biochemistry

identification conducted were fermentation glucose test, hydrolysis starch and gelatin test, motility test, catalase test, methyl red test, voges-proskauer test, and triple sugar iron agar test.

II. Sterilization temperature i.e.:

1) Sea water sample was collected using water sampler
2) Using Bushnell-Haas, mineral salts was added to the sea water sample. Four kind of medium were prepared : 10 ml sea water sample in 100 ml Bushnell-Haas mineral salts ; 30 ml sea water sample in 100 ml Bushnell-Haas mineral salts ; 60 ml sea water sample in 100 ml Bushnell-Haas mineral salts; 90 ml sea water sample in 100 ml Bushnell-Haas mineral salts. Those media were shaken every one hour and incubated for 16 hour.
3) After 16 hour of incubation, every medium was tested using microscope, to know which medium contain more bacteria. As much as 20 ml sample was taken and put in tube, cover with aluminium foil, and put in autoclave until temperature reach 100^0C. The same procedure was done with different temperature sterilization i.e 109^0C, 116^0C, 121^0C, 126^0C.
4) Isolation and growth test bacteria: a) 1 ml sample for each treatment was pour to petri dish in Bushnell-Haas mineral salts agar media, dispense 5 drop fuel diesel, then incubated. b) Examine growth bacteria colonies in 2 x 24 hour

III. Procedure for the addition of urea

Degradation capacity bacteria test with different concentration of urea: to 100 ml Bushnell-Haas mineral salts, it was added with mixed 3 ml fuel diesel and urea fertilizer in 1 ppm, 2 ppm, 3 ppm, 4 ppm, 5 ppm. Bacteria were poured in 1 ml test medium. Incubated for 14 x 24 hour and shake every 24 hour. After that, titration to measure result product degradation was conducted. Measurement of product degradation was done with standard NaOH and phenolptalein (PP).

Data analysis technique: 1) descriptive for isolation bacteria
2) Multivariate analysis for sterilization temperature
3) Varians analysis one way for urea added

RESULTS AND DISCUSSION

Based on characterization results, there are five genus of bacteria found, i.e : (1) *Bacillus* (E and G2 isolate), (2) *Pseudomonas* (D and G1 isolate), (3) *Acetobacter* (H isolate), (4) *Halomonas* (F isolate), and (5) *Neisseria* (A, B, and C2 isolate). From the research results it can be concluded that bacteria diversity for degradation of oil in Celukan Bawang harbour are classified in middle grade. Macroscopic examination found that colony shapes are irregular and spreading, round, round with raised margin, round with radiating margin, L-form, filamentous. Based on margins there are: irregular, wavy, lobate, smooth margin of colonies. Based on elevations, the colonies were flat, convex, drop-like. Based on the colour, it was found that all colonies are white. Based on Gram staining it was found that the bacteria are gram negative bacteria and only two isolate are gram positive bacteria. Environment pH: 6.4 – 7.1. In acid fast staining, all bacteria are not acid (negative). In capsule staining some isolate have capsule, and some do not have capsule. In spore staining, few of them have endospore, and some of them as vegetative cell. Based on biochemistry test, for glucose fermentation test: all bacteria show positive with glucose, and for maltose and sucrose test only D isolate cannot ferment. For lactose test: D, E, H isolates can fermentation lactose. For hydrolysis starch test: D, F, G isolates cannot hydrolyse starch and for gelatin hydrolysis test: D, F, G isolate can hydrolysis gelatine. For motility test and catalase test, all isolates showed positive results. This shows that all isolates can move and produce catalase enzyme. For methyl red test: Voges Proskauer, and H_2S, some isolates showed positive and some negative results.

Based on data analysis it was found that $F_{calculated}$ = 84.867 > F table = 2.62. It means that there are significant effect of sterilization temperature toward the number of bacteria colony and n-octanoic acid concentration. The difference was due to the denaturation of protein at high temperature. Physical treatment of colonies at different temperatures affect degree of diesel fuel degradation. The degree of diesel fuel degradation decreased if temperature sterilization was high. The number of bacteria colonies started to decrease from control, 100^0, 109^0C, 116^0C, 121^0C, 126^0C. The higher the temperature, the less colonies bacteria grow.

Based on analysis it was found that calculated statistic value was (21.29) > table value (11.07), therefore H_o is rejected. This means that there is significant difference of urea addition on isolation media towards bacteria capacity to degrade petroleum. This study also shown that the treatment group produced the highest octanoic acid i.e 13.90 mg at added 2 ppm urea. Less octanoic acid amount gradually from 1 ppm, 3 ppm, 4 ppm, and 5 ppm. The concentration of urea added vary from 1 ppm, 2 ppm, 3 ppm, 4 ppm. Octanoic acid amount increase from the addition of urea in concentration of 1 ppm until 2 ppm, after that the octanoic acid levels decreased.

There are five genus of bacteria found in this study, i.e: (1) *Bacillus* (E and G2 isolate), (2) *Pseudomonas* (D and G1 isolates), (3) *Acetobacter* (H isolate), (4) *Halomonas* (F isolate), and (5) *Neisseria* (A, B, and C2 isolate). From the research it can be concluded that bacteria diversity for the degradation of oil in Celukan Bawang harbour is classified in middle grade.

Based on data analysis was found that $F_{calculated}$ = 84.867 > F table = 2.62. It means that there are significant effect of sterilization temperature significantly toward number of bacteria colony and n-octanoic acid concentration. The difference due to denaturation of protein at high temperature.

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MITOCHONDRIAL DNA CYTOCHROME OXYDASE II (COII) SEQUENCES ANALYSIS OF BALI STARLING IN WEST BALI AND NUSA PENIDA CAPTIVITY

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ABSTRACT

Molecular technique can be applied in learning the population structure and to plan the effective conservation strategy for birds, especially for endangered birds such as *Bali Starlings* (*Leucopsar rothschildi*). This research is aimed to understand the population genetic structure, relationship and genetic diversity of Bali Starlings in captivity which executes the program of releasing them into the wild. This study used 8 growing feather samples from birds of different individuals collected from West Bali National Park (TNBB) Captivity and from Begawan Giri Foundation Nusa Penida, each birds belong to different captive maternal origins. Mitochondrial DNA (mtDNA) was isolated by DNA Isolation Kit (Invitrogen) followed by Polymerase Chain Reaction (PCR) technique. DNA sequence analysis of the results is done using the MEGA4 program. The results of showed three genetic variations in Bali Starling samples which were investigated based on nucleotide sequence differences. Genetic distance average was 0,2%. Data in this study showed that genetic diversity is very low in the sample Bali starlings from TNBB captivity and from Begawan Giri Foundation. In this research also found three different haplotipe from two registered haplotipe at the Gene Bank.

Keywords: *Leucopsar rothschildi, PCR*, *mitochondrial DNA,* , *cytochrome oxydase* II, h*aplotype*

INTRODUCTION

The Bali starling (*Leucopsar Rothschild)* represents a monotypic genus, which is endemic to the island of Bali Indonesia. Habitat destruction and capturing for the pet trade brought the species to the verge of extinction (Balen *et al*, 2000). On account of its restricted range, it's extremely small numbers in the wild and pressures on the last free ranging birds, the Bali starling is considered critically endangered according to the newest International Union for Conservation of Nature (IUCN) threat categories (Collar *et al*., 1994).

Mitochondrial DNA (mtDNA) analysis has been employed in the evolutionary study of animal species for more than 30 years (Avise and Walker, 1999). Analysis of mitochondrial DNA (mtDNA) sequences was successfully applied for investigation of genetic divergence in animal populations and the taxa of different ranks. Among the unique features of mtDNA, differing it from nuclear DNA, the most important for phylogenetic reconstructions are high evolutionary rate, maternal inheritance, the absence of recombination, and different evolutionary rates of the genes belonging to mitochondrial genome (Kozyrenko *et al.*, 2009) Cytochrome oxydase II (COII) is one of the 13 protein-coding genes in mtDNA which is involved in electron transport and oxidative phosphorylation of mitochondria. This area can also be used as molecular markers for rapid and accurate identification of species of animals and to discover new species of tree origin (Hebert et al., 2003). Mitochondrial COII genes used to generate a phylogenetic hypothesis for the Palearctic–Oriental starlings and mynas (genera *Sturnus*,

Acridotheres, *Leucopsar*, *Creatophora* and *Fregilupus*: Sturnidae). The result indicate that the group has undergone a rapid diversification in Asia since the late Miocene (Zuccon *et al*., 2008).

Maintaining genetic diversity is a major issue in conservation biology. Despite the rapid increase in numbers of birds under intensive management, the paucity of genetic diversity remains a real threat to the species. In this study, we demonstrate the study of the genetic diversity of Bali starling by analyzing sequence variation of mtDNA COII of captive populations in West Bali National Park captivity.

MATERIALS AND METHODS

A total of 8 growing feather samples were collected from 6 Bali starling birds from West Bali National Park and 2 from Begawan Giri Foundation Nusa Penida captivity. With clean forceps, one growing feather is plucked with a single motion from the wing or from the tail of bird. A 0.5–1 cm section was cut from the terminal portion of the feather quill and placed in a 1.5 ml sterile Eppendorf tube with transport medium containing NaCl in dimethyl sulfoxide (DMSO). To avoid possible contamination, the feathers are plucked only with forceps, and the base must not be touched. Samples can be stored for several months at room temperature (or more preferably at 4^0C) before DNA extraction.

The genomic DNA were isolated and suspended using a PureLynk™ Genomic DNA purification kit (Invitrogen®) and stored at -20ºC. Successful DNA isolation was confirmed by running 4 uL of genomic DNA in a 1% Agarose gel and fragment of the mtDNA was amplified by polymerase chain reaction (PCR) with foreward primers CO2-ExtF (5′-CAGGTGAAACCCCAGTACACCTC-3′) and reverse primers CO2-ExtR (5′-AGGCTAGCGCTGTTCCATAGCTTC-3′) which have length 750 bp (Zuccon *et al.*, 2008).

PCR conditions for a 10µl product using 1µl of 10ng/µl genomic DNA preparation were as follow: 1X PCR Buffer, 0.2 mM dNTP's, 0.5 µM of each primer and 0.05 Units/ul of *Taq* polymerase. After initial 5 minute denaturation step (94ºC), our PCR protocol consisted of 30 cycles of the following temperature regime: 30 seconds at 95ºC (denaturation), 40 seconds at 56ºC (annealing), and 40 seconds at 72ºC (extension). In addition, included a final extension step of 5 minutes at 72ºC was included. Along with the samples, negative (without DNA) controls were included in all PCR reactions. The amplified fragments and the negative controls were run in 1% agarose gels to confirm successful amplification and discard contamination of the reaction. Both forward and reverse strands were sequenced using a BigDye Terminator v.3.1 (Bioanalytical Instruments) and analyzed with an automated DNA sequencer (ABI 3130XL Applied Biosystem) at the Eijkman Institute, Jakarta. Software MEGA4 (Tamura *et al*., 2007) was used to generate a consensus alignment of the forward and reverse strands and the trimmed sequences were aligned by eye. Sequence parameters analyzed include polimorfic site, genetic distance and phylogenetic tree. Genetic distances calculated with Kimura 2 parameters method (Kimura, 1980). Based on the phylogenetic tree can be known to the genetic distance between species, further to note the pattern of evolution of living thing (Nei dan Kumar, 2000). Results of mtDNA COII sequences of each haplotype were compared with two reference *L. rothschildi* mtDNA COII sequences from Gene Bank (Gene Bank accession number EU552000 and EU552001).

RESULT AND DISCUSSION

The sequences result of the 8 samples Bali starlings that can be read well is 692 bp. For comparison purposes the long sequences used were adjusted to 684 standard fragments of mtDNA Bali starling that have been published by Zuccon et al., (2008). Results of analysis of mtDNA COII gene sequences of the 8 samples of Bali starling produced three polymorphic site (Table 1) and three different haplotype with the two haplotype that have been identified previously by Zuccon et al., (2008). The first haplotype is TNBB1, TNBB2, TNBB5, and BBG17. This showed that the four Bali starling were from the same female offspring because mtDNA is maternally inherited. Second haplotype were TNBB13 and TNBB8. The third haplotype were BBG11. While haplotype from sample number TNBB15 could not be determined because the two nucleotides in the position of the 8th and the 64th were not readable. The possibility of this sample following the second and third haplotype. The difference this haplotype showed that the studied samples Bali starlings have genetic variations, and comes from a different female parent with Bali starling haplotype that have been studied by Zuccon et al., (2008).

Table 1. Polimorphic site of eight Bali starling mtDNA COII gene sequences and from Gene Bank

Sample	Nucleotide position			
	8	64	436	459
TNBB1	A	C	A	C
TNBB2	•	•	•	•
TNBB5	•	•	•	•
TNBB8	•	A	G	•
TNBB13	□	A	G	•
TNBB15	□	□	G	•
BBG11	□	•	G	•
BBG17	•	•	•	•
L._rothschildi_CO2_amnh	C	•	G	T
L._rothschildi_CO2_uwbm	C	A	G	•

(-) : indicates the same base pair. (•):indicates insertion/deletion.

Analysis using MEGA4 of eight samples with the two from the Gene Bank produces an average genetic distance of 0.2%. Bali starling molecular relationship with other starlings that are found in the research and from Gene Bank displayed with phylogenetic tree (Figure 1).

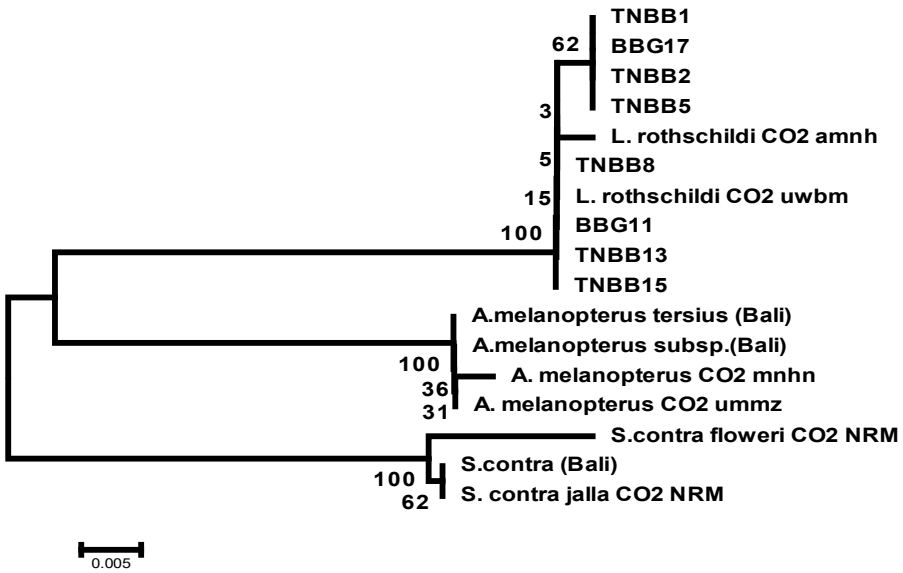

Figure 1. Neighbour joining tree of Bali starling in the study by bootstrap analysis and Kimura2-parameter method.

Branch phylogeny of Bali starlings clade indicate bootstrap probability sample (500 replications) with a high value (100%), as well as samples of *A. melanopterus* and *S. contra* (100%). This shows that all samples Bali starlings in this study are in a monophyletic group with Bali starling from Gene Bank. Phylogenetic tree analysis in this study also showed that 4 samples of Bali starlings that have a different nucleotide sequence that is TNBB1, TNBB2, TNBB5 and BBG17 are in one group and form a new sub-clade.

Further research is needed to determine the haplotype diversity of Bali starlings and to know the genetic differences between haplotype in both captivity.

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GENETIC RELATIONSHIP BETWEEN GEMBRONG GOAT, *KACANG* GOAT AND *KACANG* x ETAWAH CROSSBRED BASED ON THEIR MITOCHONDRIAL DNA

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ABSTRACT

Gembrong goat is a specific type of goat which has long hair covering its whole body including its neck and face, originated from eastern part of Bali (Karangasem). A study of this type of goat and its relationship with other local goats (Kacang and Kacang x Etawah crossbred) was carried out at Sawe village, Jembrana, Bali.. A number of 12 gembrong goats, 3 kacang goats from Kubu village, Karangasem and 3 Kacang x Etawah crossbred goats from Denpasar were used in this study. Blood samples of all goats were collected for mitochondrial DNA (mtDNA) analysis. PCR amplification of D-loop mtDNA was carried out by using CAP-F primer (5'-CGTGTATGCAAGTACATTAC -3') and CAP-R primer (5'-CTGATTAGTCATTAGTCCATC-3'). Sequencing of 550 bp (base-pairs) of mitochondrial DNA (product of PCR) only found one polymorphic site at base number 231 with two haplotypes in gembrong goat only while the other base-pairs were similar between the three goat types (Gembrong, Kacang and Kacang x Etawah crossbred) .The frequency of haplotype 1 was 83.3% and the frequency of haplotype 2 was 16.7%. It was concluded that based on their mitochondrial DNA sequences and "phylogenic analysis", the three types of goat (Gembrong,Kacang and Kacang xEtawah crossbred) had a very close genetic relationship (kinship).

Keywords: Gembrong goat, mitochondrial DNA, genetic relationship

INTRODUCTION

Gembrong goats were reported as a specific type of goat different from the indigenous breed (*Kacang* goats) and their Etawah-crossbreds (PE), which are found in Karangasem regency, eastern part of Bali Island (Matram, *et al*., 1993). This type of goat is called *Gembrong* goats because of their long hair covering their body, neck and up to their face particularly in buck (male goat). The word *gembrong* come from Balinese word which means long hair. Unfortunately, the population of this type of goats is decreasing from year to year because of the extensive management. With the extensive farming, *Gembrong* goats were kept in the field together with the other local goats. In such condition these type of goats were reported by farmers as the easiest breed to be killed by wild dogs. It is difficult to find gembrong goats nowadays in this region. In order to maintain their genetic resources, *Balai Pengkajian Teknologi Pertanian (BPTP)* of Bali tried to breed them intensively in a particular area in Sawe village, Jembrana. There are some other breeds of goat that have long hair such as Angora goats originated in central China and develop extensively in Turkey and South Africa, and Kashmiri originated in India and also found in Tibet (Devendra and McLeroy, 1982). Angora breed has long ringlets or curly hair while the Kashmiri has fine silky undercoat hair called pashmina. Australian researchers have started a selection program on their feral goats for cashmere (fine silky hair) production since 1979 (Restall and Pattie, 1991). Therefore, *Gembrong* goats seemed to have a good commercial future.

Genotyping is becoming widely practiced in animal breeding with applications using both genetic marker loci and known gene loci (Kinghorn, 2002). Puja and Sulabda (2009) studied DNA genome from hair of *Gembrong* goats. It was reported that 9 out of 10 markers used in the study were successfully amplified and the number of alleles ranged from 1 to 4 per locus of micro-satellite in *Gembrong* goat. Since very little

information could be retrieved about *Gembrong* goat, research team of Faculty of Animal Science in collaboration with *BPTP* Bali carried out a study on *Gembrong* goats including; relationship between *Gembrong* goat and local goats in Bali (*Kacang* goat and their Etawah crossbreds/PE) and their other performances such as hematology, length of their hair, body weights, body dimensions, and some reproductive aspects. However, because of lack of animals (*Gembrong* goats) number, this paper was limited to the relationship between *Gembrong* goats and other local goats (*Kacang* and PE goats) based on their mitochondrial DNA sequences and "phylogenic analysis" of their haplotypes.

MATERIALS AND METHODS

This study was carried out at *Balai Penerapan Teknologi Pertanian* (BPTP) farm in Sawe village, Jembrana, Bali since May until October 2009. A total of 18 goats consist of 12 *Gembrong* goats (7 males and 5 females) belonging to BPTP, while 3 *Kacang* goats belonging to a farmer in Kubu village, Karangasem (East of Bali) and 3 *Kacang* x Etawah crossbreds (PE) belonging to a goat collector (merchant) in Denpasar, were used in this study. Samples of blood were collected from each goat for analysis of their DNA. Amplification of mitochondria DNA (mtDNA) was carried out using Polymerase Change Reaction (PCR) technique with two primers i.e. CAP-F primer (5'-CGTGTATGCAAGTACATTAC base -3') and CAP-R primer (5'-CTGATTAGTCATTAGTCCATC-3') at the Medical and Molecular biology Laboratory, Faculty of Veterinary Science, Udayana University. DNA sequence was aligned using Clustal W in MEGA 3.1 program (Kumar *et al*., 2004). This program also calculated the frequency of haplotypes and "phylogenic tree" of the haplotypes. Haplotype means genetic variations for each individual within a species based on their nucleotide sequence. Variation of each sequence was determined by DNAsp 4.10 (Rozas, *et al*., 2003).

RESULTS AND DISCUSSION

Twelve fragments of *Gembrong* goat mtDNA with 550 bp length were successfully amplified using the PCR technique. Figure 1 shows the amplified mitochondria DNA of *Gembrong* goats. Analysis of mtDNA sequence of the 12 *Gembrong* goats, 3 kacang goats and 3 kacang x etawah crossbreds using DNAsp 4.10 found one polymorphic site at base number 231 with two haplotypes (Figure 2). Relationship between *Gembrong* goat, Kacang goat and PE (Kacang x Etawah crossbred) is shown in "phylogenic tree" (Figure 3). It appeared that *Gembrong* goat had a close relationship with *Kacang* and PE goats. Two haplotypes found in Gembrong goat where haplotype 1 had a close relationship with Capra hircus SN31 from China while haplotype 2 had a close relationship with Capra hircus HV1 from east Asia. Frequency of haplotype 1 was found 83.3 % (10 out of 12) and haplotype 2 was 16.7 % (2 out of 12).

Genetic variation of *Gembrong* goats in this study was very low. This low variation might be because of the small number of goats were found in this area and inbreeding probably the main factor. It is important to develop this goat breed urgently otherwise it will be extinct from Bali. *Gembrong* goat should be conserved in Bali because this breed of goat is the only specific breed which is found in Indonesia. As a specific local indigenous species, its genetic resources should be conserved and developed them as pure breed animal in Bali. According to Wibowo (2001), the genetic variations in a particular population may be resulted because of several factors such as natural selection, environmental factor, mutation or breeding. The long hair of *Gembrong* goats might be as a result of mutation of gene which is responsible for the hair length of the local (indigenous) goats in Bali. However, more intensive studies are needed in order to get an accurate conclusion.

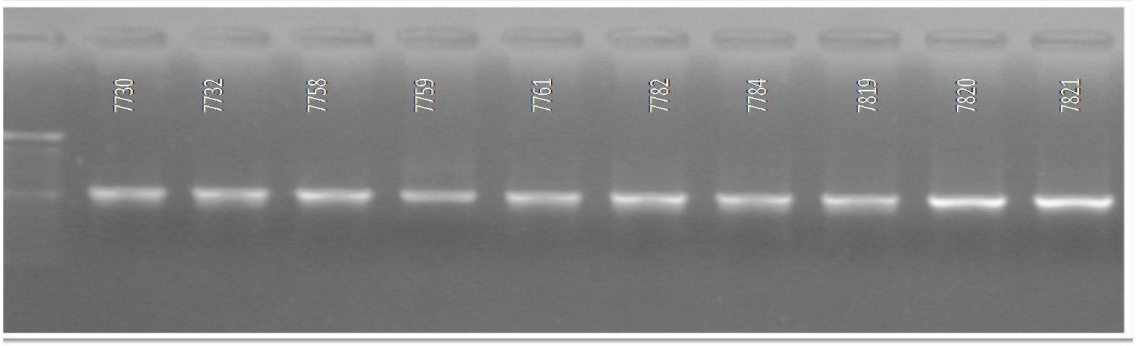

Figure 1. Amplified fragments of gembrong goat mtDNA.

```
#GJ1 ATA TAG TAC ATT AAA CGA TTT TCC ACA TGC ATA TTA AGC ACG TAT ATC
[240]
#GJ2 ... ... ... ... ... ... ... ... ... ... ... ... ..T ... ... ...
[240]
#GJ3 ... ... ... ... ... ... ... ... ... ... ... ... ..T ... ... ...
[240]
#GJ4 ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...
[240]
#GJ5 ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...
[240]
#GJ6 ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...
[240]
#GJ7 ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...
[240]
#GB1 ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...
[240]
#GB2 ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...
[240]
#GB4 ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...
[240]
#GB5 ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...
[240]
#KC1 ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...
[240]
#KC2 ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...
[240]
#KC3 ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...
[240]
#PE1 ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...
[240]
#PE2 ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...
[240]
#PE3 ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...
[240]
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Figure 2. Sequences of mitochondria DNA Gembrong goats (GJ and GB), Kacang goats (KC) and Kacang x Etawah crossbreds (PE)

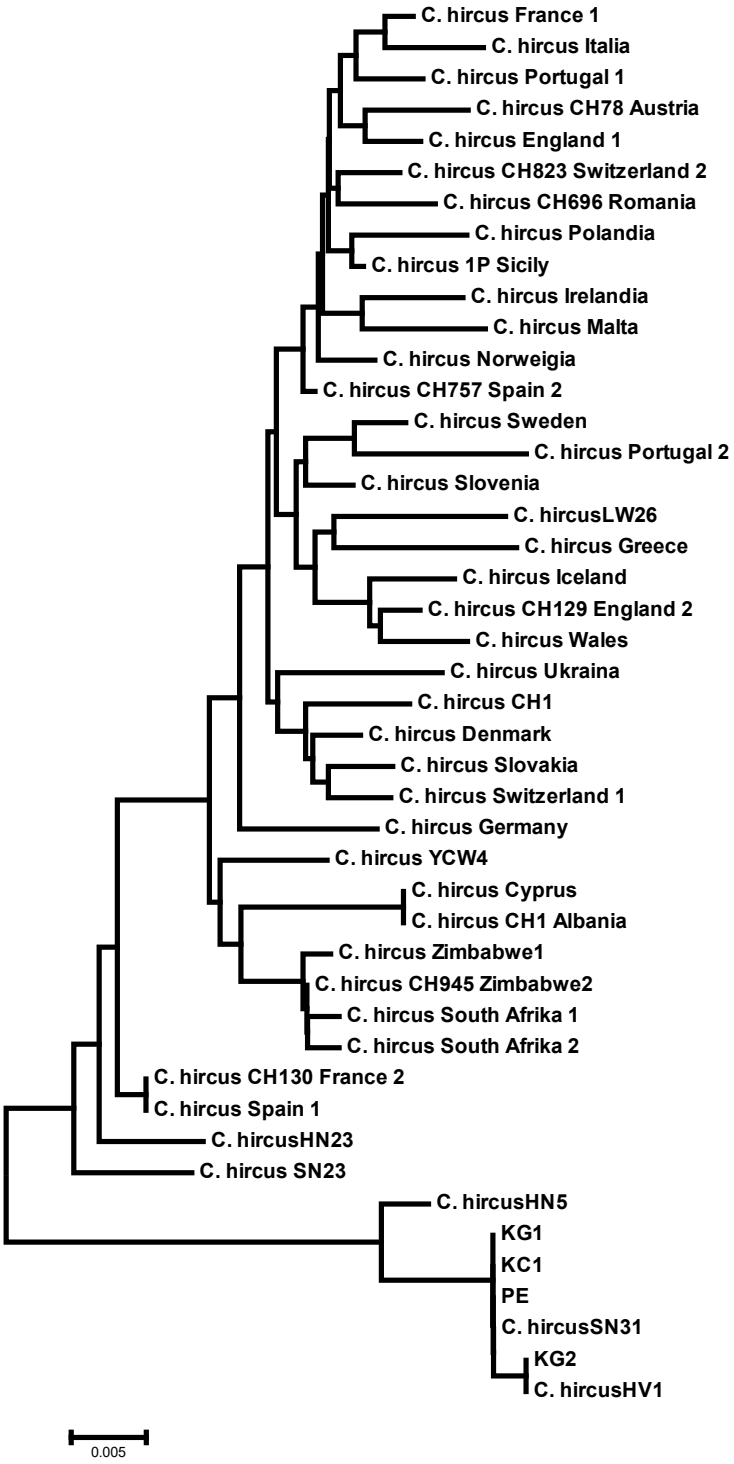

Figure3. Phylogenic tree of *Gembrong* goat (KG), *Kacang* goat (KC), *Kacang x Etawah* crossbred (PE) and several goats in the world.

CONCLUSIONS

Based on the mitochondrial DNA analysis of the three types of goat (*Gembrong, Kacang* and *Kacang x Etawah* crossbred) and analysis of their phylogenic tree, it could be concluded that *Gembrong* goat had a close genetic relationship (kinship) with the indigenous breed (*Kacang*) and *Kacang x Etawah* crossbred (PE) in Bali.

ACKNOWLEDGEMENTS

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THE POTENCY OF WEST NUSA TENGGARA SEAWEEDS AS BIOFERTILIZERS

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ABSTRACT

Concern on the impact of excessive and unwise application of synthetic fertilizers and growth hormones in agricultural system to cropping production cost, soil quality and environment has encouraged an increase usage of organic fertilizers and growth promoting substances in commercial agricultural management. Of those, many reports suggested that application of seaweed extract could promote growth and development of many plant species, reduce the dosage of synthetic fertilizer required in the cropping system (Hong *et al*., 2007, Thangaraju, 2008), as well as improve some plant response to drought, pest and diseases (Patier *et al*., 1993, Laporte, 2007). The province of West Nusa Tenggara has been suggested to be species-rich habitats for marine flora and fauna, including seaweed. At least 88 species have been identified in the regions (Sunarpi *et al*., 2005; 2006), few species commercially cultivated as hydrocolloid sources while the function of most of them remain unclear. We screened the potency of West Nusa Tenggara seaweeds as biofertilizer and identified that water-soluble extract of 15 species could stimulate germination and growth of many plant species (Sunarpi *et al*., 2007, 2008). Further analysis indicated that liquid extracts obtained from five of the 15 species identified earlier could also enhanced production of many horticultural, legume and other plant species (Sunarpi *et al*., 2009). These results suggest that many species of seaweed obtained from West Nusa Tenggara regions could be used as a biofertilizer and growth promoting substances. Despite plant growth and physiological analyses, biochemical examination is required to identify bioactive compounds which stimulate the growth responses.

Keywords: Seaweed, macro-algae, bio-fertilizer, growth promoting substances

INTRODUCTION

In recent years, there is an increase demand for an organic fertilizers in Indonesia, for example demand for NPK fertilizer increased from 96.116 tons in 2006 to 739.271 tons in 2007 (Anonim 2008). This increase was partly due to an increase in dosage used per areal. In fact, farmers use about300 – 350 kg/ha in rice paddy cultivation and use about 200-250 kg/ha for vegetable cultivation. This condition has not only increased the cropping production cost, but also decreased soil quality and caused pollution to environment. Ironically, increase in fertilizer price followed by economic loss caused by those excessive usages of inorganic fertilizers are not accompanied by an increase profit obtained by the farmers.

In order to reduce production cost, The Government of Indonesia has increased the subsidy to fertilizer from 1.5 billion rupiahs in 2006 to 1.5 trillion in 2007 (NN, 2008). This policy does not seem able to solve agricultural production problem, this policy even make fertilizer hardly available when needed by farmer as speculator tend to sell the subsidized-fertilizer overseas to get more profit. Therefore, another alternative is urgently needed to solve this problem and this could involve the use of natural bioactive stimulant which capable to increase capability of plant to absorb nutrient efficiently in order to decrease excessive usage of fertilizer.

Previous findings indicated that several fertilizer from seaweeds have been developed and used in many countries. These fertilizers were able to improve plant capability to absorb nutrients which thus promote plant growth and development and decrease the need for in organic fertilizers (Hong *et al*., 2007, Thangaraju, 2008), as well as improve some plant response to drought, pest and diseases (Patier *et al*., 1993, Laporte, 2007).

We have previously reported that there are at least 88 seaweed species identified in West Nusa Tenggara Sea and coast (Sunarpi et al., 2005, 2006). Of those, 26 species contain hydrocolloid while functions of most of them remain unclear. This paper will, therefore, report our investigation in screening West Nusa Tenggara seaweed as biofertilizers.

MATERIALS AND METHODS

Preparation of growth medium and planting. Each experiment was undertaken in plastic houses. Plants were grown in 10-L capacity pot, supplemented with 8 L medium (soil, sand, manure; 1:1:1 (v/v)), inorganic fertilizers added, hand watered daily and maintained for two weeks before being treated with seaweed extracts.

Samples collection. Seaweed samples used in this report were collected from coastal areas of West Nusa Tenggara. Most samples were found to inhabit and collected from Western and Southern coast of Lombok. Samples were collected at the same day, cleaned with running fresh-water, weighted and stored in -80°C until required.

Seaweed extraction. Samples were extracted by adding extraction buffer (1:1, w/v), as appropriate, and grinding onto find slurry, The slurry was filtered and transferred into chilled tubes, centrifuged at the top speed for 10 mins. Supernatant (designated as a 100% extract) was transferred into falcon tubes, diluted as required then freshly used for the experiment.

Germination experiment. Germination experiments were undertaken for 10 days in the Laboratory. Seeds were incubated in 10% of representative seaweed extracts for 12 hours with shaking then 100 seeds placed in a germination plate (10 x 12 cm^2) overlaid with several layers of aquadest-wetted cotton and placed in the dark. Measurement of germination rate and primary root length were undertaken daily.

Plastic-house experiment. Several experiments were undertaken in the plastic houses in Jatisela, Mataram. Each plastic house was design with dimensions of 4 m wide, 7 m length and 3 m height and top-covered with a layer of clear plastic (5 mm of thickness) while allowing for appropriate air circulation inside the house. Each set of experiments was designed by completely-randomized design (CRD) at which either one or more factors were examined, as appropriate and made in triplicate.

Treatment with seaweed extracts. Freshly prepared extract was diluted with aquadesh as appropriate, tween-20 (0.01%, v/v) added and mixed before being sprayed throughout the plants in the morning. Unless otherwise stated, treatment was started from two weeks after replanting to pot, in weekly interval, and treatment stop one week before termination of experiment. In regard to different growth stages application, treatment was started according to growing stages of the plant assessed. For vegetative stage, plant was sprayed from two-weeks old until plant started to flower while for generative-stage treatment, application of seaweed extract was started from the time of flowering and continued until growth ceased.

Variables assessed. Variables measured in each experiment including plant height, leaf number, leaf area, time required for flowering, no of flower, no of fruit, total weight of fruits and dry biomass.

Graph and Statistical analysis. All graphs presented were constructed using Microsoft Excel software and data analyzed for variance differences.

RESULTS AND DISCUSSIONS

a. Screening potential seaweed species for biofertilizer

Initial screening was started by identification of seaweed species found in West Nusa Tenggara sea and coast followed by morphological characterization. This then identified 26 species as source of hydrocolloid (including the known-commercially cultivated species) such as carrageen, agar and alginate (Sunarpi et al., 2005, 2006). The rest, mostly green and brown seaweeds, was screened for theirs potency as fertilizer. Supernatant of water extract from each species was used in imbibitions media for germination or sprayed frequently onto several plant species. Of those, ten species capable to promote seed germination, plant growth and/or increase yield were selected for further characterisation (Table 1, Figure 1).

Table 1. Potential seaweed species for fertilizer

No	Class	Species	Group
1.	Chlorophyta	*Ulva ferticulata*	Green algae
2.	Chlorophyta	*Ulva fasciata*	Green algae
3.	Phaeophyta	*Turbinaria murayana*	Brown algae
4.	Phaeophyta	*Turbinaria ornate*	Brown algae
5.	Phaeophyta	*Sargassum aquifolium*	Brown algae
6.	Phaeophyta	*Sargassum sp1*	Brown algae
7.	Phaeophyta	*Sargassum sp2*	Brown algae
8.	Phaeophyta	*Padina sp.*	Brown algae
9.	Phaeophyta	*Hydroclthrus clantartus*	Brown algae
10.	Phaeophyta	*Hormopisa sp.*	Brown algae

Figure 1. The potential ten seaweed species for fertilizers

b. Screening for extraction buffer and concentration

Initially, water soluble extract was used for the screening. However, many substances including plant hormones are insoluble in water and therefore will not be available in the water extract. Therefore, several extraction methods were tested, then capability of diluted fractions as stimulator of seed germination, plant growth and yield was tested on many plant species. Generally, additional of water and HBS extracts promoted seed germination and/or stimulated growth and yield of many plant species. Examples of those are shown in Figures 2 and Figure 3.

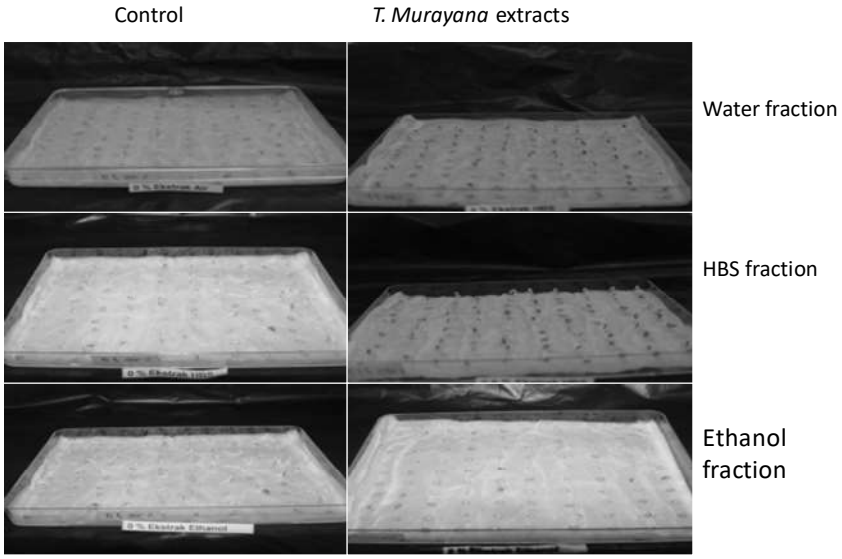

Figure 2. Germination of sesame seeds following imbibitions in water (left panels) and different fractions of *Turbinaria muriana* extracts (right panels).

As indicated in the Figure 2, water and HBS extract ,but not ethanol extract, of *Turbinaria muriyana* were able to promote sesame seed germination by shorten the time required for seed germination, thus seedlings attained longer primary roots compared to control (Sunarpi et al., 2006). The ethanol fraction, however, did not promote and even decreased time and germination rate of seeds tested (data not shown). In this experiment, only one concentration (5 %) was tested and the promoting or inhibition effect examined may be concentration dependency. Therefore, ranges concentration of seaweed extracts were tested on seed germination, plant growth and yields, and again ethanol extract was unable to promote seed germination and plant growth (Figure 3) while the promoting effect of the two other extracts was concentration dependency.

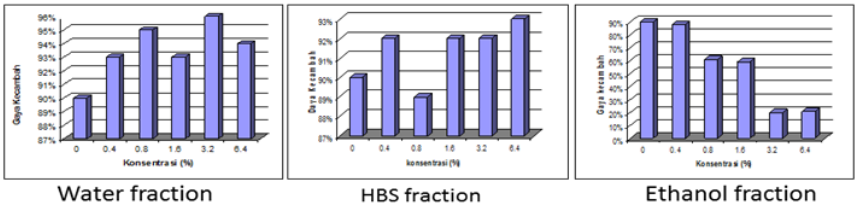

Figure 3. Germination rate of bean seeds in different concentration of water fraction, HBS fraction and Ethanol fraction of *Sargassum aquifolium.*

These results indicated that water and HBS extracts may contain growth promoting substances while ethanol may contain growth inhibition substances. As there were similar patterns of stimulation indicated by both water and HBS extracts, further characterization attempts were undertaken using water as the solvent. Water is easily available thus will be more convenient for direct application, in particular, by farmers. Further characterization indicated that growth promoting effect of water fraction from the ten seaweed extracts were not only concentration but also species dependant (example in Figure 4). Most of the species tested increased growth and yields when sprayed with seaweed extract with concentration at or below 6%.

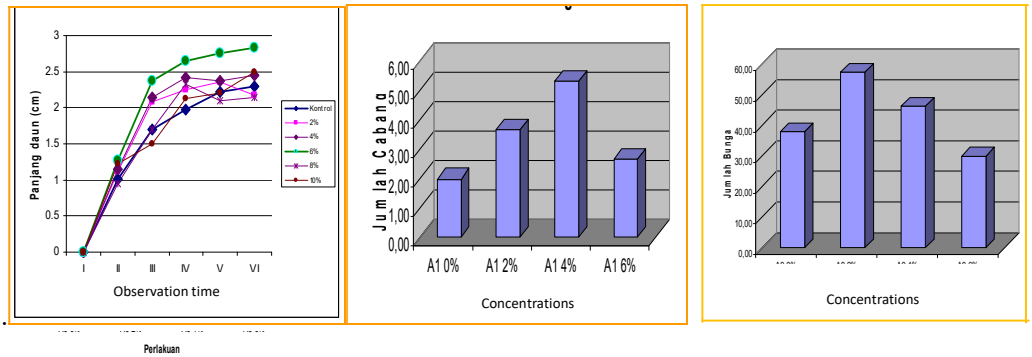

Figure 4. Growth and yield of spinach and tomato sprayed with different concentration water extract of seaweed. Growth of spinach spread with *Hydrochlarus sp.* (left), tomato spread with *Turbinaria murayana* (middle) and yield of tomato spread with *Ulva ferticulata.*

c. Application time

Previous results indicated selected promoting capability of each species which was concentration and developmental stages dependency. To confirm this, other sets of experiment were undertaken. For this experiment, one concentration of extract was used (4 %) and selected as previous experiment show this was the best concentration for promoting growth and yield. In the experiment, extracts were sprayed at three different stages of plant development: vegetative stage only, both vegetative and generative stages or at generative stage only. In this paper, an example with tomato is shown (Figure 5).

As indicated in Figure 5, application of any extract from the ten species during vegetative stage only did not significantly altered tomato growth. Significant increase in tomato growth was observed when extract from either *Sargassum sp.1, Sargassum sp. 2, Ulva festiculata* or *Hormopisa* was applied during both vegetative and generative stages. Interestingly, when applied during generative stage only, extract *Sargassum sp.1* or *Sargassum sp. 2* could also increase the number of flower in common with *Turbinaria murayana*. Similar results were also observed with other plant species including red paper, rice and sticky rice (data not shown).

Overall, the results indicated promoting effect of the ten seaweed extracts on seed germination, plant growth, development and yields. This promoting capacity is probably due to growth promoting substance contained in the extracts. Selective capacity of each species to promote certain stages of plant development in selective species indicates differential properties of the species. Many reports suggest that many species of red and brown seaweeds particularly utilize many plant growth regulator such as auxin, sitokinin and giberellin (Tay et al., 1986; Thangaraju, 2008; Prasad et al., 2010). In addition, several red and brown seaweeds also contained micro nutrients, oligoshaccarides or enzyme D-glycananes which also promoted growth of many plant species (Petit et. al., 2005; Laporte et al., 2007; Prasad et al., 2010). In these report Brown seaweeds studied were included *Kappaphycus alvarezii, Sargassum wightii Grev., Ascophyllum nodosum, Ascophyllum nodosum.* In our system, not only Brown seaweeds but also two green seaweeds observed indicating the growth promoting properties. However, it is still unclear why and how extracts from those species could selectively promote germination, plant growth and development. Biochemical analysis is underway to analyse bioactive compounds contained by each species. Various formulas are being investigated in different cultivating system including hydroponics. This will allow for formulation of seaweed extracts specifically design for different crop species.

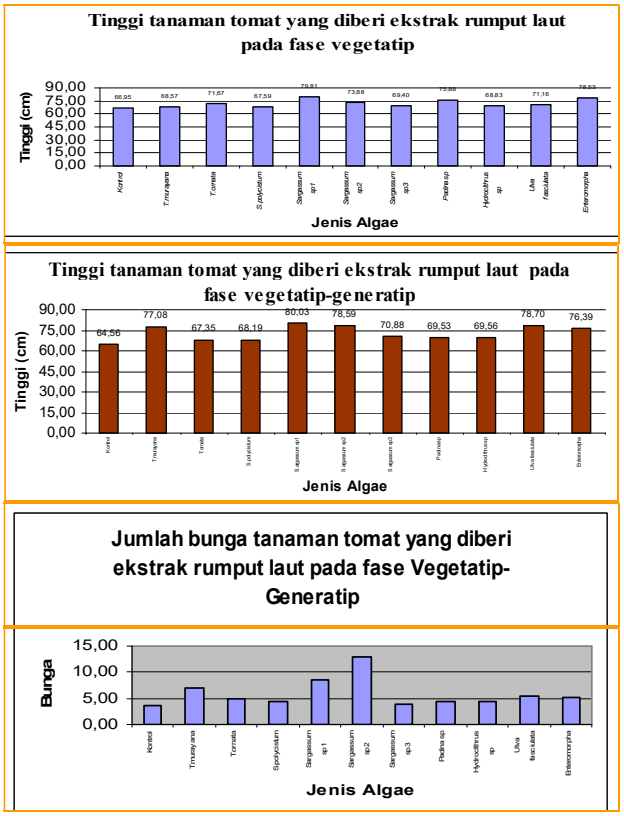

Figure 5. Effect of different seaweed species and application time on growth and yield of tomato. Species effect to plant height when sprayed during vegetative stage only (top), species effect to plant height when sprayed during both vegetative and generative stages (middle) and species effect when sprayed during generative stages only.

CONCLUSION

Potential seaweed species from West Nusa Tenggara to be developed as biofertilizer mostly fall in the Brown seaweed class (*Phaeophyta*). Different extract of seaweed species selectively promoted different stages of plant growth and development and this promotion was concentration and plant species dependent.

ACKNOWLEDGMENTS

The project is supported by research grants from West Nusa Tenggara Authority, DP2M Dikti and University of Mataram.

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SELECTION OF PANCREATIC LIKE AMYLASE PRODUCING LACTIC ACID BACTERIA AND PARTIAL CHARACTERIZATION OF THE ENZYME

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α-amylase is one of important enzymes in biotechnology. Some Lactic Acid Bacteria (LAB) have been reported to produce α-amylase and have been Generally Recognized as Safe (GRAS) microorganisms. The aim of this experiment was to select LAB of our Laboratory collection that was able to produce α-amylase and to characterize some of the enzyme properties. There were 14 out of 40 isolates tested that had capability to produce α-amylase.Among 14 isolates. It had been selected 3 isolates that had relatively high amylolytic activity namely Pediococcus pentosoceus IF0 12230, Isolat Db-3 018 and Lactobacillus delbrueckii JCM 1012 with amylolytic activity of 0.5579 U/mL minute, 0,56U/mL minute and 0,43 U/mL. The optimum pH of the enzyme produced by all selected isolates was at pH 6.0 whereas the the optimum temperature of the enzyme of Pediococcus pentosaceus IFO 12230, and Lactobacillus delbrueckii JCM 1012 was observed at 37oC whilst optimum temperature of the isolate Db-3 enzyme was at 36oC.

Keywords: Amylase, lactic acid bacteria

ORAL PRESENTATIONS:

HEALTH

ROLE OUTER MEMBRANE PROTEIN 53 kDa *Salmonella typhi* JEMBER ISOLATED AS PROTEIN HEMAGLUTININ AND ADHESIN

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ABSTRACT

Salmonella typhi is an obligate pathogen that usually found in clinical specimen from typhoid fever patient. The pathogenic mechanism of bacteria are not fully elucidated especially its potential activity of the outer membrane protein (OMP) as hemaglutinin and adhesion molecule. After identification, bacterial isolate of outer membrane protein fraction 12,5 % SDS-PAGE were used to isolate OMP followed by hemaglutinin test and invitro adhesion test. The study showed that the 53 kDa protein of *S. typhi* was a hemaglutinin protein that could agglutinate , mouse Wistar erythrocytes. The 53 kDa OMP was also adhesion protein showed by its activity to adherence to Wistar mice's enterocyt. The increase dose of 53 kDa OMP will decrease the mount of *S. typhi* bacteria to adherence to Wistar mice's enterocyt. Outer membrane protein 53 kDa *S. typhi* as hemagglutinin and adhesion protein .

Keywords: *Salmonella typhi*, OMP, adhesin, hemagglutinin

INTRODUCTION

Typhoid fever is an acute systemic illness caused by Salmonella typhi. The organism are noncapsulated, nonsporulating, facultative anaerobic bacilli, which have characteristic flagellar, somatic and outer coat antigens[1].

The first stage of microbial infection is adherence or attachment to a eukaryotic cell or tissue surface requires the participation of two factors : a receptor and an adhesin. The bacterial adhesins is typically a macromolecular component of bacterial cell surface which interacts with the host cell receptor. The mechanisms of adherence may involve two steps: non-specific adherence , reversible attachment of the bacterium to the eukaryotic surface and specific adherence, reversible permanent attachment of the microorganism to the surface. Reversible attachment is mediated by fimbria, permanent attachment is mediated by outer membrane proteins (OMP).[2]

Adhesion molecule is a bacterial virulence factor and in some bacteria hemagglutinin protein functions as an adhesion molecule. Molecular weight varies between a bacterial adhesin with other bacteria. *Acinobacter baumanni* have hemaglutinatinin molecule and is also an adhesin molecule.[3] Likewise, the bacterium the bacterium Vibrio cholera has a hemagglutinin molecule that also acts as an adhesin.[4] The purpose of this study is to prove the existence of hemagglutinin protein that functions as an adhesin, particularly OMP 53 kDa from the bacterium *S. typhi*.

MATERIALS AND METHODS

Subcultures of *S. typhi*

Bacteria to be used is S. typhi strains Jember from a local patient typhoid fever, method used a prescribed by Ehara, ie growth media that enrich TCG pili *S. typhi.* This media containing trioproline 0.02%, 0.3% $NaHCO_3$, 0.15% bactotrypton, 0.2% yeast extract, 0.5% NaCl 2% bacto agar and 1 mM EGTA. Media to be made in the capacity of 250 ml bottles are titled as much as 50 ml of agar. S. typhi are grown on Brain Heart

Infusion (BHI) are incubated at 37^0C for 4 hours. Then as many as 10 ml suspension of bacteria is put into each bottle containing of TCG media. Furthermore incubation performed at a temperature of 37^0C for 2x 24 hours.

Isolation OMP S. Typhi

Isolation method with modification Evan's. Modification on the part of the sample used is the deposition of pili on withholding treatment rounds last, pellet suspended with PBS pH 7,4 until its volume reached 15 times, then added n-octyl B D-glucopyranoside (NOG) concentration reached 0,5%, then homogenized using vortek full speed for 1 minute. Then centrifuged at 12.000 rpm for 30 minutes the temperature of 4ºC. Supernatant fluid was taken, and then dialysed. Fluids used for dialysis in the first 24 hours, ie d H_2O and at 24 hours both PBS pH 7,4.

***Sodium Dodecyl Sulfate Polyacrilamide Gel Electroforesis* (SDS-PAGE)**

Monitoring molecular weight was performed using SDS-PAGE. Protein samples were heated 100 ºC for 5 minutes in a buffer solution containing 5 mm Tris HCl pH 6.8, 2- mercapto ethanol, 5% w/v sodium dodecyl sulfate 2.5% , v/v 10% glycerol with bromophenol blue tracer color. Then minislab gel 12.5% selected with tracking the gel 4%. 25 mV voltage used [6].

0MP Purification

The SDS-PAGE gel OMP collection, straight cut its gel desired molecular weight and a piece of tape is to be collected and included in the membrane dialysis fluid using eletrophoresis buffer, running buffer. Electrophoresis is then performed using flow electrophoresis apparatus frontal 125 mV for 25 minutes. The result of electrophoresis performed dialysis fluid with PBS buffer pH 7.4 for 2x24 hours 2 1 liter and replaced two times. Dialysated fluid was performed hemaglutination test.

Hemagglutination test

Hemagglutination test was done according to instruction from Li[7]. Concentration of sample dilution was made ½ on microplateV, where each 50 ul volume wells. Each well added a suspension of red blood concentration of 0.5% volume mice werre the same. Then it was shaken with rotator plate for 1 minute. Subsequently it was placed in room temperature for 1 hour. The amount of the titer was determined by observation of red blood agglutination at the lowest dilution.

Adhesion Test

Adhesion test S. Typhi was cultured in lactose broth at s temperature of second Bcteria harvested by using cebtrifugation 6000 rpm for 10 minutes at 4 ºC. Sediment suspended with PBS and bacterial content of 10^8/ml made using spectrophotometer with a wavelength 0f 600 nm. Furthermore, the protein dose outer membrane preparation made of each 0 ul, 25ul, 50ul,100ul, 200ul, 400ul, and 800ul in 300 ul PBS. Furthermore , each dose added to a suspension of 300 ul epithelium and shaken slowly at shaking waterbath at a temperature of 37 ºC for 30 minutes. Then in each mixture is added 300 ul of bacterial suspension. Mixtures were incubated with shaking incubator for 30 minutes at temperatute at 37 ºC. Subsequently centrifuged at 1500 rpm and 4 ºC for 3 minutes, then sediment washed two times with PBS. Then the deposition was taken and made smear on glass objects and painted with Gram stain. Preparations were observed with 1000 times magnification microscope and counted the number of bacteria that is adheren epithelial cells. Adhesion index is the average number of bacteria that adhere on teh epithelium, is calculated for each observation on 100 epithelial.

RESULTS AND DISCUSSION

Isolation OMP do after cutting of pili. To predict the moleculer weight OMP , was done SDS PAGE with results as shown in Figure 1.

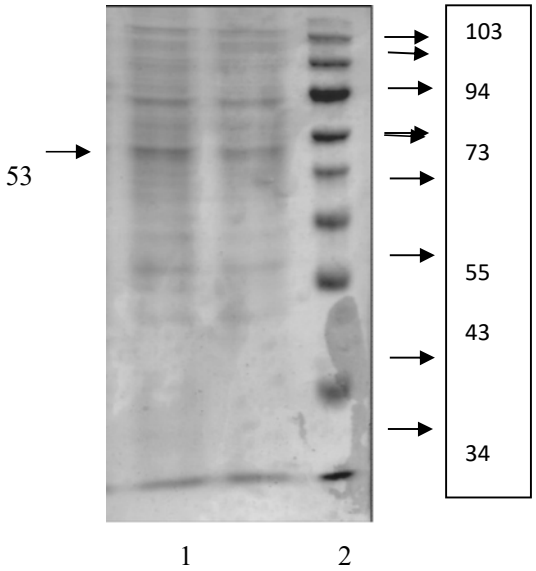

Figure 1. The SDS-PAGE OMP *S. typhi* .1. OMP *S. typhi* 2. Protein tracer

Protein profile on SDS-PAGE of OMP S. typhi showed a prominent protein with a molecular weight 53 kDa. Afterwards, the protein is cut, electroelucided, and dialysed, resulting in a protein solution. Furthermore from this result, a hemagglutination test is executed on the erythrocytes of mice that the 53 kDA omp. This protein is able to inhibit agglutination of mice (Table 1).

Table 1. Hemaglutination test result of 53 kDa OMP from *S. typhi* with gradual dilution

	Dilution									
Sample	1x	2x	3x	4x	5x	6x	7x	8x	9x	10x
OMP53kDa	+	+	+	+	+	+	+	+	-	-

After hemaglutinin protein adhesion test are done with variable molecule weight, resulted in appearance of a bacteria stick to vesica urinary epithelial cell of rabbit at considerable amount, as seen at Fig. 2.

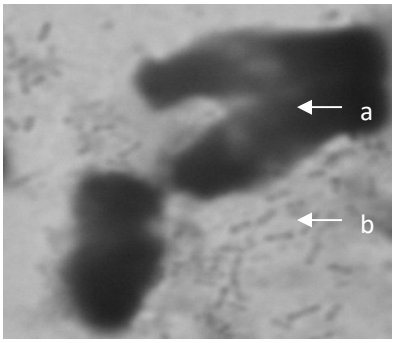

Fig 2. Adhesion *S. typhi* in enterocyt wistar mouse. a). Enterocyt wistar mouse b). *S. typhi*

When adhesion inhibition test are done with 53 kDa OMP resulted in decrease of amount of bacteriae sticking to vesica urinaria epithelial cell compared to protein-unsaluted cell as seen at Fig.3

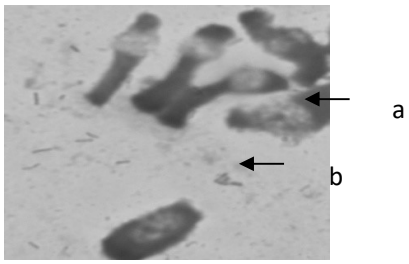

Fig 3. Adhesion of S. typhi saluted with 53kDa OMP with consentrated at 200 ul.
a). Enterocyt wistar mouse b). *S. typhi*

The average result of adhesion test to vesica urinary epithelial cell of rabbit, either it saluted or unsaluted with 39kDa OMP with gradually increased dose, starting from 800 µl to 12,5 µl shown at table 2.

Table 2. Average result of adhesion test S. Typhi to enterocyt wistar mouse

Dosis protein	Indeks Adhesi
0	6367
25	6333
50	5967
100	5512
200	1233
400	533

The result shows a decreased amount of bacteriae adherence to vesica urinaria epithelial cell after saluted with 53 kDa OMP. And the decreased amount of bacteriae adherence to epithelial cell related with increased amount of 53 kDa OMP dosage.

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POLYMERASE CHAIN REACTION RESTRICTION FRAGMENT LENGTH POLYMORPHISM FOR BETA GLOBIN GENE MUTATION DETECTION AT SUNDANESE PEOPLE

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ABSTRACT

Thalassemia is an inherited blood disorder and causing moderate or severe anemia. Anemia is associated with decreased hemoglobin level and number of erythrocytes decreased compared with normal. Globin chain mutation that can occur in the alpha chain or beta chain and appears in individuals who have homozygous or heterozygous gene. Beta thalassemia is caused by mutations in chromosome 11 that affects the entire production of beta chains such as transcription, translation, and stability of the production of beta globin chains. Detection of beta-globin gene mutations can be detected by using PCR RFLP. PCR is a technique that to be come the amplification of specific DNA segments is carried out in vitro. PCR RFLP analysis is a technique by distinguishing restriction fragment length. Restriction enzyme will recognize the area and the introduction of these restriction enzymes cut the DNA, resulting in a specific fragment length. If the cuts are missing, the fragments will be formed with different length. Enzymes used for mutation detection is Cac8I, BSI, MNL, SFC, and BFA. The first restriction enzyme used is CaC8I for detection of beta globin gene mutation IVS1-nt5. This type of mutation is the most common variations in Sundanese.

Keywords: thalassemia, beta-globin gene, PCR RFLP, restriction enzymes.

INTRODUCTION

Beta thalassemia major is a disorder that is homozygous beta thalassemia. The disease is often called Cooley anemia. Beta thalassemia major is the worst form of all types of thalassemia because the clinical manifestations usually appear after the first 4-6 months of life. Patients experiencing severe anemia with a hematocrit less than 20% so dependent on the provision of blood transfusion. Symptoms that often occur in the form of an enlarged spleen and liver, stunted growth, and changes in bone. Bone changes caused by the hyperactivity of bone marrow resulting in excess bone growth in the frontal, parietal, and protrusif maxillary zigomatikus. Changes in this form produces a typical face, ie *Facies Cooley / thalassemia facies.*

Thalassemia has been identified as having a high frequency in the subtropical region, their distribution extends from southern Europe to Southeast Asia. The main distribution area includes the border the Mediterranean Sea, most of Africa, the Middle East, Indian Sub-continent, and Southeast Asia, including Indonesia, with the incidence of 50-20%. Some areas in Southeast Asia as many as 40% of the population has one or more of the thalassemia gene. In North America there are probably 750 to 1,000 patients with homozygous beta thalassemia and only 15 to 20 new cases diagnosed each year.

Each region has a place of origin thalassemia trait beta-globin gene mutation specific. As in previous research in the Sunda populations the most common mutation is homozygous IVS1-nt5. Handling can be made easier by knowing the patient's origin or race of patients. In developed countries such as Italy, the diagnosis of thalassemia gene is not new.

One method of detection of beta-globin gene mutations that can be used is the polymerase chain reaction restriction fragment length polymorphism (PCR-RFLP). RFLP analysis is a technique by distinguishing restriction fragment length. Restriction enzyme

will recognize the area and the introduction of these restriction enzymes cut the DNA, resulting in a specific fragment length. If the cuts are missing, the fragments will be formed with different length. So there are areas that remain and the area polimorfis for each restriction enzyme. The fragments are then seperate by electrophoresis technique, small fragments will move farther than larger fragments. One or two fragments can be visualized by a *probe,* which is a single molecule of DNA pieces that complement to one or more nucleotides of the restriction fragments and labeled radioactive or fluorescent. RFLP analysis was first developed by Wyman and White, which they characterize the first time polimorfis DNA loci by calculating the results of restriction fragment length variable, later known as *Restriction Fragment Length polymorphism.* This techniq then developed by Jeffrey, A et al, known as DNA *fingerprinting,* they analyze the myoglobin gene, and found the existence of a region comprising a sequence of 33 base pairs are repeated (core repeat) as much as four times in the *intervening sequences* (introns), sequence repeat *(tandem repeat)* is referred to as the hypervariable region minisatelit and because the number of repeat sequences vary in a locus or between them.
This study aims to find other means of detection of beta globin gene mutations using PCR-RFLP method.

MATERIALS AND METHODS

The number of subjects who meet inclusion and exclusion criteria were 66 patients. To all patients performed the data collection includes examining blood samples from 4 ml for DNA analysis, PCR and RFLP.

DNA Analysis

Analysis of DNA consists of DNA isolation and PCR-RFLP. DNA was isolated from blood using the DNA Isolation Kit from Pharmacia, and then 200 ng of DNA was used as template for PCR.

***Polymerase Chain Reaction* (PCR) and *Restriction Fragment LengthPolymorphism* (RFLP)**

Primers used for thalassemia mutations detection are:
TLF62028 ComC *forward:* ACCTCACCCTGGAGCCAC
TLR62320 *reverse:* CTATTGGTCTCCTTAAACCTGTCTTGTAACCTTGCTA
PCR mixture for the inspection of the beta thalassemia mutation major (total volume 100 µl) consisted of 8 µL of PCR buffer solution (0.25 M KCl, 0.05 M Tris HCl pH 8.4, 1.5 mM MgCl, and 0.0005% gelatin). 2 µL dNTPs (10 mM) (consisting of dATP, dCTP, dGTP, dTTP), 0.6 µL *forward and reverse* primer (40 pmol). 0,5 µL *Taq Polimerase* (5 unit/µL). Mixture centrifuged for 20 seconds with a speed of 13 000 rpm. The result of the mixture added to 300 ng DNA template (template) in 1x TE buffer.
Tube containing the PCR mixture incorporated into the PCR machine (Corbette) with initial denaturation of the following conditions at a temperature of 94 ^{0} C for five minutes made for a cycle, after it went into denaturation cycle conditions at a temperature of 94 ^{0} C for one minute, a temperature annealing 67 ^{0} C for one minute, and extension at a temperature of 72 ^{0} C for 30 seconds.
Step is performed as many as 40 cycles and final extension step was continued at a temperature of 72 ^{0} C for 3 minutes made for a cycle.
Restriction enzymes are used to check the variation of beta-globin gene mutations can be seen in Table 1. Examined the mutation is a common mutation found in the Indonesian population.
The selection restriction enzymes used are based on research conducted by Pramoonjago *et al* (1999). This enzyme is Cac8I, BSI, MNL, SFC, and BFA. The first restriction

enzyme used for detection of gene mutation CaC8I beta-globin IVS1 nt5. This type of mutation is the most common variations in Sundanese.

Table 1. Restriction enzyme, Incubation Conditions, and Results of fragment beta globin gene mutation

Mutation Type	Reagent		Incubation Conditions	Fragment Restriction Results		
	Buffer	Enzyme		Normal (Bp)	Homozygous (Bp)	Heterozygot (Bp)
IVS1-nt5	NEB 3	Cac8I	37 ^{0} C for 24 hours	293	257 , 36	293, 257 , 36
IVS1-nt2	NEB 3	Cac8I	37 ^{0} C for 24 hours	293	250 , 43	293, 250 , 43
IVS1-nt1	NEB 3	Bsl	55 ^{0} C for four hours	29, 22, 175 , 45	29, 22, 240	29, 22, 175, 45, 240
HbE	NEB 2 BSA	Mnl	37 ^{0} C for 24 hours	12, 37, 106, 16, 60, 62	12, 37, 106, 16, 122	12, 37, 106, 16, 60, 62, 122
Cd15	BSA NEB 4	Sfc	37 ^{0} C for 24 hours	293	202, 91	293, 202, 91
Cd 17	NEB 4	Bfa	37 ^{0} C for 24 hours	24, 114, 155	24, 114, 72, 83	24, 114, 155, 72, 83

RESULTS AND DISCUSSION

Analysis of beta globin gene mutation Diteksi

All blood samples were examined through a process of research, DNA isolation, PCR and RFLP. DNA was isolated and then performed PCR using primers Com C to obtain DNA fragments in which there is a base target for beta-globin gene mutations. PCR product obtained is a fragment of beta-globin gene variation, which has size 293 bp. Prior to conducting further testing stage, the PCR products are inspected prior existence electrophoresis method that can be seen in Figure 2.

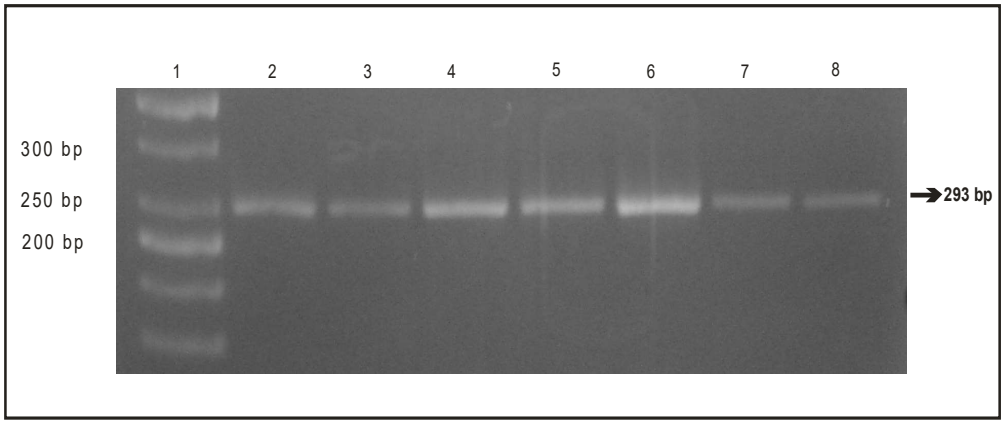

Figure 1. Electrophoresis of PCR product of Beta Globin Gene Mutation
Lane 1: markers DNAΦX174Hae III
Lane 2 – 8 : PCR Product at 293 bp

Analysis of beta globin gene mutation was only performed on the patient group. The result is then performed PCR product with restriction enzyme cuts listed in Table 1 by using RFLP method. Cutting using restriction enzymes show the results in Table 2.

Table 2. Percentage variation of beta globin gene mutation

Mutation	Amount	Percentage (%)
Homozygous IVS1-nt5	14	21.21
Homozygous IVS1-NT1	5	7.57
Heterozygous IVS1-nt5/IVS1-nt1	19	28.78
Heterozygous IVS1-nt1/Cd26	1	1.51
Heterozygous IVS1-nt5/Cd26	12	18.18
Heterozygous Cd15	6	9.09
Heterozygous Cd26	6	9.09
Homozygous Cd 26	3	4.54
Total	66	100

Table 2 shows the percentage variation of the beta globin gene mutation. Variations IVS1-nt5/IVS1-nt1 heterozygous mutation is the largest amount (28.78%), IVS1-nt5 homozygotes (21.21%), IVS1-nt5/Cd26 heterozygous (18.18%), heterozygous Cd15 (9.09 %), Cd26 heterozygous (9.09%), homozygous Cd26 (4.54%), and IVS1-nt1/Cd26 heterozygous (1.51%).

Beta thalassemia major is a single-gene disorders that are often found in Indonesia. In Malaysia was found less than 4.5% as heterozygous career. Until now, the beta thalassemia major include blood disorders in the group that still need attention.

Beta thalassemia mutation is determined by the location of major mutations of beta globin chains. Samples obtained in this study almost all come from the Sundanesse people. It has been known from previous studies that the mutation rate is the most widely Sunda IVS1-nt5. More than 140 types of mutations have been found in the population in the world.

In Table 2 shows that the mutation is heterozygous IVS1-nt5/IVS1-nt1 highest group. This is according to research conducted by Setianingsih (1998) which showed that mutations IVS1-nt5 (à G C) is the highest population among a group of beta thalassemia major. The entry into the land of Sunda thalassemia originating from Asia Pacific, because of the mutation obtained from the results of this study support previous research results.

The results Orkin et al (1982) in the LE Lie-injo., Et al (1989) showed that the mutant allele on chromosome has a strong equilibrium with a specific pattern of DNA polymorphism of the restriction referred to as beta-globin gene haplotype groups. Beta-globin gene may consist of a beta zero ($B^{0)}$ B +, *compound heterozygote.*

Detection of the beta globin gene mutation is an effort to enforce the prenatal diagnostic *screening.*. PCR-RFLP method is a reliable method to determine the type of mutation. Use of restriction enzymes would recognize the area where the presence of mutations specific cuts. A gene is said polymorphic if it found there were two or more different alleles in one locus of the gene or in other words in the 100 chromosomes examined, the frequency of rare alleles (polymorphic allele) were found more than 1% (0.01), while the less common allele frequency than 99%. DNA polymorphisms can be identified by sequencing the entire DNA segment or identification of the specific restriction enzyme sites.

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INFLUENZA H3 VIRUS AND HUMAN META PNEUMOVIRUS (HMPV) DETECTED IN PATIENTS WITH ACUTE RESPIRATORY INFECTIONS IN MOEWARDI HOSPITAL SURAKARTA, INDONESIA

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ABSTRACT

Since March 2010 an active surveillance has been performed by collecting respiratory specimens from the acute and or severe respiratory infection (ARI/SARI) patients of Pulmology Department of Moewardi Hospital Surakarta to build a molecular epidemiology database of human respiratory viruses in Moewardi Hospital Surakarta. In March – May 2010, 32 patients were enrolled in this study. All respiratory specimens (nasal and throat swab) were addressed for Influenza A virus, Influenza A H1 virus, Influenza H3 virus, Influenza H5 virus, Influenza B virus, Human ParaInfluenza Virus (HPIV) 1, HPIV 2, HPIV 3, HPIV 4, Respiratory Syncytial Virus (RSV) A, RSV B, Human Rhinovirus, Enterovirus, Human Coronavirus (HCoV)-OC43, HCoV-SARS, HCoV-229E, Human Metapneumovirus (HMPV), Human Bocavirus and Adenovirus, by multiplex nested PCR or RT-PCR. The data presents preliminary data results from on going molecular epidemiology study of human respiratory viruses in Moewardi Hospital Surakarta. The Influenza H3 Virus RNA and the HMPV RNA were detected in 9.4 % (3/32) and 6.3 % (2/32) samples, respectively. No virus co-infection was found in all samples. The correlation between etiological findings with the clinical data also has been analysed. The positive PCR products are going to be directly sequenced and analysed. We detected Influenza H3 Virus and HMPV in patients with acute respiratory infections in Moewardi Hospital Surakarta, Indonesia. For the best of our knowledge, this is the first report of HMPV detected in Indonesia and that of also in tropical countries.

INTRODUCTION

Most studies of human respiratory viral activity patterns have focused on children, and large-scale epidemiological investigations of respiratory virus infections in adults have rarely been conducted. In an attempt to characterize the respiratory virus infections and to provide insights into the etiology and clinical associations of respiratory viruses in adult acute respiratory infections, a molecular epidemiology study was conducted with adults suspected of having acute respiratory infections in Surakarta, Indonesia from March 2010 until March 2011. In this paper we present the data from an on going study of molecular epidemiology study of human respiratory viruses in Moewardi Hospital Surakarta.

MATERIALS AND METHODS

Patients and Clinical Specimens

The data presented here was resulted from patients from March 2010 to May 2010 at the Department of Pulmology Dr. Moewardi Hospital Surakarta, Indonesia. All adult patients with acute respiratory disease visited the Pulmology Department of Moewardi Hospital were offered to involved in the study after diagnosed as ILI (Influenza like illness), ARI (Acute Respiratory Infection) or SARI (Severe Acute Respiratory Infection) (World

Health Organization, 2009). To include the potential viral ARTIs and to exclude typical bacterial infections, patients enrolled in the study were selected by physicians according to the following criteria: >18 years of age, with respiratory symptoms such as cough or wheezing, acute fever (body temperature >38 °C), and normal or low leukocyte count, with or without radiological pulmonary abnormalities. Nose and throat swabs were collected from each patient, and the two swabs were pooled in BD™ Universal Viral Transport System (Becton and Dickinson, USA) and immediately transferred to Biomedical Laboratory, Faculty of Medicine, Sebelas Maret University Surakarta, then aliquoted and stored in -80 °C. A total of 32 specimens from 32 patients were collected and tested.

Nucleic Acid Extraction

Total nucleic acids (DNA and RNA) were extracted from 200 □L of each specimen (VTM) using the Purelink Viral DNA RNA kit (Invitrogen Carlsbad, CA), according to the manufacturer's instructions.

Molecular Detection of Respiratory Viruses

The presence of Influenza A virus, Influenza A H1 virus, Influenza H3 virus, Influenza H5 virus, Influenza B virus, Human ParaInfluenza Virus 1, Human ParaInfluenza Virus 2, Human ParaInfluenza Virus 3, Human ParaInfluenza Virus 4, Respiratory Syncytial Virus A, Respiratory Syncytial Virus B, Human Rhinovirus, Enterovirus, Human Coronavirus-OC43 (HCoV-OC43), Human Coronavirus-SARS (HCoV-SARS), Human Coronavirus-229E (HCoV-229E), Human Metapneumovirus, Human Bocavirus and Adenovirus, was determined by multiplex nested PCR or RT-PCR, as previously described (Leung, et al., 2009). Briefly, two multiplex nested RT-PCRs were used for the simultaneous detection of Influenza A virus, Influenza A H1 virus, Influenza H3 virus, Influenza H5 virus, Influenza B virus (Group I), as well as Human ParaInfluenza Virus 1, Human ParaInfluenza Virus 2, Human ParaInfluenza Virus 3, and Human ParaInfluenza Virus 4 (Group II), Respiratory Syncytial Virus A, Respiratory Syncytial Virus B, Human Rhinovirus, Enterovirus (Group III), and Human Coronavirus-OC43 (HCoV-OC43), Human Coronavirus-SARS (HCoV-SARS), Human Coronavirus-229E (HCoV-229E), and Human Metapneumovirus (Group IV), respectively. Human Bocavirus and Adenovirus were detected using multiplex nested PCR (Group V). Blank VTM was used as a negative control, and 100 copies of invariant □-actin gene were added to lysis buffer as internal controls to exclude inhibitors for nucleic acid extraction and PCR. Each RT-PCR amplification were performed using the SSIII FIRST-STRAND SUPER MIX and PLATINUM PCR SUPERMIX Kit (Invitrogen). PCR products were analysed by electrophoresis in 1.5 % agarose gel containing ethidium bromide and visualized under UV light.

RESULTS AND DISCUSSION

The investigation was performed on working days from March to May 2010. Specimens were collected from both females and male, 31 % (10/32) and 69 % (22/32), respectively. The clinical diagnosis consisted of 54 % (17/32) cases of pneumonia, 19 % (6/32) cases of asthma exacerbation, 9 % (3/32) cases of chronic obstructive pulmonary disease (COPD), 9 % (3/32) cases of bronchopneumonia, 6 % (2/32) cases of ILI, and 3 % (1/32) cases of ARI, respectively.

Respiratory samples from 5 (15.6 %) patients were found to be positive for one virus, and those from 27 (84.4 %) patients were negative for all respiratory viruses tested. Pathogens in such patients as these need to be further investigated. Influenza H3 virus and Human Metapneumovirus (HMPV) were detected in 9.4 % (3/32) and 6.3% (2/32) samples, respectively.

The accurate and rapid analysis of a broad range of viral agents is critical for etiological investigations. In this study, multiple viral agents, including Influenza A virus, Influenza A H1 virus, Influenza H3 virus, Influenza H5 virus, Influenza B virus, Human ParaInfluenza Virus 1, Human ParaInfluenza Virus 2, Human ParaInfluenza Virus 3, Human ParaInfluenza Virus 4, Respiratory Syncytial Virus A, Respiratory Syncytial Virus B, Human Rhinovirus, Enterovirus, Human Coronavirus-OC43 (HCoV-OC43), Human Coronavirus-SARS (HCoV-SARS), Human Coronavirus-229E (HCoV-229E), Human Metapneumovirus, Human Bocavirus and Adenovirus, were analysed with the goal of providing comprehensive data for viral infection in adults with acute respiratory infections. To our knowledge, this is the most comprehensive investigation of respiratory virus infections in Indonesia in adults with acute respiratory infections. The overall detection rate of respiratory viruses in this study was 15.6 %. However, these results may underestimate the role of virus infection, because viral loads in nose and throat swabs, as used in this study, are usually lower than those in aspirate or lavage. Furthermore, infections from as yet unknown viruses may be responsible for some acute respiratory infections. The prevalence of bacterial acute respiratory infections was not investigated in this study.

Influenza virus has been related to Lower Track Respiratory Infection (LTRI). Many reports shows that the suspected patients with Influenza virus show signs and symptoms like fever (37.1 ºC – 38.4 ºC), myalgia, malaise, cough, headache, and rhinitis. In the other hand, these viruses can deliver the patients into primer pneumonia with acute exacerbation, secondary bacterial pneumonia, otitis media, even to a septic state (Agoes, et.al., 2008; Fiore, et al., 2008 ; Yuwono, et.al., 2008; Sedyaningsih, et.al., 2007).

Our result was consistent with previous finding. Three patients with positive diagnosis of Influenza H3 have clinical signs and symptoms consisted of fever (>38 °C), wheezing, cough, myalgia and malaise. They were diagnosed with ILI, ARI, and pneumonia, respectively. All three patients also developed acute asthma exacerbation. The three influenza H3 patients got antibiotic (as the drugs treatment) and oxygen (as the supportive treatment) and completely recover.

Since its discovery in 2001, Human Metapneumo Virus (HMPV) has been detected in all four seasons' country (Fauchier, et al., 2005) and was becoming an important respiratory virus as Respiratory Syncytial Virus. The clinical appearances of patients infected with HMPV is not differ from those infected with RSV, such as cough (90 %), fever (52 %), wheezing (52 %), ronchi (20 %), and vomit (20 %). HMPV always linked to an acute lower tract respiratory infection (ALTRI), which can deliver the patients into condition like bronchiolitis (59 %), cough followed by stuffy breath (18 %), asthma with exacerbation (14 %), and pneumonia (8 %). Usually, the patient with HMPV infection, also have another co-infection with other respiratory virus (Collins and Crowe, 2007; Milder and Arnold, 2009). In our study, two patients positive with HMPV, showed clinical signs and symptoms as described above (fever, wheezing, ronchi, and cough) and no co-infection with other respiratory virus had been found. The clinical diagnoses were pneumonia and only one patient delivered into septic status. All patients had antibiotic and oxygen treatment. One patient completely recovered but the patient with septic status did not continue the treatment.

In summary, the spectrum, age distribution and clinical associations of respiratory virus infections in adults with acute respiratory infections were analysed in this study. The findings provide baseline data for evaluating the burden of respiratory virus infection in adults. As the number of patients was limited in this study, further studies should be undertaken and longer surveillance periods should provide more information concerning the epidemiology of respiratory virus infections, and their relationships with clinical outcomes, especially in Indonesia.

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HEPATITIS C VIRUS 1A AND 1C IN NARCOTIC DRUGS USERS IMPRISONED IN WOMEN PRISON SEMARANG, INDONESIA

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ABSTRACT

Narcotic drug users especially that of the injecting drug users (IDUs) are being associated with high risk of Hepatitis C Virus (HCV) infection. However, at present there is no molecular epidemiological data about HCV in narcotic drug users in Indonesia especially that of imprisoned in Women Prison Semarang. To role out the molecular data, all narcotic drug users (86 persons) imprisoned in Women Prison Semarang were enrolled in this study. Plasma were collected and addressed for serological assay. The nucleic acid was extracted from the anti-HCV positive samples. The RT-PCR nested was performed to detect part of NS5B region of the HCV genome. The positive PCR products were directly sequenced and phylogenetic analysed. The data presents preliminary data results from on going molecular epidemiology study of human blood borne viruses in Central of Java. Anti-HCV positive was found in 24.4 % (21/86) of total samples. The HCV RNA was detected in 4 out of 21 anti-HCV positive samples. Based on 366 bases of the NS5B sequences, the HCV strains were classified into genotypes 1. The HCV 1a (75 %) was the most prevalent, followed by subtypes 1c (25 %). These results were quite different to all previous reports about HCV molecular epidemiology data in hepatitis patients in Indonesia. Results indicate the discrepancy molecular epidemiology data of HCV found in hepatitis comparing to that of the non hepatitis patients community.

Keywords: *HCV, Narcotic Drug Users, IDUs, Prison, Prisoners, Indonesia*

INTRODUCTION

Globally, 170 million people (3 % of the world's population) are chronically infected with HCV and 3-4 million are newly infected each year. A precise estimation of the incidence of HCV infection is difficult to determine because most acute infections are asymptomatic and available assays do not distinguish between acute and chronic infection. Surveillance and reporting systems are inadequate in many countries and can underestimate the incidence of acute hepatitis C (AHC). Patients with acute HCV infection are generally asymptomatic, which renders diagnosis difficult and results in under-reporting. Currently, intravenous drug use, unprotected sex with multiple partners, and viral exposure during medical procedures, such as surgery, dialysis and dental treatment, are factors associated with the highest degree of risk for HCV infection. The risk factors accounting for the bulk of HCV transmission worldwide are injection drug use in developed countries and blood transfusions from unscreened blood donors, unsafe therapeutic injections, and other iatrogenic routes in developing countries (Low, et al., 2008; Santantonio, et al., 2008).

Because genotypes 1 and 4 are more resistant than genotypes 2 and 3 to the current standard of care, pegylated interferon and ribavirin combination therapy, most treatment protocols require genotype information to tailor dose and duration of treatment. Therefore, molecular epidemiology studies in communities are also important to prepare the adequate therapy for the hepatitis C patients in the future. Subsequent molecular epidemiology studies have revealed great HCV diversity in certain regions of sub-

Saharan Africa and in south and south-east Asia. Most newly described variants originate from specific geographical regions. For example, infections in western Africa are predominantly genotype 2, whereas those in central Africa are genotypes 1 and 4. Genotypes 3 and 6 show similar genetic diversity in south and eastern Asia. In contrast, the most common variants found in Western countries (1a and 1b in genotype 1; 2a, 2b, and 2c in genotype have become widely distributed over the past 50–70 years as a result of transmission through blood transfusion and other invasive medical procedures and of needle sharing by injection drug users (IDUs). Subtypes 1a, 1b, 2a, 2b, 3a, and 4a are likely to be the descendants of HCV variants from endemic areas that "seeded" these new, rapidly expanding transmission networks (Kuiken and Simmonds, 2009).

The correctional facility is already known as a high risk place for virus transmission including of HIV and HCV. The seroprevalence rates in correctional facilities were 19 -30 % for HCV (Adjei et al., 2006; Solomon et al., 2004). Recent publication shows about 38 % - 90 % prisoner incarcerated in correctional facilities with anti-HIV positive are also infected with HCV (Pontali and Ferrari, 2008; Hennessey et al., 2009). However, in Indonesia, for the best of our knowledge, there is no publication about HCV in incarcerated prisoners in Indonesia especially that of the narcotic drugs users (NDUs). Based on these conditions we performed active surveillance in prisoners incarcerated in correctional facilities in Indonesia, and for our first step, we focused on NDUs. This report present our data about HCV in NDUs incarcerated in Women Prison Semarang in Central of Java Indonesia.

MATERIALS AND METHODS

Subjects and Plasma Samples

Plasma samples and epidemiological data were collected from all NDUs (n= 86) imprisoned in Women Prison Semarang. All respondents were having no hepatitis sign(s) and or symptom(s). This study was approved by the Indonesian Department of Law and Human Right Central of Java branch and the institutional ethical review board of Faculty of Medicine Sebelas Maret University, Indonesia. All the procedures were conducted according to the principles of the Declaration of Helsinki.

Serological Tests

Plasma was separated from whole blood with EDTA and subjected to OrthoHCV Ab PA II assay (Fujirebio Inc, Tokyo, Japan) for the detection of anti-HCV antibody. Assays were performed according to the manufacturer's instructions.

RNA Extraction, Reverse Transcription, and Polymerase Chain Reaction (PCR)

HCV-RNA was extracted from 100 □l of plasma using SMITEST EX-R&D (Genome Science Laboratories, Fukushima, Japan), and reverse-transcribed according to SuperScriptIII First-Strand cDNA Synthesis SuperMix protocol with random hexamer (Invitrogen, Carlsbad, CA). A part of HCV NS5B region was amplified by nested PCR with primers, hep31b/hep32 in the first round, and hep33b/hep34b in the second round as described before (White et al., 2004). Nested PCR was performed with 50 □l reaction mixture Platinum PCR SuperMix (Invitrogen).

Determination of Nucleotide Sequences and Phylogenetic analysis

The PCR products were subjected to the determination of nucleotide sequences directly with the primers of hep33b and hep34b for HCV NS5B region. The tested sequences were aligned with HCV sequences retrieved from Genbank/EMBL/DDBJ database by ClustalW with subsequent inspection and manual modification (Thompson et al., 1994). The frequency of nucleotide substitution in each base of the sequences was estimated by the Kimura two-parameter method. A phylogenetic tree was constructed by the neighbor-

joining method, and its reliability was estimated by 1000 bootstrap replications. The profile of the tree was visualized with the program of MEGA 4 (Tamura et al., 2007).

RESULTS AND DISCUSSION

Molecular epidemiology and seroprevalence study of HCV have been done in Indonesia by other groups. In pregnant women in Bali, the seroprevalence of HCV is very low (Surya et al., 2005). In general population a seroprevalence study in Sangehe-Talaud showed 0.2 % plasma reactive for the anti-HCV (Achwan et al., 2007) while in Jakarta was about 3.9 % (Akbar et al., 1997). The anti-HCV in healthy blood donors was 2.1 % - 17.8 % (Handajani et al., 2000; Soetjipto et al., 1996; Sulaiman et al., 1995) while anti-HCV prevalence in children with hematological diseases was about 39 % (Chanpong et al., 2002). Estimated anti-HCV was positive in 56.9 % of hemophilia patients in Indonesia (Timan et al., 2002). The most prevalent subtype HCV was 1b, followed by 3k, 2a, 1a, 1c, and 2e, respectively. Moreover, the subtype of 1d, 2b, 2e, 2f, 3a, 3b, 3g, and 4a could be found in hepatitis and or hepatoma samples collected from several hospitals in Indonesia (Hotta et al., 1997; Inoue et al., 2000; Utama et al., 2009). However, for the best of our knowledge, there is no data about molecular epidemiology of HCV in NDUs jailed or detained in correctional facilities in Indonesia yet. In our opinion, the NDUs community in the correctional facilities is one of the high risk communities for HCV infection, and they may represent the real condition of HCV molecular epidemiology in the general community. In our study, in NDUs jailed in Women Prison Semarang, we found that the HCV 1a (75 %) was the most prevalent, followed by subtype 1c (25 %). The predominant of HCV 1a found in this study was quite different to all previous reports about HCV molecular epidemiology data in hepatitis patients in Indonesia. These findings warrant the further careful observation of the HCV infection trend among narcotic drugs users in Indonesia. At present, our group continue analyzing the molecular epidemiology data retrieved from other correctional facilities in Central of Java.

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IDENTIFICATION OF DRUG RELATED PROBLEMS AT SANGLAH HOSPITAL BALI

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ABSTRACT

The background and objectives of this research is firstly to introduce pharmaceutical care in practice is by identifying drug related problems (DRP) in clinical setting. Studies showed that DRP potentially occurred in patients who received polypharmacy. Additionally, data on drug used study particularly DRP at the hospital is very limited. With a consideration that identification of DRP is one way to introduce clinical pharmacy at Sanglah Hospital, a study on identification of DRP at Sanglah Hospital is a necessity. The methods include; a cross sectional study on drug related problems has been conducted at Sanglah Hospital. This is a descriptive study on identifying the most frequent DRP based on PCNE DRP classification. Data was collected from Januari to March 2010 in VIP room at Sanglah Hospital. The patients were selected based on the number of the medication they were on (more than 5) and the number of doctors treated (more than 2 doctors). Results show that there were 23 patients met the criteria of selection. Seven women and sixteen men were identified in the study. Most of the patients were at their 50s. The average number of medications prescribed was 8. Based on the PCNE DRP classification on the type of drug problems, of the 23 patients, it was identified that 29 of DRP cases were result from drug choice problems. 15 DRP cases were related to dosing problems, 7 DRP cases were associated with drug interaction. It can be concluded that the study on identification of DRP at Sanglah Hospital showed that the most frequent DRP identified was related to drug choice problems.

INTRODUCTION

A variety of new drugs are available in the market at the moment, it is because the huge development of technology in medicine. As the number of medications available is increasing, the risk of polypharmacy where patients received more than 5 medications at a time also increases. Studies also show that when patients received more than 5 medications the risk of Drug Related Problems (DRPs) is also increasing. Nowadays, patients are more aware and knowledgeable on their medications. They often need to know what the medications they are on, what they are for, and why they have to take the medicine. The patients expect to receive the best outcome of their treatment. In Indonesia, pharmacists tend to be overtrained but underutilized. Pharmacists learnt medical subjects such as pharmacology, anatomy, patophysiology, and how to interpret simple laboratory test result, besides they also learnt about their core knowledge such as pharmacotherapy, pharmacokinetics, pharmacodynamics as well as drug development. With this knowledge, pharmacists should have done something further than what they have done in clinic which is more to the logistics and drug distributions.

Last year, Indonesian government has recognized pharmacist's role as health provider with the release of a government policy which clearly mentioned about the role of pharmacist which should be implemented as pharmaceutical care (PC). Pharmaceutical care is a comprehensive care given by pharmacist to individual patients to ensure the patients received the appropriate medication for the right indication with the right dose at the right time with minimum side effects. There are three main activities pharmacists should conduct at the clinic in order to commence pharmaceutical care; assessment, care plan, and evaluation. In the assessment process, the first step pharmacists should have done is to identify drug related problems, secondly, care plan, where the pharmacists should be able to provide care plan on management of the problems identified, and lastly, evaluation is to evaluate the recommendation or counseling given and to record or document their activities. To pharmacist as professions, this would be a new

breakthrough yet it would also a challenge to be faced. The role of pharmacists is significantly different from that of doctors and nurses. Doctors task are to diagnose the patients illness, nurses to do daily care to the patients, whereas pharmacists task are to ensure the patients received the drug therapy which are effective, safe and minimum cost.

As it has been mentioned earlier, in order to commence pharmaceutical care the first step to adopt it is by doing identification drug related problems. Since Sanglah Hospital is the biggest hospital in Bali and is also the teaching hospital, in collaboration with pharmacists at the hospital, where they also need to have data on how drugs have been taken by the patients and how pharmacist has done their work at clinic, thus conducting a study on identifying drug related problems at Sanglah Hospital is a necessity.

The objectives of this research are to identify the most frequent drug related problems at VIP room Sanglah Hospital Bali and to introduce pharmacist's work at the hospital

METHODS

This was a cross sectional study which was conducted at VIP room Sanglah Hospital in Bali from January to March 2010. The inclusion criteria were the patients received more than 5 medications at a time and they were treated by more than 2 doctors. Data was collected from Patients Medical Record (PMR) by collecting patients' demography (age, sex), their current drug treatment both regular and irregular medications, and important laboratory findings. Drug Related Problems were identified using PCNE (Pharmaceutical Care Network in Europe) Drug Classification. This was used because this classification is more details and concise and it is commonly used in research or study. The classification of DRPs based on PCNE Classifications was as the following table. The DRPs collected were than analysed descriptively. The results of this study have also been discussed to other pharmacists at the hospital.

Primary Domain	Code V4	Problem
1. Adverse reactions Patient suffers from an adverse drug event	P1.1 P1.2 P1.3	Side effect suffered (non-allergic) Side effect suffered (allergic) Toxic effects suffered
2. Drug choice problem Patient gets or is going to get a wrong (or no drug) drug for his/her disease and/or condition	P2.1 P2.2 P2.3 P2.4 P2.5 *P2.6*	Inappropriate drug (not most appropriate for indication) Inappropriate drug form (not most appropriate for indication) Inappropriate duplication of therapeutic group or active ingredient Contra-indication for drug (incl. Pregnancy/breast feeding) No clear indication for drug use *No drug prescribed but clear indication*
3. Dosing problem Patient gets more or less than the amount of drug he/she requires	P3.1 P3.2 P3.3 P3.4	Drug dose too low or dosage regime not frequent enough Drug dose too high or dosage regime too frequent Duration of treatment too short Duration of treatment too long
4. Drug use problem Wrong or no drug taken/administered	P4.1 P4.2	Drug not taken/administered at all Wrong drug taken/administered
5. Interactions There is a manifest or potential drug-drug or drug-food interaction	P5.1 P5.2	Potential interaction Manifest interaction
6. Others	P6.1 P6.2 P6.3 P6.4	Patient dissatisfied with therapy despite taking drug(s) correctly Insufficient awareness of health and diseases (possibly leading to future problems) Unclear complaints. Further clarification necessary Therapy failure (reason unknown)

RESULTS

We recruited 23 patients met the criteria of selection. There were 7 women and 16 men in their age of more than 50 years old. The patients' diagnosis varies from chronic kidney disease, cardiovascular disease, pneumonia, until cancerous patients. The average numbers of medications received by the patients were 8. According to the PCNE Drug Related Problems (DRPs) classification, it was found that there were 29 DRPs related to drug choice problems, 15 were related to drug dosing problems, there were 7 DRPs associated with potential drug interactions, and 2 DRPs resulted from the patients experienced adverse drug reactions.

Of the 29 DRPs due to drug choice problems, the DRPs were felt into 5 categories. Three DRPs because patients received medications without indication, an example of this was silimarin was used by a patient where this drug has not been used widely in Indonesia. The DRP resulted from lack of evidence of the medication. Other seven DRPs were resulted from duplication of therapeutic groups or active drugs, for example, a 75 years old woman received two therapeutic group of laxantias (one was stool softener and the other was osmotic laxantia) at a time, the appropriate medication for constipation in elderly would be osmotic laxantia[1]. 5 DRPs were associated with patients received medications which were contraindicated to them, one of the examples was a patient with renal impairment received allupurinol which is excreted in the renal, this may result in worsening the renal function[2]. The other 8 DRPs were in relation to no clear indication for drug used, where in this study we found that a patient received fluconazole without indication. Another 6 DRPs were associated with no drug prescribed but clear indication, a good example of this was a patient with unstable angina pectoris, where based on the latest evidence, he should received beta blocker but this patient did not get it. The review study showed that beta blocker reduced mortality rate by 15%[3].

Of the DRPs related to Dosing problems, there were 13 DRPs were resulted from the patients received the drug dose too high or too frequent for example, omeprazole which should be administered once daily, they received twice daily. Seven DRPs were due to potential drug interaction which may result in ineffective drug therapy. For example, sucralfat and ciprofloxacin, where there should be at least one hourly separation of administration, they were taken at the same time. The ciprofloxacin administered might be ineffective, because, sucralfat reduces ciprofloxacin absorption in the gastric[2]. The last two DRPs was identified when the patient was recognized to suffer from adverse drug event after they were on 14 days of Celebrex (celecoxib-a cox II inhibitor) which may result in gastritis, a fact, which was shown, the patient were then on antacid afterward.

However, there was also limitation to this study. Ideally, in order to identify drug related problems in clinic, there should be collaborations with doctors who prescribed the medications, so that the pharmacist, would be able to identify the problems objectively. Besides, the number of patients were too small, thus, we could not be able to conduct further analysis on the results. Further study is needed to review the medication received by the patients more rationally in the future, hopefully, that would introduce the pharmacist's work at clinic as health provider.

CONCLUSION

The most frequent drug related problems identified at Sanglah Hospital was drug choice problems, the problems were mostly associated with inappropriate duplication of drug used and no clear indication for drug received by the patients. Further study is needed in order to commence pharmaceutical care at the hospital, so that the patients would receive the best outcome of their treatment.

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FIBRIN GLUE: NEW ADDHESIVE SUBSTANT FOR FIXATION ON PTERYGIUM SURGERY

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ABSTRACT

The aims of this research is to report the use of fibrin glue as a fixation on Pterygium surgery and to find out the advantages of the fibrin glue regarding the surgery time, postoperative condition and tissue adhesiveness In Sanglah General Hospital Denpasar Bali. The methods use include serial case report of 4 patients with nasal primary pterygium who undergone pterygium surgery with conjunctival autograft and use fibrin glue as the fixation instead of suturing. The surgery time, postoperative pain and discomfort and the adhesiveness of the graft were evaluated and followed up. Result showed that the surgery times were shorter than standard pterygium surgey with suturing, the pain and discomfort after the operation were minimal and the grafts were attached well. It can be concluded that the use of fibrin glue in pterygium surgery could reduce the surgery time as well as patient pain and discomfort and give better attachment of the conunctiva.

Keywords: Pterygium, conjunctival autograft, fibrin glue, thrombin, fibrinogen.

INTRODUCTION

Pterygium is a disfiguring and potentially blinding disease that in the advanced stages can require complex surgery for full visual rehabilitation.[1] Pterygium is fibrovascular wing of conjunctival tissue extending onto the cornea. [2] Pterygium generally situated on the nasal side, although sometimes occurs both nasal and temporally and rarely only on the temporal side. [3]

The prevalence rates of pterygium obtained for a number of populations vary widely. Indonesia as a tropical islands and equatorial country has a high prevalence rate of a pterygium. In Riau, Sumatra the prevalence rate of pterygium in adults over 21 was 10%.[1]

Surgical removal is the treatment of choice. Indications for surgery include visual impairment, restriction of ocular motility, chronic inflammation, and cosmetic concerns. [2] The most concerned problem in pterigium surgery is the prevention of recurrence. Over the past few decades, several surgery methods for pterygium have emerged, ranging from bare sclera procedure without microscopy to very complex approaches such as amniotic membrane transplantation and lamellar keratoplasty. [5]

The transplant of conjunctival autograft seems to be a preferable method, giving both the low recurrence rate and high safety.The most common method of autograft fixation is by means of suturing.[5] In our Institute, surgical excision of pterygium is performed with this technique.

However, the technique of attaching conjunctival autograft by means of suturing present several disadvantages, including complicated surgical technique, prolonged operating time, prolonged postoperative patient discomfort, and suture-related potential compilcations such as buttonholes, tissue necrosis and granuloma formation.[4, 5] Hence, it is logical to search for a material that can be applied to the eye surface just like a glue to substitute the sutures. [5]

Fibrin glue (Beriplast®) is a biological tissue adhesive composed of two components which mimic the natural fibrin formation. It has been used in neurosurgery, plastic surgery, ENT and ocular surgery. This glue has two components. One component contains fibrinogen mixed with coagulation factor XIII and aprotinin, and the other component contains thrombin and calcium chloride. Once the two components are mixed,

fibrinogen is converted into fibrin by the action of thrombin; fibrin then cross-linked by coagulation factor XIII to create a firm fibrin network, resulting in a fibrin clot. Aprotinin from bovine lungs prevent rapid fibrinolysis. [3, 4]

In the last 10 years, fibrin adhesive has been used to successfully close cataract incisions, attach soft tissue in oculoplastic surgery, attach conjunctiva in strabismus and in glaucoma surgery, threat leaking blebs and close macular hole in retinal surgeries. [2]

Fibrin glue use in pterygium surgery has been advocated by Koranyi as a new surgical technique for closure conjuctival graft. This sutureless technique called "cut-and-paste" is thought to result in reduced postoperative patient discomfort. [3]

In Indonesia especially in Bali, the used of Fibrin Glue is still not so popular compared to the used of suturing in Pterigium surgery. We would like to report the use of Fibrin Glue as a fixation on Pterygium surgery and find out the advantages of the fibrin glue regarding the surgery time, postoperative condition and tissue adhesiveness In Sanglah General Hospital Denpasar Bali.

MATERIALS AND METHODS

We reported serial case of 4 patients with Primary Pterygium who undergone pterygium surgery on July 2010 with conjunctival autograft and used of fibrin glue as a fixation for the graft instead of suturing. The patients were 2 men and 2 women, with Pterygium grade 2 – 3 on nasal and temporal side. Surgeries were performed at Instalasi Bedah Sentral (IBS) on Sanglah General Hospital.

The surgeries were done under local anesthesia by Lidocaine 2% sub conjuntivally. After standard ophthalmology sterile preparation and draping, the eye was exposed for operation using lid speculum. A Lidocaine 2% was injected into pterygium body to balloon out the conjunctiva. Then the pterygium was separated from the sclera and surrounding conjunctiva started from the head of pterygium on the cornea until half of the body. The tenons were also removed and cleared to prevent recurrence and granuloma formation. After that subconjunctival Lidocaine 2 % was injected again on the superior part of the conjunctiva to make a donor graft. The donor graft was excised 1-2 mm larger relative to the graft bed. Then the graft was flipped over cornea and excision was made from limbal attachment. The free graft then was placed on top of cornea and immediately flipped over and spread out onto bare sclera. During the pterygium removal and graft making, the assistant were preparing the mixing of the glue. The Fibrin glue then injected below the graft and excess glue and graft was removed and trimmed. Antibiotic and steroid eye drop was instilled at the end of surgery and pressure patch was applied to restrict the eyeball from movement or blinking.

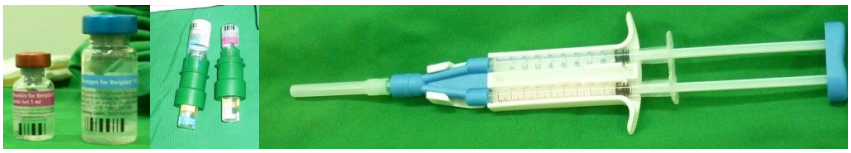
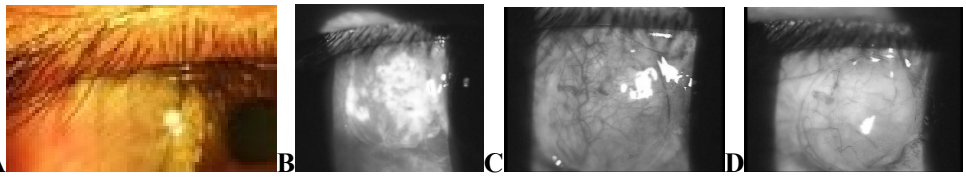


Fig.1. The Fibrin Glue. The Red one contained of thrombin and the Blue one contained Fibrinogen. The Mixture was placed on duplosyringe



A B C D

Fig.2A. Pterygium grade 2 on temporal side; 2B.one day after surgery; 2C.one week after surgery; 2D. One month after surgery. The conjunctival hyperemia was decreased.

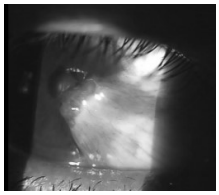
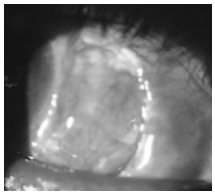
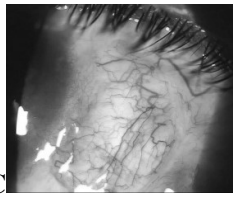
A  B  C 

Fig3.3A.Patient K with Pterygium grade 3 on nasal side; 3B one week after surgery; 3C one month after surgery.The conjunctiva was already white and clear.

RESULTS AND DISCUSSION

The surgery times for all patients were ranging from 20 – 25 minutes. It is faster when we compared to our standard pterygium surgery using vicryl 8.0 sutures for the fixation, that ranging from 40 – 60 minutes. Koranyi et al and Uy et al reported that fibrin glue could be used to attach the conjuntival autografts instead of sutures with reducing operating time and post operative discomfort.[3] The different sizes of the pterygium area encroaching onto the cornea and the co-operation of the patient would lead to differences in the required surgery time for dissection from cornea and making the graft. [5]

From the examination on day 1, all the patients have no difficulties in opening their eye, and no pain after the operation were felt as the patients of suturing graft usually felt. The patients just complain about redness and slight foreign body sensation on their eye regarding the inflammation on the graft we made and the complaint were disappeared after 1 week post operative. After 1 month post operative, the nasal side all looks like the surrounding conjunctiva, white and clear. Jin Jiang et al reported that postoperative subjective symptoms were much less with Fibrin glue used compared to suture used, especially in terms of foreign body sensation, which is the most common complaint of patients. In the fibrin glue group, by the third day there was only a very slight sensation reported. [5]

The entire graft was completely attached to underlying tissues from the first day after the surgery and there were no detachment at the part of the graft that we usually found in suturing patients. The Fibrin glue provides extensive contact with the underlying tissues and may thus inhibit the fibroblasts of the nasal tenon's tissue from proliferating towards the cornea, at the same time encouraging earlier vascularization of the graft, that lead to faster recovery and lower recurrence rate. [3,5]

Fibrin glue reduces the total surgical time because time required to place sutures is saved. The use of glue has been found to lower the risk of post-operative wound infection, contrary to conventional suturing. This can be attributed to accumulation of mucous and debris in sutures which may act as a nidus for infection. However, there is no data available to substantiate the low incidence of post operative reaction and infection. [6]

We conclude that using fibrin glue in pterygium surgery reduces surgery time, as well as patient pain and discomfort and give better attachment of the conjunctiva. All of these advantages were lead to better postoperative satisfaction for patient. Larger size study to know the efficacy of fibrin glue in Udayana University / Sanglah General Hospital is on process. Hopefully the Fibrin Glue could be the choice as an alternative of suturing for Pterygium Surgery.

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CHARACTERISTIC OF CAROTID INTIMA-MEDIA THICKNESS OF PREDIALYSIS CHRONIC KIDNEY DISEASE PATIENTS IN SANGLAH GENERAL HOSPITAL- A PRELIMINARY STUDY

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ABSTRACT

The objective of this research is to determine characteristic of carotid IMT as an indicator of subclinical atherosclerosis in predialysis CKD patients. BACKGROUND: Patients with CKD had high risk to develop cardiovascular disease. Extracranial carotid IMT was a valid marker of subclinical atherosclerosis. There was no routine examination of carotid IMT in predialysis CKD patients before, thus needed more investigation to get the data about subclinical atherosclerosis that happened in CKD patient, especially in Bali. METHODS: Descriptive cross sectional study of 43 predialysis CKD patients. IMT measurement was done at the common carotid and carotid bifurcation. Mean IMT was compared to the normal value to determine the presence of subclinical aterosclerosis. RESULT: Mean of the right, left and total carotid IMT was 0.69 ± 0.17 mm, 0.73 ± 0.17 mm, and 0.72 ± 0.17 mm, respectively. Most of subjects (77%) had subclinical atheroslerosis, that seen in CKD stage II-V. Thirty percents of the subjects had carotid plaque. Distribution of non calsified plaques were found in the higher eGFR group compare to calsified plaque. CONCLUSION : Most of the predialysis CKD subjects had subclinical atherosclerosis and it started in the early stage of CKD.

Keyword: predialysis chronic kidney disease, carotid intima-media thickness

INTRODUCTION

Accelerated cardiovascular disease is a frequent complication of chronic kidney disease (CKD).(1) Accelerated atherosclerosis can lead to increased prevalence of coronary artery disease, heart failure, stroke, and peripheral arterial disease. The high death rates of patients with CKD may reflect accelerated rates of atherosclerotic vascular disease and congestive heart failure.(2)

Extracranial carotid intima-media thickness (IMT) has been considered as a valid marker of subclinical atherosclerosis because it can represent early stage of atherosclerosis process, highly correlates with risk factors of atherosclerosis, and shows decreasing thickness after receiving treatment. Carotid IMT also acts as an indicator of atherosclerotic process in other vessels, and becomes a predictor of stroke and myocard infarct.(3,4) Detection of subclinical atherosclerosis in CKD patients is important because it can lead an appropriate treatment and also prevention from further cardiovascular complication.(1) Increase of carotid IMT can be measured using ultrasound, which is non-invasive, non ionizing and inexpensive radiological examination, but can give an accurate and reproducible measurement.(5)

So far, there was no routine examination of carotid IMT in predialysis CKD patients, especially in Bali. In the other hand, it is very important to determine whether a patient with CKD has already had atherosclerosis or not. The goal of this preliminary study is to determine the characteristic of carotid intima-media thickness (IMT) and to know about the distribution of subclinical atherosclerosis that already happened in predialysis CKD patients.

MATERIALS AND METHODS

This study was a descriptive cross sectional study of 18-65 year-old predialysis CKD patients in Sanglah General Hospital, Denpasar, Bali, and the protocol has already been approved by local ethical committee on research (Research and Development Unit of Udayana School of Medicine-Sanglah General Hospital).

Criteria and stage of CKD were based on the criteria of National Kidney Foundation /Kidney Disease Outcome Quality Initiative (2002).[11] Subjects met the criteria of predialysis when they did not need nor undergo dialysis for the treatment. Estimated Glomerular Fitration Rate (eGFR) was based on Cockcroft-Gault formula, that classified into five stage of CKD. Subjects excluded from the study if they had history of kidney transplantation, neck surgery or radiotherapy (that will potentially making a difficulty in evaluating carotid artery), smoking, undergone haemodialysis, and stroke.

Carotid IMT defined as a distance from edge of intima-lumen to the most far edge of tunica media-adventitia, not including arterial plaque.[7] Carotid IMT was evaluated using B-Mode ultrasound (Logiq-5; GE) with linear high resolution transducer (7,5 MHz) by a radiologist. Subject was in the supine position, the head turned toward the opposite site of the transducer. Visualization of the carotid wall was adequate if it could display two parallel-echogenic lines that separated by a anechoic structure between those two echogenic parallel lines.The distance between those two echogenic lines represented the thickness of intima-media complex.[5] Plaque defined as a protruded focal area toward the vessel lumen, and the thickness of this protrusion should be more than 50 % of the thickness of the surrounding wall.[4] Measurement of carotid IMT was done manually, based on technique reported by Ohya et al.[8], with modification by added IMT measurement of the bifurcation. IMT of the arterial far wall was measured using caliper. Carotid IMT was measured at eight location: three points at common carotid artery (1 cm, 2 cm, and 3 cm from carotid bifurcation) also at the bifurcation, that involved both right and left carotid artery. Measurement was done at the maximum thickness. The first 11 carotid IMT was examined by two radiologist separately to test interobserver variability, and coefficient correlation were excellent (r *interobserver* = 0.9 and r *intraobserver* = 0.9; $p<0.05$, respectively).

Criteria of subclinical atherosclerosis if IMT was higher than normal value for age, without any clinical symptom. Normal value of IMT for age was adapted from equation : IMT = (0,009 x year age) + 0,116.[7] Subject was interviewed to get their previous history and excluded when they met exclusion criteria. Physical examination was performed for measurement of blood pressure, heart rate, body weight and body height. Laboratory examination included fasting and post prandial blood sugar, also lipid profile. Carotid ultrasound was done for subject who met the inclusion criteria.

Descriptive analysis was done to get mean and standard deviation of carotid IMT in predialysis CKD patient, percentage of subclinical aterosclerosis, the presence and characteristic of carotid plaque.

RESULTS AND DISCUSSION

From this preliminary study during periode of May 2009 until August 2010, we found 43 predialysis CKD subject (38 male and 5 female), mean age 54 ± 6.45 year. One subject (2%) had eGFR >90 mL/minute/1,73m^2 or CKD stage I, 11 subjects (26%) had eGFR 60-89 mL/minute/1,73m^2 or CKD stage II, 18 subjects (42%) had eGFR 30-59 mL/minute/1,73m^2 or CKD stage III, 10 subject (23%) had eGFR 15-29 mL/minute/1,73m^2 or CKD stage IV, and 3 subject (7%) had eGFR <15 mL/minute/1,73m^2 or CKD stage V.

Initial process of atherosclerosis can be described by increase in carotid IMT.[7] Most of investigators determine IMT only from carotid far wall, but the other also combine with measurement of the near wall. Near wall visualization very depend on the *gain setting*, but combination of IMT measurement on its near and far wall possibly can increase the measurement precision without decreasing its validity.[11] In the most of study, IMT measurement was done only at the common carotid artery, while IMT measurement of internal carotid artery and carotid bulb was not often because of difficulty in visualizing the wall of internal carotid artery and carotid bulb. Difficulty in visualizing carotid wall can cause missing of many IMT picture, so that increase intraobserver and interobserver variability. Although difficult to visualized, atherosclerotic lesion happened early in the internal carotid artery and carotid bifurcation. Based on it, IMT measurement in this study was done in the common carotid artery adapted a methode by Ohya et al [8], with modification by adding IMT measurement of carotid bifurcation.

Our data showed that mean IMT for right carotid artery was 0.69 ± 0.17 mm, left carotid artery was 0.73 ± 0.17 mm, and total (right and left carotid artery) was 0.72 ± 0.17 mm. These result was lower than study by Rosas et al in USA, who performed a cohort study in 195 CKD patients. Their study involved only CKD stage II, III, and IV, with subject's mean age was 60 ± 9,5 year. They found that mean IMT of right carotid was 0.75 mm, left carotid was 0,79 mm and mean of IMT total was 0.77 mm.[9] This difference in result could be caused by differences in body size and ethnic between Bali people and American people. Szeto et al in their cohort study on 203 Chinese patients (mean age : 53,8 ± 10,9 year) with predialysis stage III and IV CKD, found that mean carotid IMT was 0.808 ± 0.196 mm.[10] Although our study and Szeto's study involved only predialysis CKD patients, our study involved wider range of CKD stages (we included all stages of CKD), that possibly influenced mean our IMT value.

From 43 subjects, we found that 10 subjects (23%) had normal carotid IMT value, and 33 subjects (77%) had abnormal carotid IMT value or in the condition of subclinical atherosclerosis. One of pathophysiology mechanism of cardiovascular disturbance in CKD is endothel disfunction. Decrease of the nitrite oxide bioavailability that caused by increase of the oxidative stress in the vessel wall is one of the factor that involved in CKD-related endothelial disfunction.[1] Condition that also has correlation with CKD such as diabetes, obesity and hypertension, and its own kidney disfunction, can stimulate activation of renin-angiotensin, oxidative stress, and increase asymmetric dimethylarginine (ADMA).Those condition can cause mild inflammation that associate with increase serum inflammatory biomarker. Through activation of renin-angiotensin system that happens in many type of kidney disease, Angiotensin II will stimulate NAD(P)H oxidase, that cause superoxide anion formation and play a role in endothel disfunction and remodelling process of vascular structure.[1] Those pathophysiology mechanisms can explain why we found most of subjects in this study had subclinical atherosclerosis.

Desbien and colleagues reported that decrease in the kidney function had association with rapid changes in carotid IMT, with mean carotid IMT changes was 0,02 ± 0.11 mm/ year.[12] The progressive increase in IMT can start its development in the predialysis stage [13]. Preston and colleagues [14] suggested that changes in the arterial wall started in the early stages of CKD and that it is associated with traditional cardiovascular risk factors, such as LDL and HDL levels. Similar with previous study, our study also found that subclinical atherosclerosis started to happen in the early stage of CKD group with eGFR 60-89 mL/minute/1,73m^2 or CKD stage II.

Calsified atherosclerotic often happen in CKD patient.[1] In this study, we found that carotid plaque was seen in 13 (30%) subjects with mean of plaque thickness was 1,93

± 0,53 mm. Calsified plaque was found in the group with lower eGFR (60-80 mL/minute/1,73m^2) compared to non calsified plaque (> 90 mL/menit/1,73m^2)

Calsification was found in the atherosclerotic plaque, tunica media, smooth muscle or elastic lamina of blood vessel. Several mechanisms of vascular calsification in CKD are passive precipitation of calcium and phosphate in relation with high level of extracelluler calcium and phosphate, influence of osteogenic transformation and hydroxiapatite formation inductor, and deficiency of calsification inhibitor e.g fetuin-A.[1,15] This possibly could explain why in our study calsified plaque was found in the group with lower eGFR.

Seventy-seven percents of the predialysis CKD subjects had higher IMT value and was suffered from subclinical atherosclerosis, and subclinical atherosclerosis seemed happen in the early stage of CKD.

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MOLECULAR ANALYSIS OF NS4B PROTEIN OF HEPATITIS C VIRUS SUBTYPE 1A

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ABSTRACT

The hepatitis C virus (HCV) non-structural (NS) protein 4B is known for protein-protein interactions with virus and host cell factors. However, at present only little is known about the corresponding protein binding sites and underlying molecular mechanisms. NS4B proteins of the yellow fever and dengue viruses also have similar topology with that of HCV NS4B indicating a common function for NS4B proteins in Flaviviridae. To predict and more understanding the molecular pattern of HCV NS4B we are performing bioinformatics study of HCV NS4B starting from NS4B of HCV subtype 1a. We aligned and performed molecular analysis of all HCV 1a NS4B complete coding sequences reported in GenBank. In total, 395 sequences were retrieved and aligned by ClustalW. The nucleotide and amino acid consensus sequences were revealed for further analysis using appropriate software. Phylogenetic analysis either by nucleotide or amino acid analysis has also been performed. We revealed the nucleotide and amino acid sequence consensus of HCV 1a NS4B. We found only aa 4-6, 8, 10, 14-17, 19-21, 23-24, 28, 32-33, 37, 47, 50-51, 53-57, 59-60, 62, 64-74, 79, 81-84, 86, 88-90, 95-96, 99-100, 102-105, 107, 109-113, 115-116, 119-121, 123, 126, 134, 136, 140, 144, 147, 151, 153, 155, 157, 159, 161, 164, 167-168, 171-174, 177-178, 180-186, 189-190, 192-198, 200-225, 227-230, 233-234, 237-238, 241-242, 244-246, 249, 251, 253, and 261 were found have no variation. The phylogenetic tree of all HCV 1a NS4B sequences was constructed using MEGA 4.0 software. Overall, the aa sequence of the NS4B protein of HCV 1a was highly conserved, indicating an important role for replication in vivo. However, amino acid variations may have relevant changes of physicochemical properties so that influence the replication efficiency. The amino acid variations found in the present report need further study.

INTRODUCTION

Hepatitis C Virus (HCV) is known as a major causative agent of liver disease. The incidence of HCV infection in worldwide population reached 3 %, which means 170 million people now infected with HCV (Alter, 2007; Holmberg, 2009). In addition, some reports show that 70-85 % infection cases of HCV last persistent. Only 20-25 % of patients with HCV infection can be cured completely, while others at high risk to reach a terminal condition (Lemon, et al., 2007).

HCV genome has a single stranded positive sense RNA along approximately 9600 base pairs. This virus has long Open Reading Frame (ORF) flanked by 5'-untranslated region (UTR) and 3'-UTR. This ORF can be functionally divided into three regions: the N-terminal, center, and C-terminal. N-terminal region associated with the region producing the structural protein: core protein (core/ C), envelope glycoprotein 1 and 2 (E1 and E2). Central region consists of proteins p7 and NS2. C-terminal region consists of non-structural proteins (NS3, NS4A, NS4B, NS5A, and NS5B) needed in the replication of RNA (Chevaliez and Pawlotsky, 2006; Lemon et al., 2007).

HCV is classified into 6 genotypes and each genotype was divided into several subtypes written in alphabet letters. HCV genotype and subtype determination is based on molecular analysis of the gene. Gene typically used in the determination

of HCV genotype is the genes considered to have potential for development of therapeutic targets and conserved well, which are the structural genes and NS5B gene which has polymerase activity (Sharma, 2010; Murayama, et al., 2010). However, recent publications reported the non structural genes also have promising potential for target of therapy, i.e. NS4B gene. NS4B has the capability to induce the replication site of viruses. If the NS4B activity can be inhibited, then the replication site will not be formed and HCV replication will fail (Lemon, et al., 2010). In point of virus pathogenesis, HCV NS4B also interesting for object study. HCV NS4B protein is already known important for protein interactions with virus and host cell factors. However, the corresponding protein binding sites and underlying molecular mechanisms have not been revealed yet. The similarity of yellow fever and dengue viruses NS4B proteins with that of HCV NS4B indicating a common function for NS4B proteins in Flaviviridae.

Since 2010, our group start to construct HCV cDNA library from all HCV genotypes isolated from blood specimens collected in Central Java Indonesia started from HCV 1a. To predict and more understanding the molecular pattern of HCV NS4B we are performing bioinformatics study of all HCV NS4B complete gene deposited in GenBank. Here we present our current analysis results of the NS4B gene of HCV 1a.

MATERIALS AND METHODS

Complete HCV NS4B Sequence

First, we downloaded all HCV NS4B sequence deposited in GenBank. We then manually selected and grouped depend on their genotype and subtype, followed by manually screening for their complete coding sequence. For this report, we only analyzed the complete coding sequence of NS4B from HCV 1a.

Aligment and Molecular Analysis of HCV 1a NS4B Sequence

In total, 395 sequences with complete coding sequence were retrieved from GenBank. The sequences then were aligned by ClustalW, for both of nucleotide and amino acid sequences, using MEGA4 (Tamura, et al., 2007). From this alignment we revealed the nucleotide and amino acid consensus sequences. We also performed phylogenetic analysis base on their nucleotide and amino acid sequences. The tested sequences were aligned by ClustalW with subsequent inspection and manual modification (Thompson, et al., 1994). The frequency of nucleotide substitution in each base of the sequences was estimated by the Kimura two-parameters method. A phylogenetic tree was constructed by neighbour-joining method, and its reliability was estimated by 1000 bootstrap replication. The profile of the tree was visualized with program of MEGA 4 (Tamura, et al., 2007).

RESULTS AND DISCUSSIONS

Different hepatitis C virus (HCV) proteins have been associated with resistance to therapy. However, the exact mechanisms of virus-mediated resistance are not completely understood. The importance of amino acid (aa) variations within the HCV nonstructural (NS)4B protein for replication efficiency and viral decline during the therapy is unknown. To solve this problem we try to perform a bioinformatic study starting with an aligmnent of all HCV NS4B complete coding sequence deposited in GenBank.

Hepatitis C virus (HCV) replicates its genome in a membrane-associated replication complex induces by its nonstructural protein 4B. NS4B also interacts with other viral nonstructural proteins and has been reported to bind viral RNA. In addition, it was found to harbour an NTPase activity and have a role in viral assembly. HCV NS4B it

self is an integral membrane protein and has been predicted to comprise four transmembrane segments in its central part. The four predicted transmembrane segments of NS4B are amino acids 72 to 92, 101 to 121, 136 to 156, and 172 to 197. The N-terminal part comprises two amphipathic alpha-helices of which the second has the potential to transverse the membrane bilayer, likely upon oligomerisation. The C-terminal part (amino acid residues 229 to 253) comprises a predicted highly conserved amphipathic alpha-helix, a membrane-associated amphipathic alpha-helix that mediates membrane association and is involved in the formation of a functional HCV replication complex and two reported palmitoylation sites (reviewed in Gouttenoire, et al. 2010).

HCV NS4B targeting and retention in the ER results from the concerted action of several NS4B structural elements. The first two transmembrane domains (TMs), within the NS4B N-terminal half (residues 1-130) were found to mediate association of the NS4B-GFP chimeras with ER membranes. Both TM1 and TM2 are required for ER anchoring and retention but are not sufficient for ER retention. Sequences upstream of TM1 are also required. These include two putative amphipathic alpha-helices and a Leucine Rich Repeat-like motif, a sequence highly conserved in all HCV genotypes, including that of HCV 1a. The N-terminal 55 peptidic sequence, containing the 1st amphipathic helix, mediates association of the 55N-GFP chimera with cellular membranes including the ER, but is dispensable for ER targeting of the entire NS4B molecule. The C-terminal 70 peptidic sequence can associate with membranes positive for ER markers in the absence of any predicted TMs (Boleti, et al., 2010).

In the N-terminal portion of NS4B there is a determinant for membrane association represented by amino acids (aa) 40 to 69 comprises an amphipathic alpha-helix extending from aa 42 to 66. This amphipathic alpha-helix has the potential to transverse the phospholipid bilayer as a transmembrane segment, likely upon oligomerization.

Alanine substitution of the fully conserved aromatic residues on the hydrophobic helix side abrogated membrane association of the segment comprising aa 40 to 69 and disrupted the formation of a functional replication complex (Gouttenoire J, et al., 2009). A putative amphipathic helix on amino acid residues 1 to 26 of the NS4B N terminus is crucial for virus replication (Elazar, et al., 2004). HCV NS4B already known has luminal loop around aa 112 (Lundin M, et al., 2006). In addition, a nucleotide-binding motif located between the second and third transmembrane domains of NS4B (residues 129 to 135 of NS4B) has also been identified (Einav, et al., 2004).

NS4B has lipid modifications (palmitoylation) on two cysteine residues (cysteines 257 and 261) at the C-terminal end. The main palmitoylation sites are mapped within the C-terminal cytoplasmic region (amino acids [aa] 198 to 261). Cys257 is the main palmitoylation site while Cys261 is also palmitoylated to a certain extent. Siteouttenoire-specific mutagenesis of these cysteine residues on individual NS4B proteins and on an HCV subgenomic replicon showed that the lipid modifications, particularly of Cys261, are important for protein-protein interaction in the formation of the HCV RNA replication complex. Cysteine 261 is well conserved, partly because cysteine 261 is also the recognition site for NS3 protease cleavage. Cys261, but not Cys257, of NS4B is indispensable for subgenomic-replicon replication. NS4B can undergo polymerization. The main polymerization determinants were mapped in the N-terminal cytosolic domain of NS4B protein; however, the lipid modifications on the C terminus also facilitate the polymerization process. The lipid modification and the polymerization activity could be two properties of NS4B important for its induction of the specialized membrane structure involved in viral RNA replication. Cysteine 257, the main palmitoylation site, is well conserved in genotypes 1, 2, and 4. In genotypes 3, 5, and 6, aa residue 257 is tyrosine or threonine (Yu, et al., 2006). Both tyrosine and threonine have a hydroxyl group. Other than cysteine residues, palmitoylation may occur on serine or threonine residues to form an acyloxyester (Shi, et al., 2003).

In our study, only aa 4-6, 8, 10, 14-17, 19-21, 23-24, 28, 32-33, 37, 47, 50-51, 53-57, 59-60, 62, 64-74, 79, 81-84, 86, 88-90, 95-96, 99-100, 102-105, 107, 109-113, 115-116, 119-121, 123, 126, 134, 136, 140, 144, 147, 151, 153, 155, 157, 159, 161, 164, 167-168, 171-174, 177-178, 180-186, 189-190, 192-198, 200-225, 227-230, 233-234, 237-238, 241-242, 244-246, 249, 251, 253, and 261 were found have no variation. The amino acid variations may have relevant changes of physicochemical properties so that influence the replication efficiency and or viral pathogenesis. The amino acid variations found in the present report need further study.

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IN VITRO RELEASE PROPERTIES OF IBUPROFEN-LOADED MICROSPHERES BASED ON BLENDS OF POLY(LACTIC ACID) AND POLY(ε-CAPROLACTONE) USING POLYVINYLALCOHOL AS EMULSIFIER

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Microencapsulation of ibuprofen in polyblend (poly(lactic acid) and poly(ε-caprolactone) has been studied by using the oil-in-water emulsification solvent-evaporation technique. Methylene chloride was used as the dispersed medium and water as the dispersing medium. The effects of formulation variables including PLA : PCL ratio and emulsifier (polyvinyl alcohol) concentration on the entrapment efficiency were examined. The ibuprofen release rate from the prepared microspheres was also studied by using the dissolution test, and the surface structures of microcapsules after dissolution were observed by using scanning electron microscope (SEM). The results showed that the microspheres prepared from the blend of PLA and PCL are generally spherical. It was also found that the efficiency of ibuprofen microcapsules increased with increasing the composition of PCL in polyblend (PLA with PCL). In vitro drug release profiles for 6 hours, which were performed in an intestinal-like medium (pH 7.2), showed high dissolution profiles of microcapsules made from the blend of PLA and PCL with a ratio of 9 : 1. The surface structure of microspheres after dissolution process showed opening of pores on the surface of microspheres.

Keywords: Ibuprofen, polyblend, poly (lactic acid), poly (ε-caprolactone), emulsification.

STUDY ABOUT INDONESIAN BLUE BOTLE JELLYFISH (*Physalia Phisalis*)VENOM FROM THE WATERS OF PAPUMA JEMBER

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The recent bloom of an indonesian poisonous jellyfish venom has caused a danger to se bathers and fishery damages in the waters of Indonesia. By using light microscope, th species of poisonous Indonesian Jellyfish was identified. The present study investigated thactivities of crude venom extract of indonesian poisonous jellyfish using ex vivo, in vitro, an in vivo study. The protein component was separated by using SDS-PAGE. The Jellyfis venom showed an irritation and hemolytic activities in both fresh venom and boiled venom suggesting its possible that jellyfish venom has many components of toxic or non-toxi which can be dependent on their specific cellular target. At the present, it is not clear whic component of jellyfish venom is responsible for the vascular irritation effect observed in th present study. It can be one of the above or unidentified one yet. To clarify this, furthe studies will be needed in the near future.

Keyword: Jellyfish; crude venom extract; ex vivo; in vitro; in vivo.

INFECTIVITY OF LYTIC PHAGE TO EPEC (ENTEROPATHOGENIC ESCHERICHIA COLI) FROM DIARRHEAL PATIENTS IN INDONESIA

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Some EPEC isolated from diarrheal patients in Indonesia were resistant to tetracycline and ampicilline. The purpose of this study was to isolate and determine the potential infectivity of the phage strains that can reduce EPEC population. Phages were isolated from waste water, all phages isolated tested their ability to form plaque, identified their morphology, their infectivity determined, the impact of phage lyses to cell morphology of EPEC were observed. Four isolated phages specific for EPEC strain. Other nonpathogenic strains of E. coli are not lyses by phage. This indicates that susceptibility of bacterial strains to phage attack depends on the specificity of receptor molecules on the surface of EPEC to the phage. Electron microscope showed that the phage has a hexagonal head (81.56 nm and 103.11 nm in lengthwidth). EPEC cells are destroyed faster at 25 minutes after infection the phage. Many cells EPEC surface is bound by phage and some cells showed lysed. At 30 minutes after phage infection, EPEC cells mostly destroyed and the population of EPEC cells were significantly reduced after five hours of phage infections. By added 200 µl of phage, decline higher to EPEC population. The results of this research may provide alternative solutions to overcome the problem of diarrhea with the application of phage-friendly environment (biocontrole).

Keyword: EPEC, antibiotic resistance, lytic phage, diarrhea disease, biocontrole

ANTIBACTERIAL EFFECT OF LACTOFERRIN AND LACTOFERRIN HYDROLYZATE ON *Enterobacter sakazakii*

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Enterobacter sakazakiiis an opportunistic pathogen widely found in the environment. It iscaused oflife-threatening neonatal sepsis and meningitis complicatedby the development of brain abscesses.Enterobacter sakazakii also kill infected infants and has been associated with powdered formula. E. sakazakii grows very rapidly in reconstituted infant formulae kept at room temperature. It is particularly well adapted to growth at temperatures around 37 – 44°C.Lactoferrin and lactoferrin hydrolyzate has been known to have antibacterial effects on some pathogenic bacteria. However, testing the antibacterial effect of lactoferrin and lactoferrin hydrolizate againts bacteria E.sakazakii has never been done. To evaluate the antibacterial effect of lactoferrin (LF) and lactoferrin hydrolysate (LFH) against the Enterobacter sakazakii in peptone yeast extract glucose (PYG) broth. The research used Macro Broth Dilution Method. Lactoferrin and lactoferrin hydrolizate was assayed at concentrations of 2, 4, 6, 8, 10, and 12 mg/ml. Determination of antibacterial effect of lactoferrin and lactoferrin hydrolyzate to the bacteria E.sakazakii based on The MIC (Minimum Inhibitory Concentration). The inhibitory was determined by the difference of absorbance before and after incubation. The absorbance oflactoferrin and lactoferrin hydrolyzate at concentrations of 2, 4, 6, 8, 10, and 12 mg/ml increase after incubation. The increased of absorbance decreases withthe high concentration of lactoferrin and lactoferrin hydrolyzate. Increased absorbance of lactoferrin was lower than lactoferrin hydrolizate.In this study the MIC (Minimum Inhibitory Concentration) was not found. The results suggest that lactoferrin and lactoferrin hydrolyzate have no antibacterial effectto the bacteriaEnterobacter sakazakii.

Keywords: antibacterial effect, lactoferrin, lactoferrin hydrolyzate, Enterobacter sakazakii, MIC

A NOVEL OF REPLACING CACO-2 CELL WITH ENTEROCYTE MICE TO DETERMINE BACTERIA ADHESION ACTIVITY IN VITRO

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Adhesion is a key factor of bacteria internalization to the host through transepithelial neutrophile cell migration. In vitro adhesion ability of bacteria is usually performe using of Caco-2 cell which is costly and need an extra care handling. This study aims to replace caco-2 cell with enterocyte mice for determining adhesion ability of bacteria. Enterocyte cell of a healthy BALB/c mice age of 3 months were isolated following the method of Nagayama (1995). Then, the cells were employed to determine adherence ability of Bifidobacterium sp towards S. typhi. A good promising results was obtained in this experiment, in which the enterocyte can be applied to determine adhesion indices of Bifidobacterium sp towards S. typhi. It was obtained thata number of 19.5 bacteria were adhered on 1 enterocyte-cell BALB/c mice, compared to the only of 15.04 of S. typhi adhered on 1 enterocyte-cell BALB/c mice. Conclusions that can be drawn from this research are the finding of enterocyte isolated from mice to replace the use of caco-2 cells for assessing adherence ability of Bifidobacterium sp towards adhesion of S. typhi. Future work that can be carried out are further researches concerning of the use of this enterocyte to performe other bacteria adhesion test.

Keywords: adhesion, internalization, enterocyte mice

POSTER PRESENTATIONS: AGRICULTURE

ORNAMENTATION STRUCTURE OF FLOWER POLLEN ON ENTHOMOPHYLI POLLINATION

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ABSTRACT

Research has been carried pollen from many plant species, with the aim to find out from the wall of pollen morphology in the interest pollination carried by insects (entomophyli) .Material taken from plants belonging to Monoicotyledoneae and Dicotyledoneae. Preparation was done by acetolysis method and observation using light microscopy. From the results showed that the pollen in flowers polination carried by insects (entomophyli) generally have a flat surface, a bit rough with the noise - kind of ornamentation.Ornamentation in the form of spine or sculpture with a different type for each species.

Key word: Pollen, Entomophyli, ornamentation

INTRODUCTION

Flowering is one aspect of plant life. Shape and color of the beautiful flowers, a neat header regular interest, and the smell of fragrant flowers that invite insects to come looking for honey .Fruit and seed crops depend on pollination results. Insects can cause pollen move from one flower to another flower. When the pollen is already carried by the fall on the stigma, it happens pollination. Once the importance of the role of insects in pollination, it is interesting to note in particular flower pollen morphological structure, as in; pollen pollination plays an important role especially in the event of conception.

The beauty of colors, flower shapes, as well as a special smell and produce honey or nectar to attract different types of insects, so the animals - these animals often visit flowers and pollinating accidentally (Arnet and Braungart, 1970).

Important role as a pollinator insects and pollen types are shown variations associated with the visited flower. Some insects are helpful in pollination are bees, flies, wasps, butterflies and beetles (Bhojwani and Bhatnagar, 1978).

Pollen produced by terrestrial plants is the privilege of development resistance of exterior walls. The outer wall is eksin that contain wax or some kind of dammar. This helps the wax layer scattering. Pollination interest carried by insects, the pollen surface ornamentation spine and there are carved images to assist in the scattering (Kapp, 1969).

MATERIALS AND METHODS

The samples taken were from the group Monocotyledoneae and Dicotyledoneae, respectively - each five species. The group of monocotyledoneae is *Lilium longiflorum, Gladiolus sp, Amaryllis sp, sp Arachnis and Vanda sp*, and the group of dicotyledoneae is *Caesalpinia pulcherrima, Crotalaria sp, Sesbania grandiflora Pers, Hibiscus rosasinensis and Nymphaea sp.* Preparation was done by acetolysis method (Soerodikoesoemo, 1983).

Pollen taken from anthers collected in a test tube (vial), which is already filled with glacial acetic acid, then left for 24 hours. Then transferred into a centrifuge tube, and centrifugation performed. The liquid was removed and replaced with glacial acetic acid with concentrated sulfuric acid with the ratio 9: 1.Heating in a water bath from room temperature to boiling. After boiling heating was stopped and settling for 15 minutes. Subsequently conducted centrifuge, then the liquid was removed and replaced with distilled water. Observation with lightmicroscope, was still dark when bleaching is

carried out using glacial acetic acid 2 cc of sodium Chlorates 2-3 drops 2-3, drops of HCl, and then centrifugation. Furthermore, the liquid removed and the sediments were washed with distilled water 2-3 times, with each laundering conducted centrifuge again. Last distilled water was replaced with glycerin jelly that has been heated and mixed with safranin dyes.Further observation with light microscope.

For the identification carried out:

- Mesurement of polar axis, and Equatorial.
-Determining of index P/E
-Determine the type of pollen (by Erdtman, 1952).
-View pollen ornamentation on the walls.

Photo shoot done using Olympus brand camera C 35 A, 100 ASA Kodak film.

RESULTS AND DISCUSSION

From 10 species observed were obtained the following results:

- Tabel 1. Average Polar axis (P) and Equatorial (E) and the index
- P / E of flowers with pollination by entomophyli.

No	Spesies	Average P axis	Average E axis	Indeks P/E
1	*Gladiolus sp*	77.42 ± 1,580	64. 15 ± 0.865	1.21 ± 1.008
2	*Amaryllis sp*	87.53 ± 1.413	74.89 ± 0.865	1.17 ± 0.006
3	*Lilium longiflorum*	108. 07 ± 0.865	91.01 ± 0.865	1.19 ± 0.002
4	*Vanda sp*	33.18 ± 1.58	30.336 ± 0.707	1.10 ± 0.026
5	*Arachnis sp*	26.68 ± 0.00	26.68 ± 0.00	1.00 ± 0.000
6	*Caesalpinia pulcherrima*	71.73 ± 1.801	68.89 ± 0.865	1.04 ± 0.013
7	*Crotalaria sp*	23.38 ± 1.322	19.28 ± 1.322	1.21 ± 0.016
8	*Sesbania grandiflora Pers*	30.97 ± 1.413	27.49 ± 1.413	1.13 ± 0.007
9	*Nymphaea sp*	35.71 ± 1.413	31.92 ± 1.713	1.12 ± 0.017
10	*Hibiscus rosasinensis*	82.40 ± 1.517	82.40 ± 1.400	1.00 ± 0.000

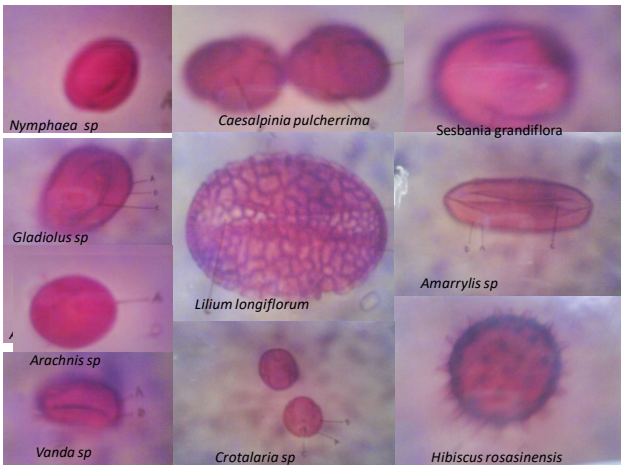

Figure 1. Ornamentation structure pollen of *Lilium longiflorum , Gladiolus sp, Amaryllis sp, sp Arachnis and Vanda sp, Caesalpinia pulcherrima, Crotalaria sp, Sesbania grandiflora Pers, Hibiscus rosasinensis and Nymphaea sp.*

Tabel 2. Pollen description of species

No	Species	Pollen Description
1	*Gladiolus sp.*	Kolpus a rather long and coiled, type subprolata, rough surface is full of warts (verucate)
2	*Amarillis sp.*	Kolpus lengthwise on a polar axis, ellipse, type subprolata, ornamentation in the form of a small spine and short on the surface
3	*Lilium longiflorum*	Kolpus lengthwise on a polar axis, ellipse, type subprolata, ornamentation in the form of a small spine - small and short on the surface
4	*Vanda sp.*	Kolpus polar axis elongates, tpe prolata sferoidal, rough surface is full of warts (verucate)
5	*Arachnis sp.*	rounded shape, oblate type sferoidal - prolata sferoidal, ornamentation is not clear
6	*Caesalpinia pulcherrima*	Having a third aperture and three porous, rough surface with warts (verucate), type prolata sferoidal.
7	*Crotalaria sp.*	Surface uneven, notched with three kolpus, type subprolata
8	*Sesbania grandiflora* Pers	rough surface is full of warts (verucate), has three kolpus, type prolata sferoidal
9	*Nymphaea sp.*	uneven surfaces, kolpus rather long, sferoidal prolata type, ornamentation is not clear
10	*Hibiscus rosasinensis*	ornamentation in the form of a long spine on the surface (echinate), oblate type sferoidal - prolata sferoidal.

In general, pollen of flowers pollination carried by insects (entomophyli) has an uneven surface, a bit rough, with exquisite ornamentation such as spines, warts and the presence of sculptures - sculpture such as nets. Type of individual - each a different species, although included in the same family, such as *Crotalaria sp, Sesbania grandiflora* Pers and *Caesalpinia pulcherima*, although all three are included in each type of the Leguminosae family is different because each type of index P / E is different. Likewise with *Vanda sp and sp Arachnis* which both belong to the family Orchidaceae, also different types. But *Gladiolus sp, sp and Lilium longiflorum Amaryllis sp*, all three including the order Liliales (Pudjoarianto and Budi Rahayu, 1977) have the same type of pollen that is subprolata. Accordingly Index P / E is to determine the type of pollen from each - each species. In Summary, Pollen on flower pollination done by insects (entomophyli) has the structure: Uneven surfaces. Slightly rough surface with ornamentation in the form of spine, the curve,warts and sculpture in the form of a net. Type of each type differs depending on the index P / E.

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WEIGHT LOSS AND RESPIRATION RATE OF SALACA FRUIT IN MODIFIED ATMOSPHERE USING POLYETHYLINE PLASTIC PACKAGING AT VARIOUS PERFORATION

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ABSTRACT

The aim's of this reaserch were to study weigh loss of Salaca fruits from consequence of modified atmosphere packaging and to study of respiration rate of Salaca fruit to preserve at modified atmosphere packaging.The treatment of this reasearch was packaging with polyethyline plastic package or passive atmosphere with thickness 0.02 mm and perforation diameter hole is 0.6 cm. The various perforation is 0, 4, 8 and 12 hole. The storage time was set at 0, 2, 4, 6, 8, 10, 12 day in room temperature.The result showed that the weigh loss of Salaca fruit was increased during the storage time. The weigh loss rate was slowly at 8 hole polyethyline plastic perforation packaging. At the begining of the storage time, the respiration rate have been increased while in the midle have been decreased. Finally, in the end of the storage time have been increased. The respiration rate was slowly at 8 and 12 hole polyethyline plastic perforation packaging.

Keywords: Modified Atmosphere, Polyethyline plastic perforation packaging, Salaca Fruit

INTRODUCTION

There is inclination that consumer be apt to food product which are fresh, practical and high quality (Daugherty, 1990; Labuza and Breene, 1989; Myers, 1989). In last decade in the U.S.A. showed that consumption of fresh food product increase in 12%, frozen food product increase by 10% however canning food product decrease by 10%. Therefore Indonesian has good opportunity of exporting fruits and vegetables. In order to increase the export value of Agro industry, fruits and vegetables should be care and handle vary well.

There is a problem that salaca is perishable and have short of time keeping. Senescence will occur after maturation and continued with damage due to the decrease of microbe defenses. Damage is usually caused by mechanical, physical, microbe, and physiological. Mechanical damage frequently is caused by chafe, peeled off, and bruised. Microbe damage is caused by infection and microbe activity. Physiological damage occurs due to internal metabolism reaction resulting in putrefaction.

Salaca (*Salacca edulis*, Reinw) is horticulture origin of Indonesia. Production was high, reaching 662546 ton in 1995 (Anon, 1995 in Agung *et al*., 1999). The cultivation areas of salaca in Indonesia are Bandung (Batujajar), Tasikmalaya (Manonjaya), Jogjakarta (Sleman), Malang, Sumatera Utara, Sulawesi Selatan, Jawa Tengah (Ambarawa and Magelang) and Bali.

The increasing production and fulfillment of demand should be balanced with post harvest treatment. This is considered that salaca as other fruits are easy of damaging. The negative environment factors along the storage cause of fast decreasing quality resulting in short storage time.

In order to increase the storage time and avoid damage, delaying the mature is performed by decreasing oxygen absorption and release of CO_2 of salaca. This can be done with atmosphere modification. Low oxygen and high CO_2 in modified atmosphere give influence in decreasing respiration rate, etilen production rate, retard the maturation and others relate to mature such as soften of fruit, change of color, lose of sugar and retard the putrefaction (Brecht, 1980; Kader, 1980).

An appropriate post harvest treatment of salak give result in added value of long storage time which mean profit for the farmer, seller and other parties. The aims of the research are: Studying changes of weight of fruit as result of storage in modified atmosphere. Studying respiration speed of salak which are stored in modified atmosphere as function of O_2.

METHODS

The research steps are preparation, respiration speed quantification, changes of weight of fruit as result of storage in modified atmosphere, descriptive and graphical analyze.

A. Treatment

The treatments are polyethylene plastic cover with thickness are 0.6 cm (positive modified atmosphere) and storage time. The perforation of polyethylene plastic cover are 0 hole (without perforation), 4 hole, 12 hole and the storage time are 0, 2, 4, 6, 8, 10, 12 days. The samples treatment and one which is uncovered and used as control, are stored in room temperature.

B. Respiration rate

a. In tight close container

Close system is used in this experiment (Hangar *et.al*., 1992 and Lee, 1997 in Song *et.al*., 1992). Three samples are put in glass container (stoples) with volume of 2,126 lt. Glass container is closed tightly (with glue/paint/vaseline to avoid leak).

In the middle of the lid, a hole cover with rubber is set to take the gas sample. This container is put in room temperature. Gas samples are collected every 2 days in 12 days. O_2 and CO_2 concentration are detected with cosmotector. Type XP-314 cosmotector is used for O_2 and XP318 for CO_2. The relieved volume in the container is also counted. These treatments are repeated for three times.

b. In polyethylene plastic

Three samples are put in a sealed 6,80 lt volume of polyethylene plastic. A hole cover with rubber is set in the bottom part of the polyethylene plastic to make easy for taking the gas sample. The edge of the hole is sealed with glue/paint/vaseline to avoid leak. And then is put in room temperature. Gas samples are collected every 2 days in 12 days. O_2 and CO_2 concentration are detected with cosmotector. Type XP-314 cosmotector is used for O_2 and XP318 for CO_2. The relieved volume in the container is also counted. These treatments are repeated for three times.

c. Weight Loss

Sample of salaca to weighted in over a particular period storage time and then in final observation as compared to initial weight before storage.

Equition to calculate weight loss, as:

$$\text{weight loss} = \frac{\text{Initial weight} - \text{Final weight}}{\text{Initial weight}} \times 100\ \%$$

RESULTS AND DISCUSSION

1. Weight Loss

Weight is a decisive factor of fresh fruits. Changes of weight of Bali's salaca show that there is a variation of weight loss of Bali's salaca in storage. The graph of weight loss changes can be observed in figure 1.

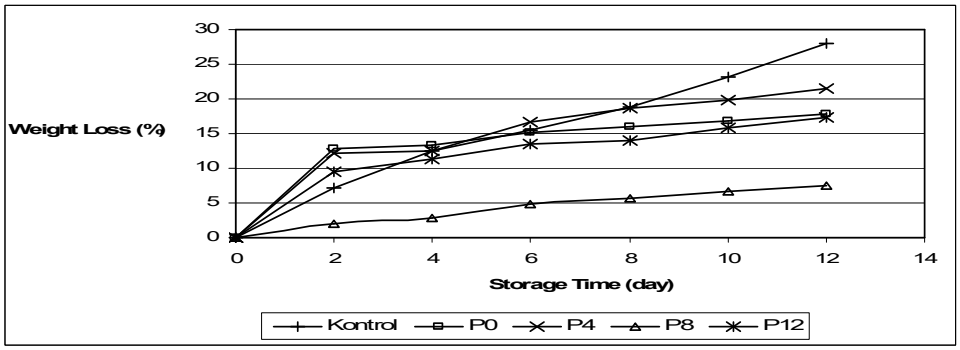

Fig 1. The Weight loss of Bali salaca fruits during the storage time

Figure 1. shows that the weight level of control of Bali's salaca and all the Bali's salaca treated with different perforation of polyethylene plastic container decrease in the storage. Decrease of weight is caused by saturated decomposing propectin to pectin enzyme activity, the solid state rate is caused by the change of cell size and turgor. The reason of weight become loss are high level of respiration activity and transpiration of the fruit cause lost of liquid therefore cell size and pressure of inside cell to the cell wall decrease (Suter, 1988).

2. Respiration Rate

The data of O_2 respiration rate of Bali's salaca in storage is presented in figure 2. Figure 2 shows that in every treatment respiration rate of O_2 changed rapidly, and then were going slowly, finally in a certain time changed rapidly until the last period of storage.

Figure 2 shows that O_2 respiration rate are increase in the beginning of storage and then decrease however increase again till the last period of storage. Decrease of respiration rate is caused by decrease of the substrate for respiration. As addition, the O_2 in the container is used by the fruits for respiration and substrate oxidation. With limitation of O_2 in the container causing the delay of chlorophyll degradation, low production of C_2H_4, decrease of production rate of ascorbate acid, change of composition of unsaturated fat and degradation of pectin is not fast as in nature. These things reflect as the delay of mature of fruit therefore storage capacity of the fruit is long (Amiarsi *et. al*., 1996).

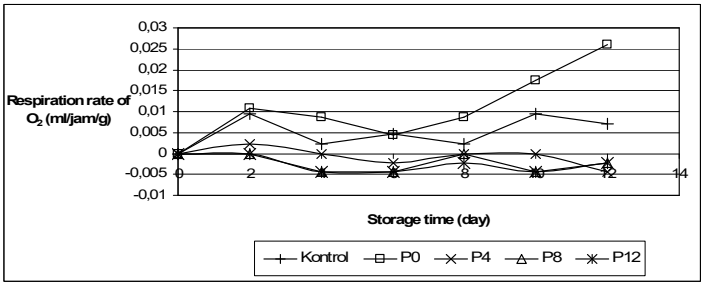

Fig 2. O_2 Respiration rate of Bali salaca fruits during the storage time

CONCLUSION

- The weight of Bali salaca fruit was decreased during the storage time.
- At the beginning of storage time, the result showed that the respiration rate of O2 have been increased while in the midle have been decreased.
- We can used polyethyline plastic packaging at perforation with 12 hole in modified atmosphere.

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SOYBEAN (*Glycine max* (L) MERRILL) *in planta* TRANSFORMATION OF SUNFLOWER ALBUMIN GENE USING *Agrobacterium tumefaciens*

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ABSTRACT

Introduction of sunflowers albumin gene into the soybean cells using *A.tumefaciens* in this research is based on the natural ability of *A.tumefaciens* which can transfer a particular DNA segment (T-DNA) of the tumor inducing (Ti) plasmid into the nucleus of infected cells where it is then stably integrated into the host genome and transcribed. The cloned albumin gene in pBI121 plasmid of *E.coli* was conjugated into *A.tumefaciens* LBA4404 using the method of triparental mating which resulting in *A.tumefaciens* LBA4404 containing pAL_{4404} and pSW_{600} plasmids. This *A.tumefaciens* was then used for transformation of soybean plants using *in planta* transformation method. As the results, transformants showed deference in branches, 35.29% more, compare to nontransformants. Transgene of 600 bp was detected by PCR analysis in transformants but not in nontransformants.

Key word: Transformation, sunflower albumin gene, *A.tumefaciens*, soybean.

INTRODUCTION

Soybean contains high level of protein, it's about 35-50% per dry weight, and therefore it is used for the protein resource of animal feed and human foods. The supply must be consistent and continue, and fulfill the quality and quantity. The quality of soybean as a resource of protein for animal and human is decided by the proportion of sistein and metionin (Sunarpi and Anderson, 1997). Any attempt to increase the sistein and metionin content in soybean protein conventionally was not succeed. The problem is caused basically by the low mobility of sulfur as organic compound in plant. In this case the biotechnological approach is considered to be the suitable way to resolve that problem by introducing *Sfa8* gene of sunflower that encodes protein with high level of sulfur. It was reported that *Sfa8* transferred into alfalfa and vicia was increasing the methionine and cysteine in vegetative and generative plant organ. However rarely reported that *Sfa8* expressed in the genitive of plant, therefore the indication of protein biosynthesis with high level of sulfur in soybean lead to the possibility of *Sfa8* gene was expressed in plant cell (Sunarpi and Anderson, 1997).

In planta transformation method is widely used now because it is no need of *in vitro* step which are need long time and expensive. *In planta* transformation method on soybean using *agrobacterium* as a vector principally target the meristematic embryo cells. The vector contain binary plasmid of transgene (*Sfa8* construct) and inoculated on seedling of soybean plants. Most of the transformants are chimera but there are also a few of transgenic plants which are the entire cell in the plant are transformed cells.

Therefore in this study, *Sfa8* gene transfer by *In planta* transformation method into soybean plant was studied with hope to obtain soybean transgenic plant which produce high quality of protein for human food and animal feed.

MATERIALS AND METHODS

Triparental mating

The triparental mating technique allows plasmids to be constructed and propagated in one strain A, and then mated with a broad-host range plasmid containing strain B.

Once the broad-host range plasmid has conjugated into strain A, it can now mobilize the engineered plasmid into another strain C.

The process require a *helper strain*, carrying a conjugative plasmid (*E.coli* JM 109 contain pBI121+600 *Sfa8*) that codes for genes required for conjugation and DNA transfer; a *donor strain*, carrying a mobilizable plasmid that can utilize the transfer functions of the conjugative plasmid (*A.tumefaciens* LBA4404 contain pAL4404); a *recipient strain*, which wish to introduce the mobilizable plasmid into (*E.coli* HB 101 contain pRK_{2013}).

***In planta* transformation method for soybean plant**

Soybean seeds were soaked in water for 15 minutes to stimulate for seedling. There after the soybean seeds growth into seedling, axilar shoot and new shoot above the cotyledon were cut and then inoculate with *A.tumefaciens* LBA_{4404} (pAL_{4404} + pSW_{600}). Soybean seedling inoculate with water were used as control and growth together in separate pot (polybag) contain sterilized sand and soil (1:1). After 1 or 2 weeks new shoot growth above the cotyledon and the leaf were used for detection of transgene (*Sfa8*) by PCR. The phenotype of transformants and control plants were also observed for the appearance since pBI121 contain growing hormone.

PCR analysis

PCR analysis for detection of transgene in soybean plants of transformant was carried out as follows. The following PCR primers were designed such that the DNA segment (760 bp) spanning *iaaM* and Tn*5* would be amplified; primers for the 1st PCR, 5' GGGGATCCATGGAAGGTTTTCGATCG 3' for forward direction, 5' GGGAATTCCC GGGTTTACATTTGGCATGG 3' for reverse direction. The genomic DNA (about 100 ng) was added to a reaction mixture of 25 µl of final volume (50 mM KCl, 10 mM Tris-HCl, pH 8.3, 1.5 mM $MgC1_2$, 200 µM dNTP, 0.2 µM each primer and 0.63 unit of γTaq polymerase (Takara Syuzou). The PCR was done at 92ºC for 30 sec once, at 92ºC for 60 s, at 60ºC for 30 s, at 72ºC for 90 s for the first 40 cycles and then 72ºC for 10 min.

RESULTS AND DISCUSSION

The pBIl2l-Hm binary vector contains NptII fragment, a GUS gene with an intron as well as a kanamycin resistance gene in its T-DNA, therefore, It was able to use assay for resistance to kanamycin to investigate the transformation of plants by this strain. As the result *A.tumefaciens* LBA_{4404}(pAL_{4404} + pSW_{600}) growth in selection media contain kanamycin showing the colony of bacterium, on the other hand the *E.coli* vector cannot grow in media selection (Fig.1).

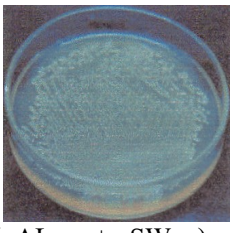

Figure 1. *A. tumefaciens* LBA_{4404} (pAL_{4404} + pSW_{600}) on selection medium (AB agar) contain kanamycin.

The difference in appearance between the control plants and the transformants (T_0) (Fig. 2) can be observed. The transformants have more branches, it was about 35.29% much more than control plants. Therefore the seeds of transformants have weight significantly difference with the soybean of control plants. The weight of randomly select

of 10 seeds from transformant and control plants showed differences about 4,624% for the transformant.

Figure 2. Difference in appearance between branch of transformed (2) and non-transformed (1) soybean plants.

PCR

For the detection of a transgene in the transformants by the LBA4404 strain harboring a pBI121-Hm binary vector with *Sfa8*, it was selected as transgene the DNA segment (600 bp) spanning the 35S promoter and GUS gene in the T-DNA region. The transgene (600 bp) was detected in the transgenic plants, whereas it was not detected in the control plants (Fig. 3). *Sfa8* gene is a specific gene and the outside gene in soybean genome. It is possible to detect it in transgenic plants but not in control plants.

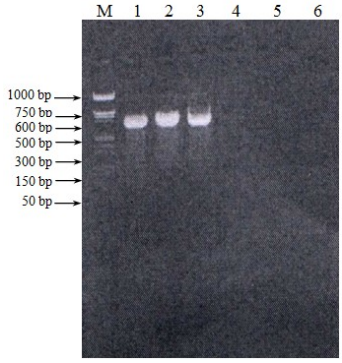

Figure 3. Detection of transgene by PCR in transformed plants, lane 1,2,3 but not in non-transformed plants, lane 4,5 and 6. M is marker.

CONCLUSION

Albumin gene of *Sfa8* from sunflower (600bp) cloned in vector plasmid of pBI121 could be transfer to *A.tumefaciens* LBA4404 by the method of triparental mating.
Genetic transformation using *A.tumefaciens* LBA4404 strain by *in planta* transformation method resulting in transgenic plant with high albumin level in the seeds.

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HOW TO USE AND TREAT THE LAND ACCORDING TO VEDIC KNOWLEDGE AND AUTHORITY

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ABSTRACT

The land in the Vedic knowledge and authority is a personality and has name. Her name is Bhumi or Devi Bhumi. The land consists of many material elements that needed by all living beings to life, such as: ether, air, fire, and water. Bhumi maintain all living beings in the material world including us or human beings, therefore, Bhumi in the Vedic scripture is considered as one of our mother, among seven of our mothers. There are seven mothers: mother who gives birth, nurse, wife of the King, brahmana's wife, spiritual master's wife, and the cow. As a mother the land should be treating nicely and use properly, we should gave high respect to her. By cultivating the land naturally, for example tillage practice using bull or cow, cow dung can be use as manure, Bhumi will feel happy and she will produce abundantly everything's that we need. We should cultivating her soil surface according to the Vedic injunction, back to nature (organic farming), without any pollutant such as chemical fertilizer and fungicide or insecticide etc. In that way, we will life in harmony with nature and certainly we will have a better future.

Key words: land (Bhumi), Vedic knowledge, material elements, living beings

WHAT IS THE LAND

According to Vedic scripture the land is called "Bhumi" or Devi Bhumi. The land consist of all elements such as: air, ether, fire, water, etc. In other word, Bhumi consist of various elements or materials that needed by all living entities to maintain their life. Bhumi maintain every living being in this material world. So that it is said in the Veda, Bhumi is one of our 7 (seven) mothers. Another six mothers are: mother who give birth, nurse, wife of king, wife of brahmana, wife of spiritual master, and cow. Philosophically, Bhumi is our mother, we are her sons and daughters, "*Mata bhumih putro'ham prthivyah*, Bhumi is my mother and I am her son."

The authority of the Vedas is unchallengeable and stands without any question and doubt. And whatever is stated in the Vedas must be accepted completely, otherwise one challenges the authority of the Vedas. The conch shell and cow dung are bone and stool of two living beings. But because they are have been recommended by the Vedas as pure, people accept them as such because of the authority of the Vedas (BBT, 1980) [**1**].

How man should use and treat the land properly according to Vedic knowledge in order to gain prosperity and life happily in this material world. Due to the land or Bhumi is our mother, we should treat her with love and respectfully according to Vedic injunction. In that way she will happy and give abundant harvest of grain and other seeds for our daily life.

THE LAND CONSIST OF EVERYTHING WE NEED FOR LIFE

The fact that almost our entire foodstuff is produce from Bhumi, the factory just for processing only. The materials for clothes and houses for example are also from the land (Bhumi), not from factory. This means that human beings absolutely depend on the land (mother Bhumi). If there is nothing has been produced from the land that we need for life, immediately we will die (disappear).

Actually everything we need for life are complete, perfect, and abundant available in the land (Bhumi). As a human being, simply cultivating the land in natural methods and just use the yield for simple life, that's all. In this way, will be there are no problem

of scarcity, lack of food, clothes and housing. We will be able to understand Vedic knowledge, that Bhumi is *sarva kama dugha mahi*, which means Bhumi consist of everything we need for life (Bhag. 1.10.4) [**2**].

Figure 1. Fruits [3]

Therefore, another name of Bhumi is Vasundhari. By simple life and high thinking, we have a lot of time for executing spiritual activities (*bhakti* service) toward Lord Krishna.

GOOD HARVEST DEPEND ON THE MERCY OF GOD

How can we understand this? Let us hear what Bhumi said: ……I have got special benediction (specific ability) from God that I can give wealth more that wealth that exist in the three worlds, when my surface was decorated by lotus feet steps of Sri Krishna which consist of pictures of: flag, thunder, stick for taming elephant, and lotus flower……. When Lord walk on my surface, I feel so happy have been touched by the dust of His lotus feet, immediately grass growing so fertile just like hair standing on end due to ecstasy" (Bhag. 1.16.33 and 35) [**4**]. It is also said in the Vedic scripture as follows: "All cities and villages on the earth surface are prosperity in all respect due to abundant of grain and vegetable. The trees full of fruits, all rivers flowing with clean water, hills and mountains rich with minerals and ocean consist of many kind of sources." This is occurring as it is due to Your glance (Bhag. 1.8.40) [**5**].

From the Vedic verses above, we can understand that Bhumi feel happy has been touched by Sri Krishna's lotus feet; therefore she was able to give so many (abundant) of all things we need. Therefore in general we can say that wealthy of human being is depend on God mercy.

HOW TO MAKE BHUMI HAPPY

Bhumi is our mother, so that is our duty to respect and treat her with love. This is the first way to make Bhumi happy. Simple life (*tapasa*) in accordance with the order of God in Vedic scripture, is the second way to make Bhumi happy. Lord Krishna is absolute. So that He is equal to His order. By simple life according to His order, Bhumi will always feeling His existent on earth, she will feel safe, happy and peaceful. If Bhumi happy, automatically she will produce abundant of what we need for maintain our body. By making Bhumi happy, automatically our life will also happy, healthy, wealthy and peaceful.

Natural life is life in line with the role in Vedic holy scripture, that is life depend on the mercy of nature (Bhumi) which is based on social order of life '*varna-ashrama-dharma*'. *Varna-ashrama* social order, is a society in which human beings is grouped into 4 (*catur* = four) social (varna) groups i.e.: 1. *Brahmana* (intellectual class of man); 2. *Ksatriya* (soldier and government class); 3. *Vaisya* (farmers and merchants); and 4. *Sudra* (workers group). The grouping into *catur-varna* is based on *guna* (character/ habitual) and *karma* (job/ occupation/ profession/ business) (Bg. 4.13) [**6**]. While executing the

duty/ job (*karma*) in accordance with each *varna*, every one also should attempt to progress spiritually by following 4 (*catur* = four) steps spiritual life (*ashrama*) i.e.: 1. *Brahmacari* (unmarried/ student/ *gurukula*); 2. *Grhastha* (married couple/ house holder); 3. *Vanaprastha* (life unattached with family); and 4. *Sannyasi* (life without attachment with material things/ mundane) (Bhag.11. 17.14) [7].

In relation with land (Bhumi) as source of job or occupation for life, agricultural land's cultivation and products distribution is done by *Vaisya* group and is help by *Sudras*. Both they life as a *grhastha* (house holder).

Land cultivation is done naturally such as tillage practices (*krsi*) by the help of bull (cow), and cow dung is use as manure (organic fertilizer). Simple organic farming like this, eternality or long-lasting and sustainability of nature (land/Bhumi) will be nicely protected.

Figure 2. Tillage practise [8].

IN THIS AGE (KALI YUGA) BHUMI IS UNFORTUNATE

People mostly lack of spiritual knowledge and think that Bhumi is just dead material things. They use and treat Bhumi roughly/ rudely, cruel/ brutal, and uncultured. As a result Bhumi is so much suffering, and in some parts of the world become a desert, without any inhabitant or living entities that exist on it.

Figure 3. Mining [9]

CONCLUSION

From the explanation about the land (Bhumi) above, it can be drawn some conclusion as follows:

- We should use the land to produce what we need just for maintain this body
- The land has an ability to produce more than enough and complete / perfect
- According to Vedic injunction, human beings should be grouped into *Varna-ashrama* social order
- The land or Bhumi is our mother
- We should treat her with love and full respect, in this way, life is in harmony with nature and we will have a better future

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POST HARVEST MANAGEMENT OF GLADIOL (*Gladiolus hybridus)* AS CUT FLOWER

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ABSTRACT

Gladiolus hybridus is one of the well known cut flower that requires proper maintenance after harvesting for the flowers and corms. The study was explored by surveying some farmers at Cipanas, West Java. The gladiolus were grown using corms (minimum 2.5 cm in diameter) as its propagative organs. Flowers can be harvested at 70-75 days after planting or while one floret at the bottom of flower stalk has opened (bloomed). Flowers were harvested every 2-3 days using sharp knife to avoid stalk injury. Farmers usually harvest the flower in the morning, collect and grade them into class A and B depend on the number of floret in the stalk. Every 10 flowers were tied up and hanging up in shady area to prevent loss of water due to transpiration. Some farmers use chemical to prolong the self life of the flower. Corms of gladiolus were harvested 1.5-2.5 months after flower harvested or while the leaves have dried. Corms were cleaned from soil and separated between corm and cormels. Both types of corms were soaked in fungicide solution 1.5 g/L for 15-30 minutes, then wind drying. Corms and cormels were kept in dark place for 2.5 month to break dormancy.

INTRODUCTION

Gladiol (*Gladiolus hybridus*) is belonged to cut flower in ornamental plants. Its flower is very attractive due to some individual flowers are arranged in one spike. The color is varied, such as white Friendship (white colour), Peter Pears and Spic and Span (orange colour). At Sindanglaya, Cipanas, West Java, Indonesia, some cultivars were cultivated to produce cut flowers. The cultivars were introduced from Netherlands and adapted to Cipanas area, such as Esta Bonita, Spic and Span, Red Mayesti, Red Beauty, Rose Delight. The others were domestic cultivars that cultivated by some farmers.

Production of cut flower required good cultivation in the field and post harvest handling before marketed to consumers or suppliers. This paper is pointed to discuss how to manage the post harvest of gladiolus as cut flower.

MATERIALS AND METHODS

Data were collected by surveying some farmers and some data were presented from Balithor Cipanas (Horticultural Research Centre) at Cipanas, West Java.

RESULTS AND DISCUSSION

Cultivar

Farmers grew some cultivars, such as Queen Occer, Albino, Golden Boy, Salem dan Dr. Mansur (Mansoer Red). The corms that were used as seed were buy or produced by the farmers themselves. Introducing cultivars were come from Netherlands in form of ready to planted corms. There were 16 cultivars which grew potentially at Cipanas.

Planting materials

There are two types of planting materials, corms and cormels as its vegetative propagation. New varieties are raised from seeds. Seeds germinate freely and the seedlings grown carefully will flower in the second year (Anonim, 2008). Farmers usually use 2.5 cm in size of corms' diameter. After 2-3 months of storage, the corms are

ready to be planted because dormancy of corms is finished. Anonim (2008) stated that large flower spikes developed after 3-4 years when the corms attain a good size.

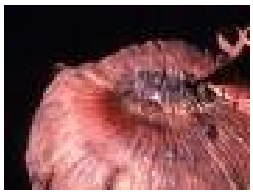

Figure 1. Corm of gladiol (Anonim, 2008)

Before planting, corms or cormels were soaked in fungicide solution such as Dithane M-45 or Antracol 1.5 g/L for 15-30 minutes to protect from soil borne disease. Gladioli corms were planted 5 to 10 cm deep into the soil, at a distance of 20x15 cm or 20x25 cm.

Figure 2. Planting Site

Plants maintenance

The gladioli plants were fertilized using 400-600 kg Urea/ha, 400-600 kg TSP/ha and 400 kg KCl/ha during planting time. Half dosage of TSP and KCl were given at planting corms and one week after harvest the flower. Urea were applied at 20 DAP (Days after planting) and 50 DAP.

Others maintenance such as watering/irrigating, application of pesticide, staking and earthing is required for the plants in the field. Anonim (2008) reported that earthing was done after 6 to 8 weeks of planting. Staking was required after the emergence of flower spikes, so the spikes may not fall down when there is a strong wind. However, staking is not necessary when the plants are grown closely or in clumps. Care should be taken not to injure the leaves at any stage as the injury is harmful to the plants (Anonim, 2008).

Harvesting

Harvesting was divided into two categories: harvest the flowers and harvest the seeds (corms and cormels). Gladioli flowers start to bloom from the bottom of the spike to the top. Flower harvesting starts at the age of plant about 70-75 DAP. Anonim (2008) reported that the flowering spikes appear in 60-90 days after planting, the flowers continue to open in succession from below upwards and the open flowers remain fresh for a number of days.

Farmers usually harvest the flower every 2-3 days in the afternoon. Flowers were cut using sharp knife from the flower stem at 10-15 cm from the soil. The leaves (4-5 leaves) were left in the field. Anonim (2008) reported that after flowering, the leaves begin to turn yellow and wither. The corms and cormels are then lifted with bamboo stick and kept in a dry shady place. The corms are thoroughly cleaned, the cormels separated and stored in cool, airy and shady place until the next planting season.

After harvesting, the flowers were collected, graded based on colour of flower and bunch in units of 10 flowers and held together by rubber band. Sereki et. al. (1994) reported that the opening and senescence of gladiolus (*Gladiolus sp.*) florets was accompanied by climacteric or non-climacteric patterns of respiration and ethylene production, depending on variety, and whether data were expressed on a fresh-weight or floret basis.

Post harvest handling by local farmer is very traditional. Nunes reported that in order to minimize these losses, studies were conducted to determine the effects of some chemicals wich promote a later senescense, through the following treatments: control sample destillated water, potassium nitrate 40 ppm, gibberellic acid 60 ppm, magnesium sulfate 40 ppm. After harvesting, the flower branches were immersed in the solutions, where they stayed till the senescense began.

Packaging

Bundles of 10-spikes each were first wrapped in banana leaves for protection from sudden temperature fluctuations, bruising and moisture loss. Then they were fasted with rubber bands, packed in bamboo basket, then ready for transportation to the consumers or supplier at around Cipanas and Jakarta, Indonesia.

Figure 3. Handling of Flowers

ACKNOWLEDGMENT

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CHROMOSOMES OBSERVATION ON CULTIVARS OF *Brassica napus* L.

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ABSTRACT

A research was conducted on chromosomes observation on cultivars of *Brassica napus* L. at Laboratory of Biotechnology, Faculty of Agriculture, Udayana University. Aim of this research was to determine the number and types of four cultivars of *B. napus*. The cultivars are *B. napus* Argyle, *B. napus* Tanami, *B. napus* Trigold and *B. napus* Boomer which were released by Canola Breeding of Western Australia. The method used was the squash method and aceto-orcein staining method. Results showed that chromosomes of *B. napus* were very small, condense and uniform. This leads to difficulty in counting chromosome number. It has been reported in several publications that the number of chromosome of B. napus is 2n=38. However, the number of chromosomes obtained from this study for *B. napus* Argyle was 2n=32; *B. napus*. Tanami 2n=36 and *B. napus* Trigold 2n=34. This differences may be due to the use of conventional technique used for chromosome prepartion in this study or the occurrence of abnormal segregation during anaphase.

INTRODUCTION

Brassica is known as vegetable crop in Indonesia including cabbage, cauliflower, and broccoli. These vegetables belong to farmily of *Brassica oleracea*. There are other Brassica species that have not been cultivated and utilised in Indonesia. In Australia, Canada, America and European countries, variant of *Brassica* species such as *B. oleracea*, *B. napus*, *B. rapa*, *B. carinata*, are known as vegetable, animal feed, source of vegetable oil and biodisel. *Brassica napus* is one of *Brassica* species that can be used for animal feed, vegetable oil for human consumption known as Canola (Mahasi and Kamundia, 2007). *Brassica napus* has been known as third oil source following coconut and soybean (Neeser, 1999).

Several new cultivars of *B. napus* were resulted from breeding program of Canola Breeding of Western Australia (CBWA), Australia. Those are *B. napus* Trigold, *B. napus* Trilogy, *B. napus* Tribune, *B. napus* Boomer, *B. napus* Tanami, *B. napus* Argyle and *B. napus* Stubby. For further study these cultivars of *B. napus* was used as material for breeding program, genetic information of those cultivars.

Evolution and relationship between Brassica species was explained in U triangle (U, 1935). The U triangle shows that the formation of three Brassica species which are *B juncea*, *B. carinata* and *B. napus*. *Brassica juncea* has chromosome number of 2n=4x=36, AABB. This species is a hybrid between *B. rapa* (2n=20, AA) and *B. nigra*. *Brassica carinata* is a cross result from of B. nigra (2n=16) and B. oleracea. It has a genome of BBCC with chromosome number of 2n=2x=34. The last species is *B. napus* with chromosome number of 2n=4x=38, AACC and it was reported a cross breed of *B. oleracea* (2n=18, CC) and *B. rapa* (Bathia *et al*., 1996).

The chromosome profiles of *B. napus* cultivars released by CBWA, however have not been reported. The information of chromosome number, ploidy level and chromosome arrangement is part of genetic information that can be used to facilitate construction of better plants (Yan, 1996). Threfore a research was conducted to evaluate chromosome number and type of four cultivar of *B. napus* released by CBWA namely *B. napus* Argyle, *B. napus* Tanami, *B. napus* Trigold and *B. napus* Boomer.

MATERIALS AND METHODS

Seeds of *B. napus* Trigold, *B. napus* Argyle dan *B. napus* Tanami, *B. napus* Boomer were donated by CBWA, Australia. Seeds were germinated in petri disk. Chromosome slides were prepared using squash method. Pretreatment with paradichlorobenzene, fixation and staining following the method of Kokubugata dan Kondo (1998). Root tips of Brassica seedling were cut in 1 – 2 cm at 6.00–7.00 a.m Central Indonesian Time. Then roots were fixed in ethanol: glacial acetic acid (3: 1) for 24 jam. Following fixation, root tips were placed on glass slide then were cut into 2mm. Hydrolysis was done using 2N HCl at 60ºC for 15 menit.

Staining was done using aceto-orcein for 10 menit. Then root tips were covered using cover glass and squashed to spread the cells. Chromosomes were observed under microscope and photographs were taken.

RESULTS AND DISCUSSION

Observation of somatic chromosomes of *B. napus* was proved difficult. This is due to the fact that *B. napus* has small size chromosomes and the chromosomes are often clustered together. The similar finding was reported by Howard (1938) who stated that the difficulty in having suitable chromosome spread for chromosome counting of *B. napus*. According to Howard (1938) that *B. napus* showed a characteristic of chromosome clumping and unclear chromosome constriction.

In this research several slides had well-spread chromosomes (Figure 1), and chromosomes count is showed in Table 1. Chromosome number of *B. napus* Boomer cannot be determined due to unavalability of clear and well-spread chromosomes. Several other slides showed chomosomes of *B. napus* at metaphase, anaphase and telophase (Figure 2)

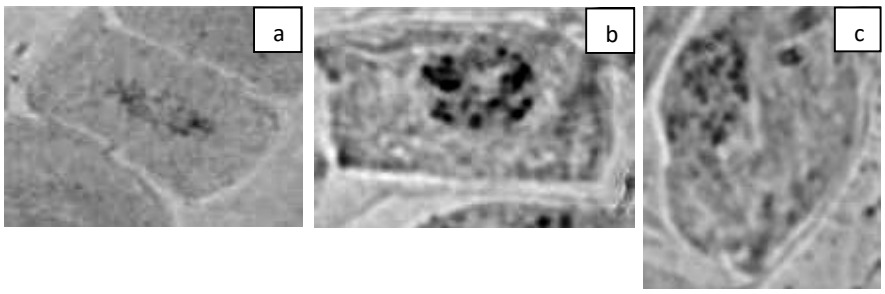

Figure 1. Chromosomes spread at late prophase or premetaphase stage of *B. napus* Argyle (a), *B. napus* Tanami (b), *B. napus* Trigold (c)

Table 1. Chromosome number of *B. napus* Argyle, *B. napus* Tanami, *B. napus* Trigold

No	***B. napus* cultivars**	**Chromosome number**				**Average**
1	*B. napus* Argyle	34	30	34	30	32
2	*B. napus* Tanami	36	38	34	34	36
3	*B. napus* Trigold	34	30	32	38	34

In this study, the chromosome number of three cultivars of *B. napus* (Argyle, Tanami and Trigold) vary from 30 to 38. While it was reported that the number of chromosome of *B. napus* is 38 (Bathia *et al*., 1996, Ahmadi *et al*., 2003). This defferences in chromosome number observed may be due to the conventional technique used that was

aceto-orcein staining. This technique failed to identify centromer position. Furthermore the chromosomes observed was in a very dense stucture and clump.

The small size of chromosome of Brassica especially *B. napus* was reported in several publications (Howard, 1938, Ahmad *et al*., 2002, Snowdon *et al*., 2002). The chromosomes are difficult to be differentiated one another due to similar morphology (Hasterok and Maluszynska, 2000). Beside the small size of chromosomes, the chromosomes of *B. napus* has very few cytogenetic signs and uniform structure (Snowdon *et al*., 2000).

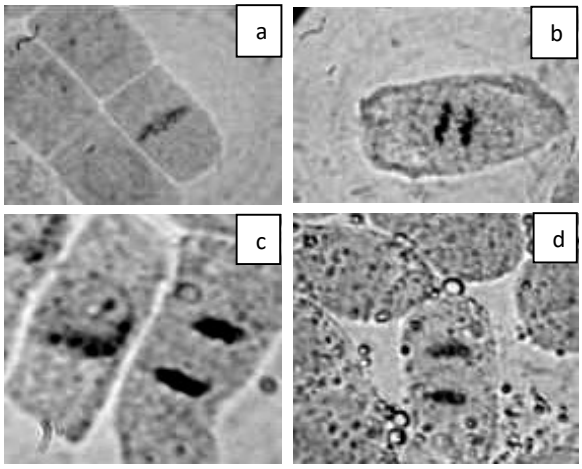

Figure 2. Chromosomes of *B. napus* Argyle at metaphase (a), chromosomes of *B. napus* Tanami at anaphase (b),chromosome of *B.napus* Trigold at telophase (c), and chromosome of *B. napus* Boomer at telophase

Other reason for the difficulty in observation of *B. napus*' chromosomes using conventional technique is that the *B. napus*' chromosomes often highly condensed. Heterochromatin is limited only at centromer region, so that the arm of chromosomes cannot be identified (Snowdon *et al.*, 2000).

The variations number of *B. napus*' chromosome could also because of the abnormality in mitosis (Nair and Ravindran, 1994). The variation of chromosome number in plants is due to abnormal segregation at anaphase stage (Nair dan Ravindran, 1994). Early segregation will drive chromosomes to enter stage of anaphase before their normal time, while other chromosomes are still at metaphase. As a result, telofase will be forced to start following chromosomes which are at late anaphase. Other chromosomes which have not been separated will move to one cell pole. This leads to different number of chromosomes in daughter cells (Nair and Ravindran, 1994).

The small size and uniform stucture of *B. napus* observation using conventional technique, there for cannot be used in identification of *B. napus* cultivars. Molecular cytogenetic technique using fluorescence hybridisation or DNA probe must be applied to identify and develop karyological analysis of *B. napus* cultivars.

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RESPONSE OF FEEDING PANCREAS EXTRACT AND RATION SUPPLEMENTED WITH PROBIOTIC ON PERFORMANCE OF BROILER (CARCASS, BLOOD SUGAR CONCENTRATION AND BLOOD LIPID PROFILE)

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ABSTRACT

Aim of this research was to study the response of carcass, blood sugar concentration and blood lipid profile by feeding pancreas extract and ration supplemented with probiotic to broiler. The experiment was assigned following a Completely Randomized Design with Factorial pattern. The first factor was four levels of pancreas extract. The second factor was four levels of probiotic Starbio: 0.0%, 0.05%, 0.10% and 0.15% (S0, S1, S2 and S3). Each treatment combination consisted of four replicates and each replicate consisted of four broilers. Carcass, blood sugar concentration, and blood lipid profile were measured. Result of the experiment on control treatment showed that final weight, carcass weight and carcass percentage were 1615 g/head, 1215 g/head, and 75.23%, respectively. Higher concentration of extract of pancreas and probiotic (Starbio) caused carcass and blood sugar concentration significantly increased ($P<0.05$), except on carcass percentage was not significant different ($P>0.05$). Blood sugar concentration on control treatment was 251 mg/dl, and extract of pancreas fed caused decreasing of blood sugar concentration significantly ($P<0.05$). Control treatment combined with Starbio increased blood sugar concentration, and combined with pancreas extract could decrease blood sugar concentration ($P<0.05$). Total cholesterol level, high density lipoprotein (HDL), and low density lipoprotein (LDL) on control treatment were 141.0 mg/dl, 27.0 mg/dl, 100.8 mg/dl respectively. Pancreas extract supplementation decreased cholesterol level significantly ($P<0.05$), but probiotic supplementation on control treatment increased cholesterol level ($P<0.05$), and its combination decreased cholesterol level significantly ($P<0.05$). It can be concluded that combination supplementation of pancreas extract and probiotic to broiler increased carcass weight but not on carcass percentage, blood sugar concentration and blood cholesterol were significantly decreased.

Key words: pancreas extract, probiotic, carcass, blood sugar concentration, blood cholesterol, broiler

INTRODUCTION

The use of local feedstuff from local feed sources need to be increased, so it can reduce the feed price, but the problem for local feedstuff from agriculture or agroindustry by-product is high content of crude fiber, so the digestibility is low (Lubis, 1992). Local feedstuff need to be supplemented with probiotic which could increase the digestibiliy of diets, so absorption and nutrient used will increase, as well as weight gain (Belawa, 2007). Nuriyasa *et al.* (1998) found that by offering *Effctive Microorganisms-4* (EM-4) into cassava tuber which contain pancreas extract could significantly increase digestibility in *in vitro* experiment. Belawa and Suwidjayana (2000) tried to offer EM-4 liquor through drinking water on chicken ration with different fibre level and showed positive respons to the digestibility and significant increase of weight gain but decrease blood cholesterol level. Livestock performance is also affected by hormones in the body. Bidura (2007) found that the offering of extract hipofisa hormones could increase weight gain but decrease body fat content. Yupardhi (1998) reported that offering steroid hormone to male and castrated cattle has no effect on feed efficiency and meat texture. Wirtha (2002) have tried offering cortisone hormone to broilers and he reported that there were no effect on weight gain, abdominal fat weight and feed conversion. Function of insulin and glucagon hormone affect the carbohydrate metabolism which are produced by α dan β cells, besides delta cell produce somatostatin to pursue insulin and glucagon hormones secretion (Harper *et al*., 2009). Offering probiotic could increase digestibility and give

positive affect to the availability of nutrient and if combine with pancreas extract hormone that would reduce blood sugar concentration, hidrolyse fat to fatty acid, reduce fat accumulation in adipose tissue, so it could reduce cholesterol content in blood and prevent hypertensi.

Based on the above problems, a research was conducted to study the response of offering pancreas extract and ration supplemented with probiotic on carcass, blood sugar concentration and blood lipid profile to broiler.

MATERIALS AND METHODS

Place and Period of Research

The research was conducted in Guwang village, Gianyar regency for six weeks. Laboratory test was conducted in Laboratorium Teknologi Hasil Ternak (Laboratory of Livestock Yield Technology) Faculty of Animal Husbandry, Udayana University, and blood sugar concentration and blood lipid profile was conducted in Laboratory of District Health, City of Denpasar for one month.

Chicken

Two hundreds fifty six heads of male broiler Strain CP 707, PT. Charoen Pokphand Jaya Farm Surabaya production, age two weeks were used in this experiment.

Ration and Drinking Water

The ration was composed based on Scott *et al*. (1982) recommendation and using yellow corn, rice bran, coconut meal, fish meal, soybean, probiotic Starbio, premix and salt. Ingredients and nutrient composition are presented in Table 1 and Table 2. Ration and drinking water were provided *ad libitum*. Source of drinking water is from local drinking water firm (PDAM).

Table 1. Ingredients Composition of Broiler Feed Age 2-6 weeks

Ingredients (%)	Treatment[1)]			
	S0	S1	S2	S3
Yellow corn	55.36	55.36	55.36	55.36
Fish meal	8.12	8.12	8.12	8.12
Rice bran	14.14	13.64	13.14	12.64
Coconut meal	9.31	9.31	9.31	9.31
Soybean	11.97	11.97	11.97	11.97
Starbio	-	0.50	1.00	1.50
Premix	0.55	0.55	0.55	0.55
NaCl	0.55	0.55	0.55	0.55
Total	100.00	100.00	100.00	100.00

Table 2. Nutrient Composition of Broiler Feed Age 2-6 weeks

Nutrient	Unit	Treatments [1)]				Standard[3)]
		S0	S1	S2	S3	
Metabolis Energy	kcal/kg	2909.21	2892.06	2883.91	2875.72	2900
Crude Protein	%	17.15	17.08	17.01	16.94	17.00
Crude Fiber	%	4.41	4.36	4.31	4.26	3-8[2)]
Ether Extract	%	6.31	6.24	6.17	6.10	5-10[2)]
Calsium (Ca)	%	0.84	0.84	0.84	0.84	0.70
Phosphor(P)	%	0.80	0.80	0.80	0.80	0.50

Notes:
[1)] Treatments:
S0: Control treatment (without Starbio)
S1: Ration contain 0.5% Starbio
S2: Ration contain 1.00% Starbio
S3: Ration contain 1.50% Starbio.

[2)] Murtidjo (1988)
[3)] Scott *et al*. (1982)

Pancreas Extract

Fourty grams of pig pancreas were smashed and added with 700 ml of water. The extract was filtered and ready to use as pancreas extract. Pancreas dosage were 0.00%; 0.05%; 0.10%; 0.15% (P0, P1, P2, dan P3).

Experimental Design

A Completely Randomized Design with factorial arrangements (4 x 4) was used in this experiment. The first factor was level of pancreas extract: 0.0%, 0.05%, 0.10% and 0.15% (P0, P1, P2, and P3). The second factor was level of probiotic Starbio: 0.0%, 0.05%, 0.10% and 0.15% (S0, S1, S2, dan S3). There were 16 treatments combination: P0S0, P0S1, P0S2, P0S3, P1S0, P1S1, P1S2, P1S3, P2S0, P2S1, P2S2, P2S3, P3S0, P3S1, P3S2, and P3S3. Each treatment combination was replicated four times and each treatment combination consist of four broilers.

Variables Measured:

Variables measured were final body weight, carcass percentage, blood sugar concentration, total blood cholesterol, high density lipoprotein (HDL) and low density lipoprotein (LDL) of blood.

RESULTS AND DISCUSSION

Carcass

The final body weight of the chickens on control treatment (P0S0) was 1615 g/head (Table 3). Offering of strabio and pancreas extract supplemented diet increased body weight significantly ($P<0.05$). Enzymes in Starbio probably increased digestibility of the ration, and pancreas extract supplementation probably increased metabolism, nutrient absorption, then increasing growth, so increased the final weight. Carcass weight on S0P0 treatment was 1215,00 g (Table 3). Offering of Starbio and pancreas extract combination in the diet increase carcass weight significantly ($P<0.05$). Carcass weight are affected by final weight and non carcass weight (Soeparno, 2005), because there is a tendency that final weight is higher as well as carcass weight higher. Carcass weight of the control was 75.23% (Table 3). Treatment combinations has no effect ($P>0.05$) on carcass percentage. Cakra (1986) stated that carcass percentage is affected by non carcass factors.

Table 3. Response Offering Probiotic Starbio and Pancreas Extract diets on Broiler Carcass

Treatments[1)]	Variables measured		
	Final body weight (g/h)	Carcass weight (g/h)	Carcass persentage (%)
S0P0	1615e[2)]	1215e	75.23a
S0P1	1648e	1248e	75.72a
S0P2	1660de	1260e	75.90a
S0P3	1675d	1275e	76.11a
S1P0	1690d	1290de	76.33a
S1P1	1750bc	1350cd	77.14a
S1P2	1760bc	1360bcd	77.27a
S1P3	1770bc	1370bc	77.40a
S2P0	1700bc	1300dc	76.47a
S2P1	1760b	1360bcd	77.27a
S2P2	1775b	1375abc	77.46a
S2P3	1835a	1435a	78.20a
S3P0	1720d	1320dc	76.74a
S3P1	1770bc	1370bc	77.40
S3P2	1785b	1385abc	77.59a
S3P3	1850a	1450a	78.37a

Blood Sugar Concentration and Blood Lipid Profile of Broiler

Blood sugar concentration of S0P0 treatment was 251 mg/dl (Table 4). Offering probiotic supplemented diets increased blood sugar concentration, but if combine with pancreas extract depressed blood sugar concentration significantly (P<0.05). Starbio contains enzymes, which related with carbohydrate metabolism, such as selulase, hemiselulase, ligninase that yielded fatty acid and amino acid. Amino acid and fatty acid could change into glucose through gluconeogenesis process (Murray *et al.,* 2006), so it are increase blood sugar concentration. Besides, pancreas extract contain insulin hormone has a role in glucose transport into all cells (except liver and erytrocyte), so stimulate in decreasing blood sugar concentration (Ngili, 2009). Blood sugar concentration on ration containing pancreas extract is lower compare with those broiler given ration containing of starbio without pancreas extract.

Table 4. Response of Offering Probiotic Starbio and Pancreas Extract in the diets to Broiler Blood Sugar Concentration and Blood Lipid Profile

Treatments[1)]	Blood sugar concentration (mg/dl)	Total Cholesterol (mg/dl)	HDL (mg/dl)	LDL (mg/dl)
S0P0	251 abcd[2)]	141c	27 a	100.8 c
S0P1	248abcd	135 d	25b	96.0 d
S0P2	234abcd	126 f	20 c	85.0 e
S0P3	177 d	114 g	20 d	78.7 g
S1P0	330 a	186 a	16 e	116.2 a
S1P1	328 a	153 b	12.2 g	115.2 a
S1P2	316 ab	142 c	10.20 g	98.6 e
S1P3	202 d	129 e	10.0 g	84.0 e
S2P0	301 abc	106 h	15.8e	81.8 f
S2P1	195 d	102 j	14.2 e	79.2 g
S2P2	158 d	76 l	12.5 g	50.47 k
S2P3	147 d	71m	11.0 g	42.5l
S3P0	301 abc	103 i	15.0 e	113 b
S3P1	251abcd	101j	14.1 ef	75.1 h
S3P2	225 bcd	92 k	13.0 fg	62.6 i
S3P3	208cd	91 k	12.4 g	58.7 j

Blood cholesterol, HDL and LDL concentration were 141.0 mg/dl, 27 mg/dl, 100.8 mg/dl respectively (Table 4) for the control treatment. Pancreas extract increased significantly (P<0.05) the blood cholesterol HDL and LDL. Decreasing in cholesterol concentration relates very closely with decreasing of blood sugar concentration as well as lipoprotein and affected by decreasing of blood cholesterol. There is a relation with insulin hormone production by pancreas extract which could decrease blood sugar concentration, so decreased cholesterol level, HDL and LDL.

CONCLUSION

From the result of this experiment it can be concluded that offering of ration supplemented uses Starbio and pancreas extract increased final weight, carcass weight, but has no effect on carcass percentage, and decreased blood sugar concentration level and blood cholesterol of broiler.

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TERATOGENIC TEST OF YOUNG PINEAPPLE FRUIT (*Ananas comosus*) ON MOUSE FETUS (*Mus musculus* L.)

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ABSTRACT

This experiment was performed to examine the effects of young pineapple (Ananas comosus) extract on fetus morphology and skeletal development if given to the pregnant mice during organogenesis period. Twenty pregnant mices were randomly divided into 4 groups. Extract was given orally by gavage with 0 (control), doses 20%, 40%, and 80%. Treatment was given at day 6 to 15 of gestation. Caesarean section to remove fetus was performed on day 18 of gestation. The observation covered morphological of the fetus (weight and length of the litter, and malformations), skeletal development (ossification number of metacarpus, metatarsus, and vertebrae caudalis), and malformations of costae, sternum, and vertebrae. Statistical analysis was performed using Anova and Duncan's Multiple Range Test. Teratogenic effect caused the decrease of fetal weight and length and the increase of hemorrhage. Ananas comosus extract also inhibited skeletal ossification (decreased ossification number of metacarpus and metatarsus) and caused costal malformation (intercostal fusion and convulated costal structure).

Keywords: *Ananas comosus*, teratogenic, skeletal development

INTRODUCTION

Pineapple (*Ananas comosus*) contains many nutrients, vitamin A, calcium, phosphorus, magnesium, iron, sodium, potassium, dextrose, sucrose, and the enzyme bromelain (a mixture of 95%-cysteine protease) which can hydrolize proteins (proteolysis) and resistant to heat (Sawano, *et al*, 2008). Potential of bromelain as painkiller, antiinflammatory, antiedema, and debridement, accelerate wound healing, and enhance the absorption of antibiotics and are very useful in postoperative healing (Orsini, 2006). Bromelain is effective as anticoagulant, fibrinolytic, antimetastatic (Maurer, 2001), antileukemia (Baez *et al*, 2007), anti-cancer, and modulate the immune system and hemostatic (Chobotova *et al*, 2010). Bromelain accelerate menstrual cycle and potential as contraceptive and abortivum. Previous research had been carried out on pregnant mice. Extract was given orally 0.2 ml on the day of gestation 2, 4, and 6 (period of implantation). The extract turned out to be embryonic lethal (embryotoxic) on the day of gestation 2 and 4 (Mulyoto, 1986).

Teratogenicity is the ability of chemical substance to cause congenital abnormalities (structural or anatomical abnormalities) in babies, minor or major malformations and functional disorders. There will be no teratogenicity testing done on human. Therefore various methods in animals continue to be developed to anticipate teratology cases. Based on many benefits of pineapple fruit while the negative effect on the implantation period in early pregnancy has been proven (Mulyoto, 1986), it is necessary to determine birth defects if the mother consumed the fruit during period of organogenesis.

MATERIALS AND METHODS

Young pineapple fruit (1.5 - 2.5 months), peeled, washed, cut into small pieces, oven dried (30-40^{0}c), smooth blend, macerated (72 hours with methanol 90%) while

shaken, then filtered with whatman paper. The solvent evaporated by rotary vacuum evaporator to obtain the crude extract (pasta) then dissolved in distilled water.

Twenty female mices (*Mus musculus* L.), virgin, age 3 months, weight 30 grams, are acclimatized for one week. Estrous cycle is determined by vaginal smear and giemsa staining. Female in estrous period were mated with the male. If the vaginal plug or remaining sperms inside vagina were found in the next morning, the next day will be determined as day 1 of gestation (Kaufmann, 1992). Animals were randomly divided into 4 groups. Extract was given orally 0.2 ml/ animal/ day by gavage with 0 (control), doses 20%, 40%, and 80% at day 6 to 15 of gestation (organogenesis period). Caesarean section to remove fetus was performed on day 18 (one day before normal birth of mice).

Fetus morphology observed the fetal length and weight, the completeness of front and rear legs, tail, and hemorrhage. Measurement of body length and weight performed after clearing fetal amniotic fluid from the wrap. Skeleton preparation was done by Alcian Blue-Alizarin Red S staining (Inouye, 1976). Skeletal development observed vertebrae, costae, sternum, metacarpus, and metatarsus. Quantitative data were analyzed by Anova and Duncan Multiple Range Test.

RESULTS AND DISCUSSION

Fetus in the womb protected by the placenta and amniotic membranes are not completely protected from the bad influence of substances that are consumed by the mother. The velocity penetrating subtances to the placental barrier depends on molecular size, solubility in fats, and the ionization degree. The influence of a teratogenic agent in the early period of pregnancy is usually lethal, so the pregnancy ended in abortion (embryotoxic agents). In the next period of organogenesis (differentiation of cells to form organs), any disruption if do not cause death, always results in produced severe congenital abnormalities (teratogenic agents). Then the next period occurs maturation of organs in size and function. Bad influence of a substance in this period usually could cause only mild congenital abnormalities or merely an aberration function (Siswosudarmo, 1998).

Effects on Fetus Morphology

Fetal weight and length tended to decrease with the increasing doses. The rate of fetuses growth and development determine variation size of the young. The mean weight of normal fetus on gestation day 18 is 1.4 grams. Disturbance of individual development within uterine causes abnormal birth weight. Decreased fetal weight and length is an indication of fetal growth restriction. Growth inhibition occurs when agents affect cell proliferation and interactions, reducing the rate of biosynthesis associated with the inhibition of synthesis of nucleic acids, proteins, or mucopolisacharides (Price and Wilson, 1984). Decreased body weight and length is lightest form of teratogenic expression and is a sensitive parameter for teratogenic testing (Yantrio *et al*, 2002).

Table 1. The Average of Fetus Weight, Length, and Hemorrhage

Treatment	Number of Mother	Average		
		Weight (grams)	Lenght (cm)	Number of Hemorrhage
K (Kontrol)	5	1.07 ± 0.10 a	24.11 ± 0.58 a	0.00 ± 0.00 a
D1 (20 %)	5	0.54 ± 0.20 b	17.60 ± 3.25 b	5.00 ± 4.64 b
D2 (40%)	5	0.56 ± 0.09 b	16.82 ± 1.61 b	7.00 ± 2.24 b
D3 (80%)	5	0.57 ± 0.19 b	17.04 ± 3.40 b	4.60 ± 4.28 b

Hemorrhage was not found in the control, but increases with the increasing doses. Hemorrhage is a discharge of blood from the blood vessels, then will accumulate in body tissues. Extract given repeatedly up to a high concentration in blood resulted osmotic imbalance. In normal condition, embryo develops inside amniotic fluid which is isotonic with body fluids. Foreign substances in tissues can alter osmotic pressure. This imbalance can be caused by osmotic pressure and viscosity of the fluid disturbances in different parts of embryo, between blood plasma and extra-capillary space or between extra-and intra-embryonic fluids. It will cause blood vessels rupture and resulting hemorrhage (Price and Wilson, 1984).

Effects on Fetus Skeletal Development

Observations showed inhibition of ossification segment of metacarpus and metatarsus to a smaller number than the control group. All control fetuses had three segment (normal fetus ossification degree) on front leg (metacarpus) and rear leg (metatarsus). Metacarpus with only one segment is most prevalent at high doses. Decreasing number of metacarpus and metatarsus ossification in particularly high-dose because blood levels of enzyme bromelain is high. The molecular weight of this enzyme may less than 600 dalton so it can pass the placental barrier. Accumulation of bromelain in the placenta will inhibit transfer of nutrients from the mother to the fetus, then inhibit the metabolism of nutrients necessary for growth and development of fetal organs. Nutrients in the blood contain minerals for calcification. Inhibition of its supply from the mother to the fetus can also disrupt osteogenesis and bone growth. No abnormalities were found in the number of costae, all fetuses have 13 costae. Deformities of intercostal fusion ("bridge-like structure") and convulated costae were found at dose 80% (figure 2). Observations also indicate no deformity and abnormal ossification on caudal vertebrae and sternum.

Table 2. The Average of Ossification Number of Metacarpus and Metatarsus Segment.

Treatment	Number of Mother	Number of Observed Fetus	Average of Ossification Number	
			Metacarpus	Metatarsus
K (Kontrol)	5	20	3.00 ± 0.00 a	3.00 ± 0.00 a
D1 (20 %)	5	20	1.10 ± 0.55 b	0.70 ± 0.66 b
D2 (40%)	5	20	0.65 ± 0.59 c	0.40 ± 0.50 c
D3 (80%)	5	20	0.50 ± 0.51 c	0.30 ± 0.47 c

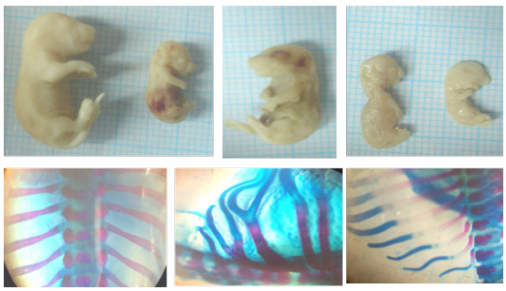

Figure 1. Fetus Morphology (ABOVE): Normal Fetus (left), Hemorrhage (1st and 2nd from the left), Abnormal Fetus/ Dwarf (1st and 2nd from the right);
Costae (BELOW): Normal Costae (left), Intercostal Fusion (middle), Convulated Costae (right). Red colour: cartilage that has undergone ossification, Blue colour: cartilage.

Costae grow as the development of vertebrae, both fused and grown together since the beginning of its formation. Separation of costae from vertebrae occurs at the beginning of ossification so that disturbances on early development of vertebrae often followed by abnormal costae (Primmet *et al*, 1988). Abnormal costae suspected from the beginning of blastema formation of the vertebrae. Fused costae may be the result of irregularly direction growth of the vertebrae's bud and in some places the distance between successive ribs were too close. Adjacent costae, when grows, likely intersect each other, so they will undergo ossification together and fused. According to Theiler (1988, in Yantrio *et al*, 2002), ossification malformations can be caused by interference of somit, chorda dorsalis, and sclerotom differentiation. Major vertebrae malformation is the result of segmentation process disturbances. Abnormal formation of vertebrae is caused by the disturbance of somit that occurs in the early development.

Young pineapple fruit (*Ananas comosus*) extract caused morphological abnormalities, inhibited skeletal ossification, and caused costal malformation on mouse fetus (*Mus musculus* L.).

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EFFECT OF DIFFERENT ENERGY – PROTEIN RATIO CONTAINING DIET ON PERFORMANCE OF *KAMPUNG* CHICKENS

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ABSTRACT

Experiments were conducted to determine whether dietary of level balance energy and protein ratio have some effect on performance of 8 weeks old *kampung* chickens. A total of 48 unsexed 3 day old *kampung* chikens were used in this experiment. A completely randomized design consisted of 4 treatments and 4 replicates and each replicate has 3 birds. The treatments were a ration with energy 3100 kkal/kg and 22% protein (A); ratio with energy 3000 kkal/kg and 20% protein (B); ratio with energy 2900 kkal /kg and protein 18% (C); ration with energy 2800 kkal /kg and 16% protein (D). The variables recorded were: feed consumption, feed conversion, final body weight, body weight gain, protein consumtion, energy consumtion, carcas weight, carcas percentage. Data obtained were analyzed using analysis of variance and followed by Duncan's multiple range test. Results of this experiment showed that effect of ration with balance energy- protein at 8 week age gave a final body weight, body weight gain, feed consumption, feed conversion, energy consumption, protein consumption, was significantly higher than (P<0,05) but carcas weight, carcas percentage, were not significant (P>0,05) among the treatments.

Key word: *Kampung* chickens, energy, protein, weight gain, feed conversion.

INTRODUCTION

Kampung chickens is kept in wide range of agro-ecological zones and has been kept as a part of Bali communities culture. They are well adapted within region since several decades. High requirement for ceremonials purposes is considered as supporting factors for development of this chicks. Currently, raising *kampung* chickens has moved toward cultural needs only to market oriented industries for meat and egg. These industries has grown sharply during last decades. These industries development has increased interest of communities to breed *kampung* chickens. The one problem encountered in developing the *kampung* chickens is low productivity (Sartika, 2006; Hassen *at al.,* 2008).

More than 6.5 millions *kampung* chickens in Indonesia and 4,521,033 chickens in Bali province (Direktorat Jendral Peternakan, 2007). The productivity of *kampung* chickens was lower under the rural management system or extensive traditional system than the intensive farming (Supraptini, 1985). This low production may be attributed to lack of improved poultry breeds, the presence of predators, the poor feeding and there is no feeding standard for different age of chickens (Sartika *at al.,* 2008; Dewi, 2010).

Nutritions are the main factors that affect the performance of the *kampung* chickens, and among those factors, the primary nutritional factors are energy and protein of the ration. According to feeding programme, balance energy – protein, mainly influence a total energy, protein and feed consumption.

There is no feeding standard for *kampung* chicken in Indonesia, however recommended requirements of laying chickens by Scott *et al*. (1982) and NRC (1994) can be used. For example, requirements of energy and protein for 2 - 8 weeks old in the range 2600 – 3100 kcal/kg and 18% - 24% (Scott *et al*., 1983). Lee *et al*. (2005) reported requairents for the energy ration is about 2600 – 3100 kcal/kg and 18% protein. The ratio of the requirements of energy - protein of *kampung* chickens in tropical areas is not been documented. So, estimation and effect of different energy and protein for *kampung* chickens is necessary to maintain the performance of the chickens. The aim of this study

was to determine the effect of energy and protein ratio on performance of 8 weeks old *Kampung* chickens.

MATERIALS AND METHODS

This experiment was carried out in Poultry Science Laboratory, University of Udayana in Peguyangan Village, District of East Denpasar, Bali for 8 week. Forty eight, 3 days old-unsex *Kampung* chickens (54.19 ± 2.70 g), were randomly assigned following a completely randomized designed (CRD) birds were allotted to 4 treatments with 4 replicates of 3 chickens each. The experiment were as follows: Metabolizable energy (ME) 3100 kcal/kg and 22% protein (A), ME 3000 kcal/kg and 20% protein (B); ME 2900 kcal/kg and 18% protein (C) and ME 2800 kcal/kg and 16% protein (D). Ration were formulated with ingredients: yellow corn, soybeen meal, coconut meal, fish meal, rice bran, coconut oil, premix, salt.

The cage used battery pens consisted of 16 cage, were made up of the stainless wire and each pen 65 cm X 50 cm X 75 cm. Feed and water were provided for *ad libitum*. At the end of the experimental period the birds were weighed and feed intake recorded for feed efficiency ratio. One bird was randomly selected from each pen were then slaughtered for carcass percentage and inner organs determination.

Feed consumption, feed conversion, final body weight, body weight gain, protein consumtion, energy consumtion, carcas weight, carcas percentage were also measured Data obtained was analyzed using analysis of variance and followed by Duncan's multiple range test (Steel and Torrie, 1993), when significant differences ($P<0.05$) amongs treatments were found.

RESULTS AND DISCUSSION

The live weight of the chickens are given in Table 1. The effect of treatments on the live weight and body weight gain were significant ($P<0.05$) for treatment A, B, C and D. The highest live body weight gain and daily weight gain were demonstrated is 441.50 g/bird and 8.08 g/bird/day. Body weight of the chicks in treatment B, C and D lower than A. Dietary energy and protein ratio significanly decreased nutrient intake and decreased performance of the chickens. In accordance with Candrawati (1999) *Kampung* chickens ages 8 weeks, given ration with energy: protein ratio (3100 kcal/kg: 22%, 2823 kcal/kg: 15.33%) gave 502 g/bird and 9.1 g/bird body weight gain.

The effect of treatments on cumulative feed consumption was not significat ($P > 0.05$) and the highest was in the chickens gave treatments A (Table 1). The rate of feed convertion ratio was significantly ($P < 0.05$), the result of this experiment lower than those reported by Hassen *et al*. (2008) feed consumption native chicken at 8 week age is 2.4 and it was significant.

Protein and energy consumption of the chickens on treatment A was 5.11 g /bird/day and 85.25 kcal/bird/day highes significantly ($P < 0.05$) than treatment B, C and D. The decreased energy and protein on the treatment can be decreased significantly feed convertion ratio of *kampung* chickens. Lowering energy and protein ration caused the lower protein digestible and protein retention and caused decreased growth the chickens. In accordance Jorgensen *et al.* (1996), the ration with high energy and protein influenced growth performance and feed convertion ratio (FCR). It is possible there for increase protein and metabolizable energy intake, used for body composition in chickens. Dewi and Sinlae (2004) reported that protein retention influenced by protein consumption and metabolizable energy in the rations. Furthermore, NRC (1994) reported that the total protein retention can be estimated from high or low production and growth of the chickens. The chickens in treatment A consumed energy 85.25 kcal/bird/day (Table 2). On the other hand, treatments B, C and D were 7.48%, 8.63% and 20.22% significantly

lower than treatment A. The results obtained in this present study suggest that chickens perform better when given higher level energy-protein ratio. This finding in agreement with Sturkie (1976) which stated that lowering energy and protein ratio affect consumption of ration and energy.

Table 1. Effect of different Energy – Protein ratio on Performance of ***Kampung*** **Chicken**

Parameters	Treatment			
	A	B	C	D
Feed consumption (g/bird/day)	18.90[a]	17.60[a]	18.59[a]	17.20 [a]
Body weight gain (g/bird/day)	441.50[a]	418.80[a]	390.01[b]	378.50 [b]
Daily weight gain (g/d/bird/day)	8.08[a]	7.65[a]	6.84[b]	6.52[b]
FCR	2.09[a]	2.16[a]	2.48[b]	2.59[b]

Table 2. Effect of different diets containing similar Energy – Protein ratio on Carcass of *Kampung* Chicken

Parameters	Treatment			
	A	B	C	D
Protein consumption (g/bird/day)	5.11[a]	4.37[b]	4.12[c]	3.31[d]
Energy consumption (Kcal/bird/day)	85.25[a]	78.87[b]	78.48[b]	70.91[c]
Weight	293.60[a]	280.85[a]	254.68[a]	248.86[a]
Carcas Percentage	66.50 [a]	67.06 [a]	65.30 [a]	65.75 [a]

Increasing energy and protein ratio in the diet caused increase in total energy retention in the body of chickens. The chicken show can consume ration with high energy and protein ratio, (A) gave the best production. Table 2 showed that in term of production this finding is in a good agreement with data from various author, who reported that there is no significant ($P > 0.05$) effect in composition/retention on carcass and carcas percentage after feeding different dietary energy and protein (Husmaini, 2004). Increasing total energy and protein ration caused increased energy retention and best production the chickens. Nieto *et al.* (1995) reported the major quality or quantity of amino acid on the ration could give higher protein retention on carcass of the chickens.

The average of carcass percentage with treatment B, is the highest but among the treatment there is no significat different ($P > 0.05$). The good quality of energy – protein ratio gave a good effect on carcass percentage (Hassen, 2008; Dewi, 2010).

From the results of this experiment it can be concluded that level of balance energy – protein ratio in the ration for *kampung* ckickens at 8 weeks of age, gave significant ($P<0,05$) effect on the final body weight, body weight gain, feed consumption, feed conversion, energy consumption, protein consumption, but not for the carcas weight, carcas percentage.

ACKNOWLEDGEMENT

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SOMATOTROPIN SUPPLEMENTATION IMPROVE SKIN AND BONE COLLAGEN CONCENTRATION ON SIX-MONTH AND ONE-YEAR OLD FEMALE RATS

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ABSTRACT

As age increases, aging symptoms such as decrease of stamina, wrinkle of skin, osteoporosis, increase of body fat and menopause will appear. These symptoms are due to the decrease of somatotropin secretion which plays a significant role in cell proliferation. The objective of this study is to investigate the effects of somatotropin supplementation on skin and bone collagen concentration of six-month and one-year old female rats. Forty eight female rats were assigned into a randomized block design with 4 factors. The first factor was age with 2 levels (6 months and 12 months). The second factor was somatotropin dosage with 2 levels (0 and 9 mg/kg body weight). The third factor was duration of injection with 2 levels (3 weeks and 6 weeks), and the fourth factor was sampling period with 2 levels (0 and 14 days after somatotropin termination). The results showed that somatotropin supplementation had significant effects on bone (P=0.014) and skin (P=0.006) collagen concentrations.

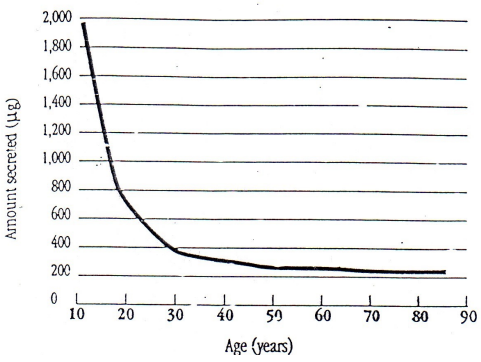
Key Words: collagen, female rat, somatotropin

INTRODUCTION

Somatotropin or Growth hormone is a hormone secreted by anterior pituitary gland that plays a significant role in cell proliferation. The hormone directly or indirectly induces mitosis, cell size and number of cell. Indirect action of this hormone is by triggering growth factors like IGF-1 (Guyton, 1995). Somatotropin through IGF-1 induces activation of protein anabolism, such as collagen and elastin (Klatz, 1998).

In childhood phase, the growth of children is generally very fast because their somatotropin concentration is very high, but as age increases, its concentration decreases slowly. This causes some aging symptoms, such as decrease of stamina, wrinkle of skin, osteopososis, increase of body fat and causes menopause Bengstsson *et el*. 2000).

Wrinkle of skin that predominantly composed of collagen occurs due to decreases of collagen concentration. Decrease of collagen concentration will bring impacts to cell permeability, and obstruction nutrient transport across the cell. As a result, cell will be aging and apaptosis will be appeared,and these influence the decrease of organs function and body as whole (Tarsio *et al*. 1998).



Decreased of somatotropin secretion related to age

MATERIALS AND METHODS

Forty eight female rats were assigned into a randomized block design with 4 factors. The first factor was age with 2 levels (6 months and 12 months). The second factor was somatotropin dosage with 2 levels (0 and 9 mg/kg body weight). The third factor was duration of injection with 2 levels (3 weeks and 6 weeks), and the fourth factor was sampling period with 2 levels (0 and 14 days after somatotropin termination)

Somatotropin was injected intramuscularly following the research design. Skin and bone are collected, cleaned from fat and other materials, dried in oven at 60 ^{o}C, grounded until become small fragmens. So we get free oil dried materials that ready for analysis.

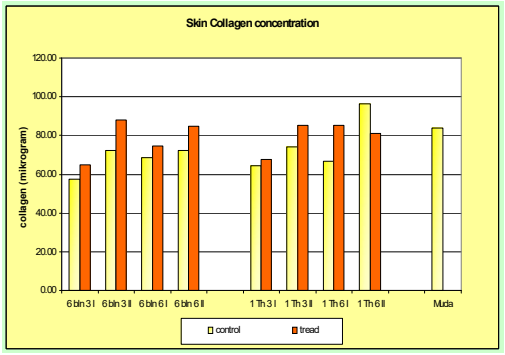
Collagen were extracted from free oil dried materials by 6N HCL solution and boiled at 130^{o}C, then collagen were stained by chloramin T, PCA, p-dimetilaminobenzaldehid and then was read by spectrophotometer.

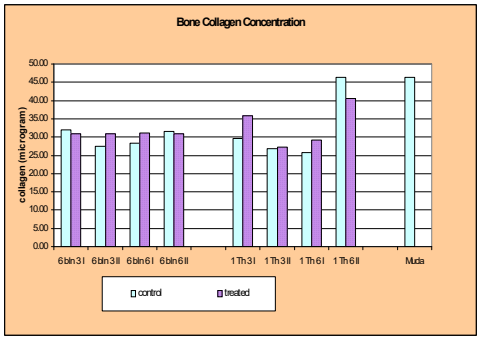
RESULTS AND DISCUSSION

Appearance of smooth skin that indicated by increase of collagen concentratin, influenced by somatotropin dose (P=0,000), duration of injectin (P=0,006), sampling period (P=0.008), and age (P= 0.047). This is due to the factorial the hormone induced protein anabolism such as on collagen and elastin (Klatz, 1998). Somatotropin dose 9 mg/ kg body weight had a significant effect to skin collagen concentration.

Somatotropin injection increased bone matrix mass that was indicated by increased of bone collagen concentration. There were interaction between somatotropin dose, injection duration, and sampling periods to the effect collagen concentration.This interactionis ware very significant.

Izzhucchi and Robin (1995) suggested that somatotropin supplementation increased bone mass caused by somatrotropin action through IGF-1 that induced osteoblast proliferation.





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BIOSORPTION OF CHROMIUM (III) ON NITRIC ACID –TREATED ALGAE *Eucheuma spinosum* BIOMASS

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ABSTRACT

Studies on biosorption of chromium (III) on nitric acid-treated algae (*Eucheuma spinosum*) adsorbent have been carried out. These studies includ determination of optimum H^+/biosorbent ratio, biosorbent acidity, isoterm and biosorption capacity. The result shows that the optimum H^+/biosorben ratio for treated *Eucheuma spinosum* algae is 3.0 mmol/g. The surface acidity of nitric acid-treated biosorbent (Rn_3) and control biosorbent (R_c) are 5.72±0.10 and 2.59±0.27 mmol/g respectively. Biosorption capacity of nitric acid-treated *Eucheuma spinosum* is larger than that of the untreated. The biosorption capacity of Rn_3 and R_c are 68.72 and 57.32 mg/g respectively.

Key Word: Biosorption, Cr(III), nitric acid-treated Algae *Eucheuma spinosum*

INTRODUCTION

Heavy metal contamination of industrial effluents is one of the significant environmental problems due to their toxic nature and accumulation throughout the food chain as non-biodegradable pollutants[1]. Trivalent chromium, Cr(III), is released to the environment by the effluents of industries such as mining, iron sheet cleaning, chrome plating, leather tanning and woods preservation [2]. The maximum levels permitted in wastewater are 5 mg/l for trivalent and 0.05 mg/l for hexavalent chromium [3]. Although chromium (III) is an essential element, it can be toxic at elevated concentrations in the environment. Cr(III) is selected instead of Cr(VI) because Cr(III) is toxic in excessive quantity and caused abnormalities in organism. Cr(III) sulphate salts are mainly used in tanning [4]. The removal of chromium employing conventional methodologies [5] like ion exchange, chemical precipitation or reverse osmosis suffer from limitations namely high operating cost, incomplete precipitation, sludge generation, ect. On other hand biosorption is receiving increasing attention as an emerging technology for the removal of heavy metals from contaminated effluents[6]. The process is based on the adsorption behaviour of certain biological materials towards organic or inorganic substances from their solution. Biosorption, a technologycally feasible and economically process, has gained increased credibility during recent years[7]. The present work investigates the potential use of nitric acid-treated algae *Eucheuma spinosum* for biosorption of trivalent chromium from aqueous solution.

MATERIALS AND METHODS

Sorbent preparation:

The algae Eucheuma spinosum was collected form the Sawangan Nusa Dua beach in Bali. The collected biomass of algae was washed with tap water several times to remove impurities and rinsed with deionized water three times. Then, the biomass was dried at 70 ^{0}C. The biomass of dry algae was ground and sieved with 500 and 250 μm siever. The particle size of biomass powder obtained was 250-500 μm. The powder of biomass was treated with nitric acid by various concentrations of 0.05; 0.10; 0.20; 0.30; and 0.40 M ($Rn_{1,5,}$ Rn_3, $Rn_{6,}$ Rn_9, and Rn_{12}). The treatment was conducted by immersing 1 g of biosorbent in 30 ml of acid solution for 20 mins while stirring. The mixture was filtered and washed with deionized water until neutral. The washed powder was dried at

70 ^{0}C and stored in desicator until used to adsorp Cr(III).

Determination of biosorbent acidity

A 0.50 g of powdered nitric acid-treated algae was dispersed into the 25 mL of 1 M NaOH solution. The mixture was strirred for two hours with magnetic stirer, and then filtered. The filtrate was titrated with 0.5 M HCl. The same procedure was carried out for the blank, solution which onlycontain 25.0 mL of 1M NaOH. The total acidity of biosorbent was calculated by substracting the amount of acid needed for sample from that of for blank.

Batch biosorption experiments

Batch biosorption experiments were carried out in 50 mL Erlenmeyer flasks containing 0.5 g powdered nitic acid-treated algae and 25 mL of 200 ppm Cr(III) solution prepared from chromium chloride salt ($CrCl_3.6H_2O$). The flasks were shaken at 200 rpm for 20 mins at room temperature. The suspensions were filtered, and the concentration of total chromium in the filtrates were analyzed using atomic absorption spectrometer. The total uptakes of chromium were calculated using the mass balance equation as follows[8].

$$qe = \frac{Ci - Ce}{m} . V$$

where *qe* is the metal ion adsorbed (mg metal ion/ g biosorbent) at equilibrium, *V* is the volume of the solution (L) , *Ci* and *Ce* are the initial and equilibrium concentrations of metal ion (mg/l) respectively, and *m* is the dry weight of the biosorbent (g).
A control experiment was carried out under the same conditions in the absence of biosorbent.The effect of pH on the biosorption Cr(III) was investigated by varying the pH of initial Cr(III) solutions in the range of 1-5. The pH solutions were adjusted using 1 M HCl or 1 M NaOH. The effect of initial concentrations of Cr(III) were investigated by varying the initial concentrations of Cr(III) solutions. The experiments were conducted using the initial concentrations of Cr(III) of 100, 200 , 300, 400, 500, 750, 1000, 1200, 1400 and 1500 ppm, under optimum conditions which were: pH = 3, 20 minuts time shaken, at room temperature.

RESULTS AND DISCUSSION

Effect of nitric acid treated on biosorbent

The effect of nitric acid treated biosorbent on sorption of chromium is shown in Fig.1. The sorption of Cr(III) by the biosorbent was maximum at the ratio of acid/biosorbent of 3mmol nitric acid for 1g of powdered algae, 3 mmol/g. Before reaching the maximum point, the sorption of chromium was low, may be because the formation of active sites is not complete, while after the maximum point it decreased may be caused by the biosorbent breakdown.

Surface acidity of biosorbent

Acidities of biosorbent Rn_3 and R_c were determined by acid-base titration method. Acid sites of biosorbent were completely neutralized with excess NaOH and the residual of OH^- was determined by titration with HCl. The surface acidity of nitric acid-treated algae and control were 5.72 and 2.59 mmol/g respectively.The present of acid may have increased the active sites by the dissolution of the impurities and the proton entraped on biomass matrix.

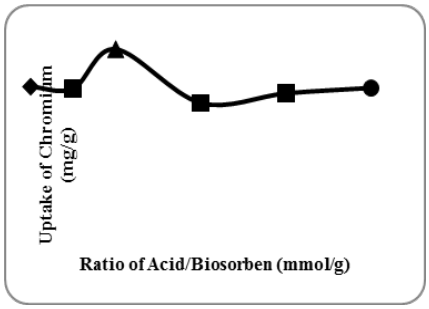

Fig.1. Efect of ratio of acid/biosorben on sorption of chromium

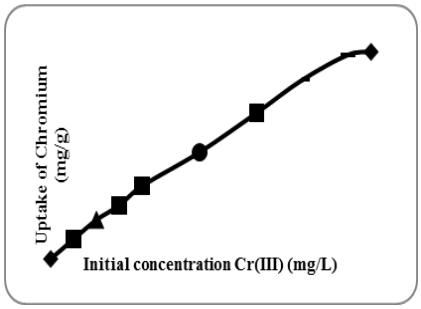

Fig.2. Effect of initial concentrations of Cr(III) on sorption of chromium

Biosorption isotherm and Capacity Determination

Determination of biosorption capacity and biosorption isotherm patterns were conducted by interacting the biosorbent with Cr(III) solution at various concentrations. Fig.2. shows the increase in the Cr(III) uptakes by nitric acid-treated algae with increasing initial concentration of Cr(III) up to 1400 ppm. At higher concentrations, the amount of Cr(III) absorbed on the biosorbent Rn_3 are relatively constant. This means that the number of active sites on the biosorbent has run out so there is no more sites to bind Cr (III). Isotherm biosorption of Cr(III) is described by ploting the concentrations of Cr(III) in equilibrium with the amount of Cr(III) absorbed, as seen in Fig.3.

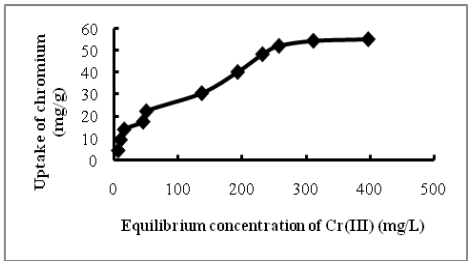

Fig.3. Biosorption isotherm for Cr(III) on nitric acid-treated algae

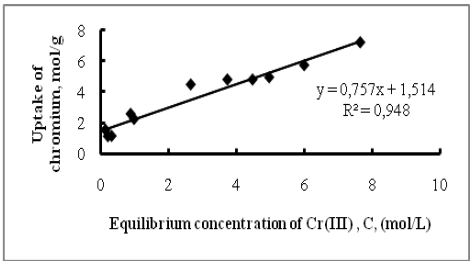

Fig.4. Curve of Langmuir's isotherm

Fig.3. shows that the biosorption isotherm pattern obtained resembles L type isotherm known as the Langmuir isotherm. Based on the equation of Langmuir's isotherm (Fig.4.) the biosorption capacity (b) of treated biosorbent (Rn_3) is 68.72 mg/g. It is larger than that of untreated biosorbent (control) as reported in previous work which is 57.3 mg/g [9].The acid- treated biosorbent has higher capacity because the acid treatment increases the formation of active sites of biosorbent. This is supported by data of surface acidity, where acid-treated biosorbent has higher surface acidity than the untreated.

CONCLUSION

The present study shows that the nitric acid-treared algae *Eucheuma spinosum* has maximum ratio acid/biosorbent of 3.0 mmol/g, with the surface acidity of 5.72±0.10 mmol/g. Biosorption capacity of nitric acid-treated *Eucheuma spinosum* biosorbent for the Cr (III) ion is 68.72 mg/g.

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APPLICATION OF ARTIFICIAL INSEMINATION TO INCREASE LITTER SIZE ON PIG

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ABSTRACT

The aim of this study was to increase the litter size of female pigs (Landrace) using Artificial Insemination (AI) in 15 female pigs. All of the Landrace have farrowed twice. The research used a completely randomized design (CRD) with three treatments: P_1 (AI on first day of estrous), P_2 (AI on second day of estrous) and P_3 (treatments combination of P_1 and P_2). AI dose of 2000-3000 x 10^6 cells/80ml. The results showed that average litter size of Landrace receiving combined treatments P_1, P_2 and P_3 were 4.40 ± 0.548; 6.80 ± 0.837; and 8.20 ± 0.837 piglets, and the average birdth weight per individual were 0.939 ± 0.028; 0.847 ± 0.174; and 0.786 ± 0.175 kg. In conclusion, the litter size of Landrace can be increased with AI program on the first day and the second day of estrous.

Keywords: Artificial insemination, estrous, litter size, Landrace.

INTRODUCTION

Reproductive performance in pigs is measured primarily by number of living piglets at birth or by the total farrowing or weaning weight of the piglets produced by the dam within one year (Anderson, 2000). Pig is well known as a polytocus animal and polyestrous throughout the year. The female has two long uterine horns (bicornuate uterine) and a negligible uterine body. Hunter (1982) recorded that age at puberty in gilts is within 5-8 months. Puberty is characterized by first estrous, ovulation of graffian follicles and release of ova capable for fertilization (Anderson, 2000; Hunter, 1982). Onset of estrous in pigs is characterized by gradual changes in behavioral pattern such as restlessness, mounting other animals, lordosis response, vulva responses such as swelling and pink-red coloring and occasionally a mucous discharge from its vulva.

According to Anderson (2000) and Hunter (1982), the normal estrous or heat cycle of the pig is 20-22 days in length, but it can range from 18-25 days. The estrous cycle can be divided into segments the period of receptivity to the male (standing heat or estrous), lasting from a few hours to several days, and the non receptive period. The average length of estrous is 1-2 days for gilts and 2-3 days for sows. The most critical factor in achieving maximum conception rates with Artificial Insemination (AI) is to inseminate females at exactly the right time. If the length of estrous is longer or shorter, the chances of picking the right time to inseminate a female are lower. Artificial Insemination is the most important single technique devised for the genetic improvement of animals, because a few select males produce enough sperm to inseminate thousands of females per year (Ax *et al.*, 2000).

In logical approach, idea of using single and double AI at different time of estrous apparently is worth to study to look for the appropriate time of mating with AI in Landrace. Using double mating on this study may give more opportunities for sperms to fertilize the ova, give better reproductive performance, especially in order to increase litter size on pig.

MATERIALS AND METHODS

The experiment was conducted using 15 female pigs (Landrace), which were in two farrow and each female pigs was raised in an individual concrete pen (battery models), completed with feed and water troughs. The pens, feed, and water troughs were cleaned daily. All female pigs were given a complete feed from PT. Charoen Pokphand with nutrients content of Protein (18%) and Metabolic Energy (3814.16 kcal). The complete feed was given to the female pigs 2.5 kg/pigs daily.

The research used a Completely Randomized Design (CRD) with three treatments: P_1 (AI on first day of estrous), P_2 (AI on second day of estrous) and P_3 (treatments combination of P_1 and P_2). AI dose of 80 ml/pigs with content of 2000-3000 x 10^6 cell of spermatozoa, and sperm motility of up to 60% (Sumardani *et al*., 2008).

The variables recorded on this study were litter size, and birth weight per-individual of the female pigs. Analysis of Variance (ANOVA) with SAS program used on this study.

RESULTS AND DISCUSIONS

Results of this study showed the averages litter size of Landrace receiving treatments P^1, P^2 and P^3 were 4.40 ± 0.548, 6.80 ± 0.837, and 8.20 ± 0.837 piglets, respectively. The averages of the birth weight per-individual were 0.939 ± 0.028, 0.847 ± 0.174, and 0.786 ± 0.175 kg, respectively (Table 1).

Table 1. Effect of time mating with AI on litter size and birth weight per-individual of Landrace.

Treatments	Litter Size (piglets)	Birth Weight per-Individual (kg)
P_1	4.40 ± 0.548^a	0.939 ± 0.028^a
P_2	6.80 ± 0.837^b	0.847 ± 0.174^b
P_3	8.20 ± 0.837^b	0.786 ± 0.175^b

P_1 = AI on first day of estrous. P_2 = AI on second day of estrous. P_3 = treatments combination of P_1 and P_2. ^{ab} values with different superscripts within the same column are differ significantly.

The lowest litter size on the Landrace receiving treatment P_1 (AI on first day of estrous) indicated that AI at that time is not good. The sperms may be relatively by too long for mating the eggs or ova from the ovary. This could disrupt the fertilization process because most of the sperms should be lost its potential to fertilize the ova. On the treatment P_2 (AI on second day of estrous) and P_3 (AI on first and second days of estrous) increased the litter size of Landrace. In this due to the fact that, the sperm may be have more opportunities to fertilize the ova at appropriate time. According to Johnson *et.al* (2000) the interval from onset of estrous to ovulation in the sow may vary from 19 to 120 hours. According to Hafez *et.al.* (2000) and Kemp & Soede (1996), the duration of estrous in pig is 48-72 hours and the timing of ovulation occurred within 35-45 hours after the onset of estrous. The optimum time for mating or insemination in pig is 10-12 hours before ovulation or early on the second day of estrous and the lifespan of ova and sperm in the female reproductive tract are 8-10 hours (Stevenson & Britt, 1981; Hunter, 1982; Anderson, 2000). So, mating or AI must be carried out before ovulation if the risk of eggs or ova ageing is to be avoided. The most critical factor in achieving maximum conception rates with AI is to inseminate females at exactly the right time. To accomplish this, the breeder must practice proper heat detection. Sows with early estrous should be inseminated later than those showing late estrous, which should receive insemination immediately after detection of estrous (Johnson *et al.*, 2000). According to Anderson (2000), the fertilization rate in pigs is usually high (>90%). Low or high ovulation rates have little or no effect on fertilization rates. Loss of the whole litter may result from fertilization failure or death of all embryos. Early embryonic death results in resorption of

the conception, whereas losses occurring after day 50 may result in abortion, fetal mummification, or delivery of stillborns at term. The conclusions of this study are the litter size of Landrace can be increased with AI program on the first days and second days of estrous.

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INHIBITION POTENCY of *Streptomyces* sp. TO PATHOGENIC FUNGI *Fusarium* sp. CAUSES STEM ROT DESEASE of *Aloe barbadensis* Mill.

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ABSTRACT

This research was done from February until April 2010 with the aim to find out inhibition potency of *Streptomyces* sp. to pathogenic fungi *Fusarium* sp. and to find out the highest percentage of inhibition potency of *Streptomyces* sp. to pathogenic fungi *Fusarium* sp. method used in this study was descriptive method and to measure the inhibition of *Streptomyces* sp. to the growth of *Fusarium* sp. is used whipp method. Twenty *Streptomyces* sp. (*Streptomyces* sp1 – *Streptomyces* sp20) samples used in this research were collection of Microbiology laboratory Department of Biology Faculty MIPA Udayana University, whereas *Fusarium* sp. used in this research is isolated from *Aloe barbadensis* plant in Gianyar regency which is suffered from stem rot desease.Data analyses results obtained; fourty *Streptomyces* sp. had potency to inhibit growth of *Fusarium* sp with difference percentages. the highest percentage that inhibited *Fusarium* sp is *Streptomyces* sp5., that is 92%, followed by *Streptomyces* sp20, *Streptomyces* sp18,S*treptomyces* sp2, *Streptomyces* sp1, *Streptomyces* sp12, *Streptomyces* sp15, *Streptomyces* sp14, *Streptomyces* sp17, *Streptomyces* sp4, *Streptomyces* sp3, *Streptomyces* sp6, *Streptomyces* sp19,S*treptomyces* sp16, there are 73%, 61.5%, 61.2%, 42%, 25%, 23.4%, 22.4%, 18.7%, 14.8%, 14.5%, 14%, 13.8%, 13.6% and 13.5% respectively. In conclusion; fourty *Streptomyces* sp. had potency to inhibit growth of *Fusarium* sp with difference percentages and the highest percentage (92%) that inhibited the growth of *Fusarium* sp. is *Streptomyces* sp5 which is collected from Suana Nusa Penida-Klungkung forest and the lowest percentage (13.5%) is *Streptomyces* sp16 which is collected from Munduk Pangejaran-Bangli forest.

Keywords; *Streptomyces* sp*, Fusarium* sp. inhibition growth.

INTRODUCTION

Aloe (*Aloe barbadensis* Miller.) has been planted in Bali since 2006 with the total area of 170 hectares. The leaves of this plant is processed into gel for further use such as pharmaceutical, food and baverage, cosmetic, etc.

Chemical fungicides are extensively used in current agriculture. However, excessive use of chemical fungicides in agriculture has led to deteriorating human health, environmental pollution, and development of pathogen resistance to fungicides. Because of the worsening problem in fungal disease control, a serious search is needed to identify alternative method for plant protection, which are less dependent on chemical and are more environmentally friendly. Many species of Actinomycetes, particularly those belonging to the genus Streptomyces, are well known as antifungal biocontrol agents that inhibit several plant pathogenis fungi.

The aim of this study is to find out inhibition potency of *Streptomyces* sp. to pathogenic fungi *Fusarium* sp. and to find out the highest percentage of inhibition potency of *Streptomyces* sp. to pathogenic fungi *Fusarium* sp.

MATERIALS AND METHODS

Method used in this study was descriptive method and to measure the inhibition of *Streptomyces* sp. to the growth of *Fusarium* sp. is used Whipp Method. Twenty *Streptomyces* sp.(*Streptomyces* sp1 – *Streptomyces* sp20) samples used in this research were collected from 10 forest in Bali proviencies and save as collection of Microbiology Laboratory Departement of Biology Faculty MIPA Udayana University. Whereas

Fusarium sp. used in this research is isolated from *Aloe barbadensis* plant in Gianyar Regency which is suffered from stem rot desease.

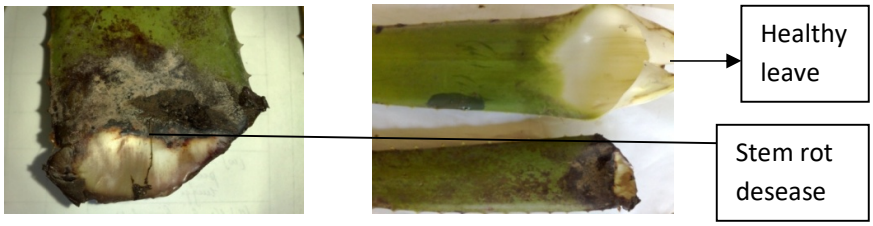

Figure 1. *Aloe barbadensis* Mill infected by *Fusarium* sp. causes stem rot desease

Figure 2. Colony of *Streptomyces* sp.2 (left); Microscopis hypha of *Streptomyces* sp.2 (right)

RESULTS

Data analyses results obtained; Fourty *Streptomyces* sp. had potency to inhibit growth of *Fusarium* sp with difference percentages. The highest percentage that inhibited *Fusarium* sp is *Streptomyces* sp5., that is 92%, followed by *Streptomyces* sp20, *Streptomyces* sp18, *Streptomyces* sp2, *Streptomyces* sp1, *Streptomyces* sp12, *Streptomyces* sp15, *Streptomyces* sp14, *Streptomyces* sp17, *Streptomyces* sp4, *Streptomyces* sp3, *Streptomyces* sp6, *Streptomyces* sp19, *Streptomyces* sp16, there are 84%, 78%, 64%, 42%, 26%, 24%, 22%, 20%, 18%, 18%, 16%, 14%, and 13% respectively.

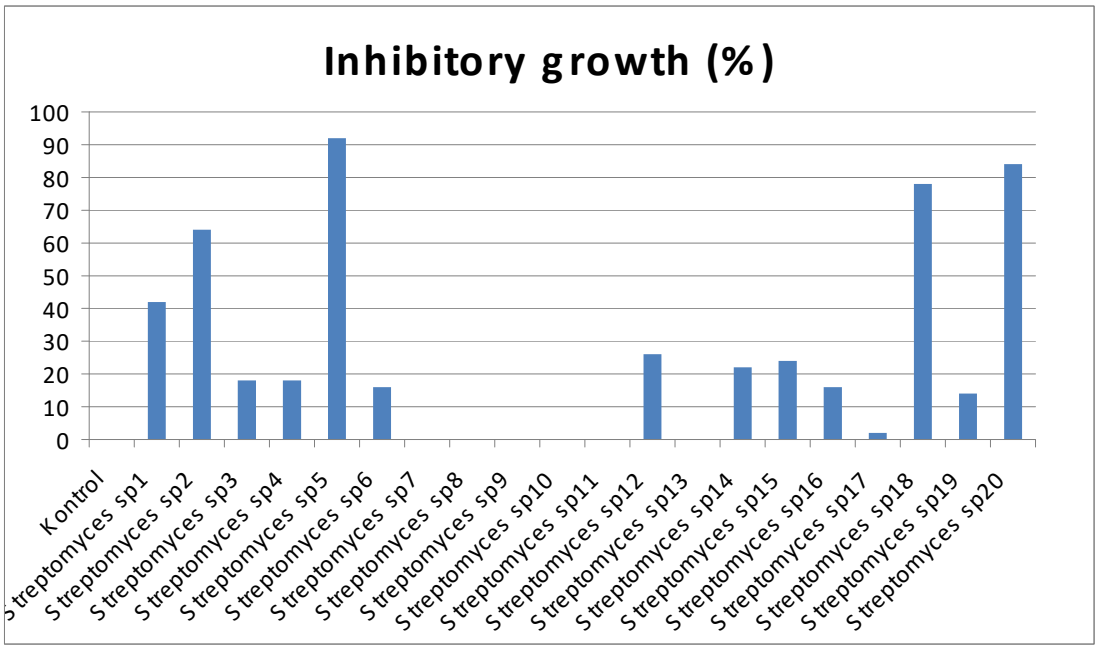

Table 1. Growth Inhibitory (%) of *Streptomyces* sp. to *Fusarium* sp.

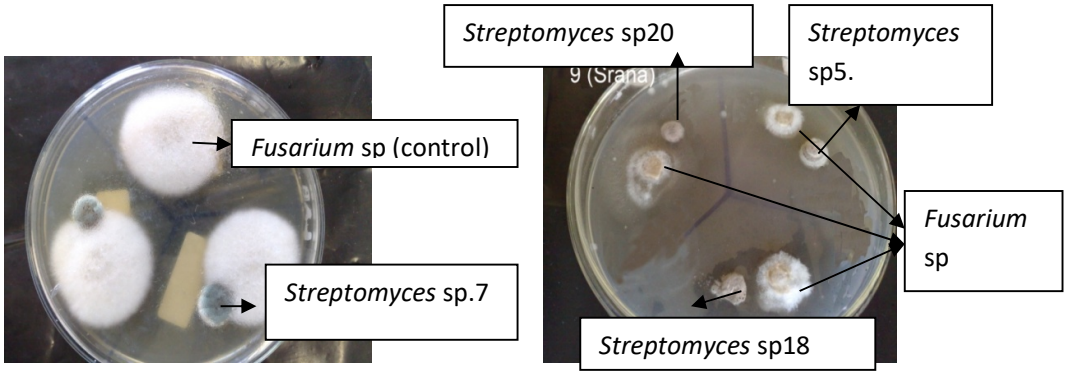

Figure 3. *Streptomyces* sp. cannot inhibit the growth of *Fusarium* sp (left);
Streptomyces sp. can inhibit the *Fusarium* sp in different percentages (right)

DISCUSSION

Streptomyces sp.5 had potency to inhibit the growth of *Fusarium* sp. causes stem rot desease of *Aloe barbadensis* Miller. *Streptomyces* sp.5 bacteria is collected from Suana forest Nusa Penida-Klungkung in which condition of the forest is still natural with biodiversity tropical plants and produces thick litter layer. The litter decomposition produces organic matter that can be used for the growth of Streptomyces. Another factor that influence the growth of Streptomyces is pH. The pH of the Suana forest is neutral (pH 6.5-7.0) and it is suitable for the growth of Streptomyces.

The antagonistic activity of Streptomyces to fungal pathogens is usually related to the production of antifungal compounds (Fguira *et al* 2005., Oudoch *et al*.,(2001). Taechowisan *et al*., (2005)) and extracellular hydrolytic enzymes (Estrada *et al*.,(1998).,Mahadevan B., and Crawford D.L. 1997). Chitinase and B-1,3-glucanase are

considered to be important hydrolytic enzymes in the lysis of fungal cell walls, as for example, cell walls of *Fusarium oxysporum*, *Sclerotinia minor* and *S.rolfsii* (El-Tarabily *et al.,*2003). In this study there is need more research about which part of the phytopatogenic fungal of *Fusarium* sp. is lysis or broken.

CONCLUSION

Fourty *Streptomyces* sp. had potency to inhibit growth of *Fusarium* sp with difference percentages and the highest percentage (92%) that inhibited the growth of *Fusarium* sp. is *Streptomyces* sp5 which is collected from Suana Nusa Penida-Klungkung forest and the lowest percentage (13,5%) is *Streptomyces* sp16 which is collected from Munduk Pangejaran-Bangli forest.

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USE OF WATER PLANT FERMENTED WITH *ASPERGILLUS NIGER* LEVELS IN DIET ON VILLAGE CHICKENS PERFORMANCE AND NUMBER OF LACTIC ACID BACTERIA IN DIGESTIVE TRACT

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ABSTRACT

This study was aimed at finding out use of water plant fermented with *Aspergillus niger* levels in diet on village chickens performance and number lactic acid bacteria in digestive tract. A completely randomized design was adopted in the study that had four treatment i.e. those offered 0, 10, 20 and 30% water plant fermentation in diet of the chickens studied. Each treatment group had four replicates and each replicate had six chickens was 21.40 – 24.36 g. Energy and protein contents of diet offered to the chickens during period of the study were 2.900 kcal ME/kg and 18% respectively. Diet in mash form and dringking water were provided *ad libitum* to the chickens during 12 weeks. Result of the study showed that inclusion of water plant fermented with *Aspergillus niger* in diets significantly ($P<0.05$) increased village chickens performance and number lactic acid bacteria digestive tract and increased *rate of passage* of diet in their digestive tract. It was concluded that use of water plant fermented *Aspergillus niger* at the range 20 – 30% in diet of the village chickens significantly changed performance and number lactic acid bacteria in digestive tract.

Keywords: water plant, *Aspergillus niger*, lactic acid bacteria, villlage chickens

INTRODUCTION

Nusa Penida - Bali Chickens is one of the local Germplasm has good potential for development, because meat chickens realative compact and inexpensive compared with other livestock and their maintenance are very simple and meat prices cheaper than other livestock.

On the other hand, the threat of a global crisis and the more narrow the availability of land, reduced food sources, so for the preservation of village chickens in semi-intensive or intensive, required the availability of quality diet and sustainable. By the way, look for local sources of feed materials in-conventional alternative, a cheap, easily available, does not compete with human needs, is expected to be able to solve problems in agriculture, namely the use natural fiber source, which comes from water plant, such as *Eichornia crassipes,* which is fermented with *Aspergillus niger.* Soeharsono (1979) reported that water plant fibers contain a relatively high crude (20:16%) and low in fat (1.10%). Crude fiber can improve the ecosystem of the digestive tract probiotics mikroflora, characteristics and bulky rations became volominus. As a result, increases intestinal peristalsis, *rate of passage* of diet, transit rations for a while in the digestive tract, thus limiting the absorption of energy, prebiotic fermentation by probiotic bacteria in ventriculus in the form of acetic acid (acetyl - CoA), which serves as a precursor for tissue cholesterol (Surono, 2004), where as bile salts are absorbed back as in the form of HDL cholesterol (High Density Lipoprotein) and stored at liver (Montgomery, 1993).

Crude fiber can lower cholesterol, filling the stomach, overcome digestive disorders, bowel cleanse and reduce by 25 g/l00g fat chicken meat (Anonymous, 1997). Thus expected to provide poultry products with lower fat levels. Syamsuhaidi (1997) reported that the use of prebiotic derived from water weeds such as duckweed from 20 to 40% in broiler chickens aged 3 to 8 weeks no significant decrease total serum cholesterol and triglycerides. The higher ratings tend to produce duckweed cholesterol lower meat. Fermentation with *Aspergillus niger* able to increase protein levels by lowering of

indigestible fiber by poultry. Sutarpa (2009) reported that given of 20-30%, a prebiotic that is fermented with *Aspergillus niger* on diet lowering cholesterol of serum and meat Merawang chickens from Sumatra. Therefore, the study is conducted on a different chicken.

This study aimed was finding out the use of water-plant fermented with *Aspergillus niger* levels in diet on village chickens performance and number lactic acid bacteria in digestive tract.

MATERIALS AND METHODS

The study was conducted in Badung - Bali for 12 weeks, the calculation of feed consumption, *rate of passage* is conducted every weeks and the number of lactic acid bacteria in the digestive tract was calculated on microscopic methods Breed (Lay and Hastowo, 1992), where as cholesterol analysis of meat held at The Laboratory Sanglah General Hospital, Denpasar.

Diet in the form of mash and given drinking water *ad libitum.* Diet with content of 2900 kcal ME/ kg and protein 18% (Scott et al., 1982), composed of material: corn dent yellow, rice bran, coconut meal, soybean meal, fish meal, water plant fermented with *Aspergillus niger* and calcium carbonate. Nutrients composition of diet (Table 1). Water plant derived from Pemogan village, Badung regency.. Before mixed with other ingredients, first water plant sun dried, then ground up into flour. Fermentation with *Aspergillus niger* conducted during 14 days, at 2% DM of water plant.

Experimental design used a Completely Randomized Design with four treatments (0, 10, 20 and 30% water plant fermented with *Aspergillus niger*), with four replications, so there are 16 postal units, and each cage cages filled with six units Village chickens, so use 96 village chickens with weight ranges from 21.40 – 24.36 g. Meat cholesterol were analyzed with methods Enzymatic Cholesterol High Performance CHOD-PAP KIT (Boehringer, 1993). The results were analysis of variance, if at significantly different between treatments (P <0.05), then followed by Duncan test (Steel and Torrie, 1993).

Table 1. Nutrients composition diet village chickens during 12 weeks of the study

Nutrient of diet Standard[1])	Water plant fermentation (%)				
	0	10	20	30	
Metabolizable energy (kcal ME/kg)	2.900,11	2.899,73	2.899,67	2.900.24	2.900,00
Crude Protein (%)	18.00	17.99	17.99	17.99	18.00
Fat (%)	6.33	5.91	5.32	4.80	6-9[2])
Crude Fiber (%)	4.82	5.94	7.07	8.30	3-8 [2])
Calcium (%)	1.03	1.22	1.21	1.24	0.60
Phosphorus (%)	0.56	0.57	0.57	0.58	0.40
Lysine (%)	1.18	1.20	1.22	1.24	0.70
Methionine (%)	0.41	0.38	0.38	0.36	0.36
Triptophan (%)	0.19	0.17	0.17	0.16	0.16

Note: I) Standard Scott et al. (1982).
2) Standard NRC (1994).

RESULTS AND DISCUSSION

Water plant is fermented with *Aspergillus niger* to increase the number of lactic acid bacteria in chicken digestive tract (P <0.05). This means that the fermentation causes the availability of nutrients for bacteria, especially lactic acid bacteria is increasing, and to increase the acidic in digestive tract, by enhancing the strength of oxidizing and

reduction of feed. The increased acidity of the gastrointestinal tract (4-5) of the optimal pH (6-7) and during fermentation produces volatile acid which also gives the antimicrobial effect in conditions of low redox potential, resulting in the reduced number of Gram-negative bacteria that have a competitive nature of the nutrients (Fardiaz, 1992), which in turn increase absorption and retention of nutrients in the body. This fact is supported by the enzyme *ß-galactosidase*, and *lactase dehydrogenase glycolase* of lactic acid bacteria that produce lactic acid and lactose. Lactic acid gave the physiological benefits: 1) to increase the digestibility of protein, 2) improve the utilization of calcium and iron, 3) increase the acidity of ventrikulus, 4) improve the ventrikulus movement, which agree with the results of the study, that the *rate of passage* become shorter (Table 2) and 5) as an energy source. Lactic acid bacteria also produce metabolite (**bacteriocin**), an antimicrobial peptide that is so called as biopreservation amino acids, various vitamins, folic acid, which have an effect on growth of chicken or daily gain significantly faster (Table 2), and flavor compounds, as diasetyl and peroxide help breakdown lactose and lactic acid as an energy source (Surono, 2004). This is showed in the results that feed consumption was reduced with the increasing use of *Aspergillus niger* as a fermentor of feed (Table 2). The results of this study is supported by the statement of Scott *et al*. (1982), that feed consumption is determined by the energy available in the feed and energy metabolism results in lactic acid bacteria in the digestive tract (Fardiaz, 1992)

Table 2. Performance, serum and meat cholesterol, *rate of passage* and lactic acid bacteria village chickens 12 weeks.

No	Response	Water plant fermentation (%)			
		0	10	20	30
1	Feed Consumption (g)	1011.26 a	1000.78 a	998.97 a	909.36 a
2	Daily Gain (g)	428.74 a	425.84 a	433.58 a	533.80 b
3	Lactic Acid Bacteria in Digestive Tract (cfu/g)	$1.9x10^6$ a	$2.5x10^6$ a	$9.3x10^7$ b	$1.1x10^7$ b
4	Serum Cholesterol (mg/dL)	158.00 a	154.00 b	153. 60 b	150.30 b
5	Meat cholesterol (mg/dg)	56.68 a	55.17 a	40.90 b	42.40 b
6	*Rate of passage* (minute)	99.40 a	94.11 a	79.88 b	81.02 b

Note: Different letters in the same row, significantly different ($P < 0.05$)

Total meat cholesterol of village chicken fed with water plant fermented with *Aspergillus niger* 20 to 30% significant ($P < 0.05$) lower than in chickens that received diet without and 10% water plant fermented with *Aspergillus niger* (Table 2). This means that the use of water plant in the diet at the level of 20 to 30% can reduce the Cholesterol village chickens. This decrease occurred because increased use of water plant, increasing crude fiber, so that the diet be voluminous and flow rate/*rate of passage* of diet increased (Table 2). Moreover, with increasing use of water plant in the diet, diet to be bulky, so that the digest value of the diet reduced. This statement is supported by Kusmarjaya (1987), which reveals that the substitution of water plant leaves on diet decreased the broiler diet digest. The second consequence of the above led to the absorption of opportunities - reduced nutrient and energy retention. As a result, it inhibited the process of acetyl-CoA formation, mevalonat, scualen, lanosterol and cholesterol, which in turn causes a decrease TG, LDL, VLDL (Sutarpa, 1998), the implications for the lower cholesterol of meat (North and Bell, 1990).

This phenomenon is consistent with the opinion of Anonymous (1997) which states that the coarse fibers lower cholesterol, overcome interference and cleaning the intestines. Sitepoe (1993) adds that, crude fiber can lower cholesterol by binding bile acids and bile salts, and then excreted with the feces. While *Aspergillus niger* as the fermenters of water plant has been able reduce natural fibers.It was concluded that use of water plant

fermented with *Aspergillus niger* at the range 20 – 30% in the diet of the village chickens significantly changed performances and number of lactic acid bacteria in the digestive tract.

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AMINO ACID COMPOSITION OF DICTYOTA PATENS

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ABSTRACT

Determination of amino acid composition of *Dictyota Patens* using HPLC method has been conducted. Dictyota Patens species contains 15 types of amino acid, those are aspartic acid (0,57%), glutamic acid (0,57%), serine (0,20), hystidin (0,06%), glycine (0,25%), threonine (0,17), arginine (0,59%), alanine(0,08%), tyrosine (0,02%), methionine (0,12%), valine (0,22%), phenilalanine (0,41%), isoleucine (0,16%), leucine (0,33%), and lycine (0,93%).

Keyword: HPLC*, Dictyota patens,* amino acid

INTRODUCTION

Seaweed is one of the biological resources found in the territory of Indonesia. Seaweeds constitute the bulk of marine plants that have a function as a food ingredient Nutritional content of seaweed found on the carbs at 39-51%, protein of 17.2 to 27.13%, fat content 0.08%, 1.5% ash content. Minerals content in seaweed namely K, Ca, P, Na, Fe, I and the content of vitamins such as vitamins A, B1, B6, B12 and C (Anonin, 2009).

Most of the seaweeds contain amino acids valine, leucine, isoleucine, methionine, phenylalanine, tryptophan threonine, and lysine which are essential amino acids. This amino acid can not be synthesized by the human body so must be obtained from outside the body such as food (anonymous, 2010)

Seaweeds that grows in the waters of Bali have been studied at the University of Udayana, but the most point to the raw materials for medicines and health products such as astaxanthin, fucoxanthin, and other carotenoid substances (hero in 2005), swantara, 2008) Research on the macro-nutrients from seaweed such as starch and protein as well amino acid will considered as the basic information in the utilization of seaweeds as food. Analysis of starch and protein as well as amino acid are expected to be useful as a base information in choosing the type of seaweed used as raw material for food.

Sampling was carried out on 20 types of seaweed on one of its water seaweed that is capable of producing seaweed is along side Sanur coast. Located in the city of Denpasar. This research was conducted to determine the type of seaweed which has a relatively high protein content amoung those some seaweeds samples taken at the same place and time. Knowing that the relatively high protein content of 20 types of seaweed, the seaweed species were analyzed again to determine the amino acids content and then identifying its species.

MATERIALS AND METHODS

Materials

The materials used in this experiment consisted of 20 species of seaweed, petroleum ether, ethanol, hydrochloric acid, sodium hydroxide, copper sulfate reagent, nitrogen, potassium borate buffer pH 10.4, the amino acid standard solution 0.5 µmol / L, and OPA reagent (ortoftaldehida), disodium tetrahydrofuran, sodium acetate and aquabidest

Equipment

The equipment used consisted of: Analytical Balance, measuring flasks, Erlenmeyer 100 mL, test tube, Uv-Vis spectrofotometers, kuvet, centrifuge,and a set of tools HPLC.

Procedure

The materials are 20 types of seaweed were taken from the ocean beach sanur. Seaweed was washed with fresh water and then dried using the oven at 40 ^{0}C. The dried material were sieved then blended with 250 μm sieve to obtain powder with a sample size of <250 μm . Furthermore, soluble protein levels analyzed **using** Biuret method of Uv-Vis and then from the highest relative content of protein amino acid Composition was analyzed using HPLC.

RESULTS AND DISCUSSION

Amino acid analysis of samples by HPLC

Analysis of amino acids results in the form as spectra of the amino acid standard solution and sample solution from species of seaweed Dictyota Patens (the highest relative content of soluble protein ,0.04%) presented in figure 1. From the results of analysis show the amino acid composition content dictyota patens seaweed on the beach Segara, Sanur on January 22, 2010 are presented in Table 1.

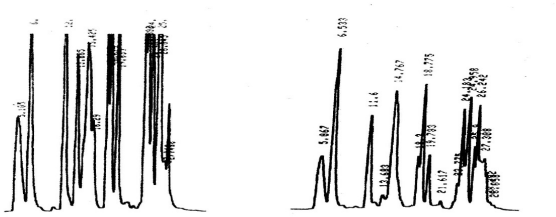


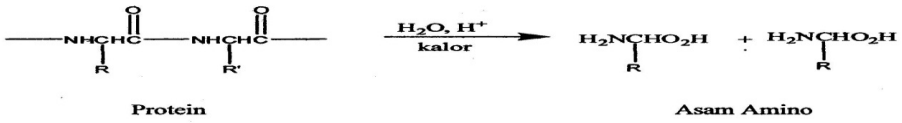
Figure 1. Spectra of standard amino acids and the sample type of seaweed

Table 1. Results Analysis of the amino acid from seaweed

No	Tr (retention time)	Amino acids	Amino acid levels in samples (% w / w)
1	5.105	Asam aspartat	0.36
2	6.905	Asam glutamat	0.57
3	12.020	Serin	0.20
4	13.885	Histidin	0.06
5	15.425	Glisin	0.25
6	16.290	Treonin	0.17
7	18.343	Arginin	0.59
8	18.873	Alanin	0.08
9	19.835	Tirosin	0.02
10	23.802	Metionin	0.12
11	24.180	valin	0.22
12	24.905	Fenilalanin	0.41
13	25.747	Isoleusin	0.16
14	26.045	Leusin	0.33
15	27.438	Lisin	0.93

The above data shows that there nine essential amino acids are arginine, histidine, isoleucine, leucine, lisisn, methionine, phenylalanine, threonine, valine, and six non-essential amino acids; aspartic acid, glutamic acid, serine, glycine, alanine and tyrosine.

Amino reaction is written as below:



Amino acids are used as N sources for the body in the formation of substances containing N and as a source of essential amino acids that can not be produced in the body or only in small quantities to meet the daily needs. A number of amino acids in the body used for the formation of proteins and be in the form of the polypeptide, co-enzyme, the basic framework of a number of compounds important in metabolism (especially vitamins, hormones and nucleic acids), an important metal ion binding required for enzymatic reactions (cofactor).

The highest soluble protein content among the 20 types of seaweed at the beach the Segara, Sanur, which is 0.04% w/w is shown by type of seaweed *Dictyota patens*. *Dictyota patens* contains 15 amino acids, namely aspartic acids, glutamic acid, serine, histidine, glycine, threonine, arginine, alanine, tyrosine, methionine, valine, phenylalanine, isoleucine, leucine, and lysin and the percentage were: 0.36%, 0.57%, 0.20%, 0.06%, 0.25%, 0.17%, 0.59%, 0.08%; 0, 02%, 0.12%, 0.22%, 0.41%, 0.16%, 0.33% and 0.93%, respectively.

It is recommended that protein analysis of other seaweeds of the Segara Beach, Sanur is needed, to identify which type of seaweed has a relatively high protein content. More analysis is needed for other nutrients of the seaweeds on the Sanur Segara Beach and other beach in Bali.

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EVALUATION OF UREA–AMMONIA TREATED RICE STRAW AS A SOURCE OF ROUGHAGE FOR GROWING GOATS

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ABSTRACT

The aim of the experiment is to study the effect of replacing green forage with urea-ammonia treated rice straw as a source of roughage for growing goat was conducted for 6 weeks at the research station of Faculty of Animal Science Udayana University. Balance simple cross over design was used in this experiment consisted of 2 treatments and 2 periods. Four goats were used with average initial body weight 17, 3 ± 0,2 kg. The 2 treatments were native grass and urea-ammonia treated rice straw for treatment R0 and R1 respectively. Roughage and water were given *ad libitum*. Concentrate was given for 1.5% of body weight and offered twice a day. Variable measured were intake and digestibility coefficient of dry matter, organic matter and crude fiber and protein intake. Data were analyzed with variance (Astuti, 1980). Results of this experiment showed that dry matter, crude protein and crude fiber intake of the goat in treatment R1and R0 was not significantly different ($p > 0.05$) as well as the digestibility coefficient of dry matter, organic matter and crude fiber. Organic matter intake of the goat in treatment R1was 15.24% lower than that of treatment R0 ($p < 0.05$). Based on the results of the experiment it can be concluded that urea- ammonia treated rice straw can be used to replace native grass as a source of roughage for growing goats as long as the feed supplemented with high quality concentrates. Further research is needed to know its affect on the reproduction performance of the goat in the longer period of time.
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Key Word: Native grass, urea rice straw treated, goat

INTRODUCTION

Goat is one of the small ruminant species kept by farmers in Bali. Goat production in Bali was insufficient to meet the needs of local people. It is due to the most of feed offered consist of low quality forage especially during dry season. Forage feed provided during the dry season consisted of predominantly low-quality native grass. It's contains 8.77% crude protein, 58.5% total digestible nutrients (TDN), and 43.44% dry matter digestibility (Nitis *et al*., 1985). Availability of native grass in dry season is lower than that of wet season. This causing the goat production decreased. Therefore it is necessary to find alternative feed ingredients, especially when grass production is decreased.

Rice straw (*Oriza sativa*) is one of the agricultural by- product that have a potential to overcome the shortage of native grass in Bali. Rice straw production in Bali reached 5.53 ton dry matter/ha/period of production (PBS Prov. Bali, 2006).Its contains 3-4% crude protein (Sutrisno, 1988), high silica and lignin, and low dry matter digestibility (Hataka, 2000; Eun *et al*., 2007). Treated rice straw with urea is an alternative way for improving the quality of rice straw as feedstuff. Treated rice with 4% urea containing 8.36% crude protein and 46.01% dry matter digestibility (Hartutik *et al.*, 1988). A single urea - ammonia treated rice straw or native grass were insufficient to meet the nutritional needs of goats although given *ad libitum.* Goat with15 kg body live weight with 50 grams live weight gain/day needs the diets with 9.6 protein and 62% TDN (Kearl, 1982). Thus, giving the urea- ammonia treated rice straw or native grass on growing goat have to be supplemented with concentrates, in other to fulfill the nutrient requirements of goats. The aim of the experiment to study the utilization of urea- ammonia treated rice straw as a source of roughage instead of native grass. Subsequently the utilization of land for planting food crops is not affects on productivity of goat in the future.

MATERIALS AND METHODS

Four goats with initial body weight 17 ± 1.2 kg were used in this experiment. Goats are kept in individual fence with a height for 50 cm above the ground floor. Rice straw treated with 4% urea (4kg of urea for 100 kg. of dry matter of rice straw). Ratio of water and rice straw dry matter is 1: 1. Urea-ammonia treated rice straw was given to the goat after incubated for two weeks. Concentrate contain 83.58 % TDN,12,50% crude protein 3,86 % crude fiber, 0.76% Ca and 0.48% P. Composition and nutrient content of urea-ammonia treated rice straw, native grass and concentrates are shown in Table 1.

The Balance Simple Cross- Over Design (Astuti, 1980) was used in this experiment consisted of two treatments, namely the utilization native grass (R0) and urea-ammonia treated rice straw (R1). However, concentrate was fed 1.5% of body weight. Each period was conducted for 28 days consist of seven day adaptation and 21 days for data collection. Total collections was conducted for seven days and conducted at last week of each period using the method of Harris (1970). Concentrates offered twice a day at 8:00 am and at 15:00 pm. Sample urea-ammonia treated rice straw, native grass and concentrate were taken every day as well as samples of rice straw, native grass and concentrate refused. Samples of feed and feed refused were dried under the sun shine for 3 days and the end the period sample of native grass in same period were pooled then 200 gram sub sample of native grass was taken for dry matter, organic matter, crude protein and crude fiber analysis, as well as for urea-ammonia treated rice straw and concentrate. Native grass, urea-treated rice straw and concentrate refused were analyzed dry matter only. Proximate analysis was carried out with method of AOAC (1990) and conducted at the Animal Nutrition Laboratory, Faculty of Animal Husbandry, Udayana University. Variable observed in this experiment is the intake and digestibility coefficients of dry matter, organic matter and crude fiber and crude protein intake. The data were analyzed with variance (Astuti, 1980). The differences among the treatments were used confidence interval at level 5% ($P < 0.05$).

Table 1. Chemical Composition of Feed Ingredient and Treatments

Chemical composition)	Diet component[1)]			Treatment[2)]	
	Native Grass	Urea–ammonia treated rice straw	Concentrate	R0	R1
Dry matter	19.88	20.26	90.58	55.23	55.42
Organic matter	92.03	91.54	94.08	93.15	92.81
Crude fiber	18.23	24.29	12.39	20.14	22.14
Crude Protein	8.77	8.36	12.50	10.64	10.43

[1)] Results from the Animal Nutrition Laboratory Faculty of Animal Husbandry, Udayana University

[2)] The ratio between native grass or urea-ammonia treated rice straw to concentrate 50%: 50% respectively.

RESULTS AND DISCUSSION

Intake and digestibility coefficients of dry matter, organic matter and crude fiber and crude protein intake of the goats R0 and R1 are shown in table 2. Intake of dry matter of the goats R1 and R0 statically was not significantly different (p> 0, 0 5). This is due to (1) urea – ammonia treated has been able to improve the quality of rice straw to be nearly equal to the quality of the native grass. The evident is from the digestibility coefficient of dry matter and organic matter of diet between R0 and R1 was not significantly different (p> 0.5). Consequently feed flow rate from the rumen to the post rumen may be a similar. This condition causing dry matter intake was not significantly different (2) Chemical composition of diet between R1 and R0 is almost a similar (Table

1) mainly crude fiber content. Crude fiber content of diet correlated with feed intake (Putra, 1992). Intake of organic matter in goat R1, 15.24%, significantly ($p < 0.05$) lower than that of R0. This is due to (1) Quantitatively dry matter intake of the goat in R1 lower than of the goat in R0. Organic matter intake correlated positively with dry matter intake (Putra ,1992). (2) The lower of organic matter content in the diet R1 (Table 2).will be affect on organic matter intake. Crude fiber intake between R0 and R1 were not significantly different ($p > 0.05$). It is caused by (1) palatability (Arora, 1995) and bulky (Hattab, 1976 in Putra 1992) of diet R0 and R1 may be almost a similar. (2) Digestibility coefficient of crude fiber on the goat R0 and R1 was not significantly different (Table 2). Consequently the rate of fiber flow in the digestive tract may be a similar.

Table 2. The Effect of Replacing Native Grass with Urea-Ammonia Treated Rice Straw on Intake and Digestibility Coefficient of Dry Matter, Organic Matter, and Crude Fiber and Crude Protein Intake of Growing Goats

Variable	Treatments		SEM
	R0	R1	
Dry matter intake (gram/ $BB^{0,75}$/day)			
- Roughage	25.37[a2)]	23.55 [a]	0.87
- Concentrate	23.64[a]	23.07 [a]	0.14
- Total	49.01 [a]	46.62 [a]	1.02
Organic matter intake (gram/ $BB^{0,75}$/day)			
- Roughage	22.76 [a]	16.51[b]	0.66
- Concentrate	21.89 [a]	21.37 [a]	0.14
- Total	44.69 [a]	37.88 [b]	0.78
Crude fiber intake(gram/ $BB^{0,75}$/day)			
- Roughage	4.63 [a]	5.72 [a]	0.21
Concentrate	2.91 [a]	2.85 [a]	0
- Total	7.54 [a]	8.57 [a]	0,.3
Crude protein intake (gram/ $BB^{0,75}$/day)			
- Roughage	0.69 [a]	0.5 [a]	0.1
- Concentrate	3.06 [a]	2.8 [a]	0.12
- Total	3.75 [a]	3.3 [a]	0.11
Digestibility coefficient of dry matter (%)	65.37 [a]	67.68 [a]	1.76
Digestibility coefficient of organic matter (%)	71.57 [a]	73.77 [a]	1.03
Digestibility coefficient of crude fiber (%)	45.44 [a]	47.02 [a]	6.67

Notes:
1) R0: Native grass *ad libitum* + concentrates 1.5% of body weigh
R1: Urea – ammontreated rice straw *ad libitum* + 1.5% of body weight
2) Value with the same letters on the same line are not significantly different ($p > 0.05$)
3) SEM: Standard Error of The Treatment Means

This condition causing feed and crude fiber intake become not significant different Crude protein intake between the goats R0, and R1 was not significantly different ($p > 0.05$). This is due to crude protein content in the diet is a similar (Table 1) and dry matter intake of the goat R1 and R0 was not significant different (Table 2). Digestibility coefficients of dry matter, organic matter and crude fiber between R1 and R0 was not significantly different ($p > .05$).This is associated with the successful of urea in providing ammonia to treat of rice straw during two weeks incubation, making it easier for rumen microbes to degradation of fiber in feed as a result digestibility coefficient of dry matter, organic matter and crude fiber increase. In line with Utomo *et al*. (1988) report that the escape lignin -cellulose bonds will increase penetration of enzymes produced by rumen micro organisms to feed as a result was increasing digestibility of rice straw. This statement is supported by Hartutik *et al*. (1988) report that the digestibility of 4% urea – ammonia treated rice straw were 46.01% while the digestibility of rice straw without urea 37.75%. Based on these results of this experiment, it can be concluded that the urea-

ammonia treated rice straw can be used to replace the native grass as a source of roughage for growing goat as long as the feed supplemented with concentrate. Further research is needed to know its effects on reproductive performance of the goats in the longer period of time

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DETERMINATION OF THE EFFECTIVENESS OF COCONUT WATER INTERACTED WITH MILK AS AN ATTEMPT TO DIVERSIFY YOGHURT PRODUCTS

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ABSTRACT

This research was conducted to determine the effectiveness of coconut water interacted with milk as an attempt to diversify the yoghurt product. The method used in this study was *Rancangan Acak Lengkap* (abbreviated to RAL) with three formulas of treatment such as: A (the interaction of milk and coconut water for a 7.5% dry yoghurt raw matter); B (the interaction of milk and coconut water for a 10% dry matter of yoghurt) and C (the interaction of milk and coconut water for a 12.5% dry matter of yoghurt). The results of the study show that formula A produced the lowest pH value ($P<0.05$) that is, 4.54, followed formula B and formula C, that is, 4.73 and 4.77, respectively. The low pH value was followed by a high total of similarity such as 0.81%; 0.76% and 0.69% ($P<0.05$). The more skim milk used in each formula, the thicker the yoghurt was. The lowest yoghurt lactose content ($P<0.05$) was found in formula A, C and B that is, 2.27%; 2.53% and 2.74%, respectively. The formulation of raw materials did not affect the amount of the bacteria and the amount of lactate acid bacteria. To use formula A for producing yogurt with the raw materials of coconut water and skim milk.

Key Words: skim milk, coconut water and yoghurt quality

INTRODUCTION

Fermented milk such as yoghurt certainly contains probiotic substance. It has been proved to provide the yoghurt product with functional values (Nagpal *et al*, 2007). Many researches were conducted to prove such functional values of the yoghurt product. Therefore, further attempts will be made to develop this product with its different characteristics. Its quality will be recognized if it matches what is intended by the consumers as the final goal. Today the awareness to consume the functional yoghurt is still low. In addition, sour taste is one of the obstructions for the yoghurt product to be accepted by consumers. Therefore, many innovations have been made to improve its taste without reducing its functional values, such as the innovation which has been done by Indratiningsih *et al* (2004) and Miwada *et al* (2008).

In general, coconut water has been a waste material. Young coconut water has been well known for a long time. It is easy to find vendors selling it along particular streets. It is sold fresh from its shell. It is getting popular as fresh drink. Apart from being fresh drink, it is also used a means of producing what is called *nata de coco*. From its specific value, that is, it tastes specific, it is also a moisture which contains nutrition; especially mineral which is highly useful to the human body. As stated by Hariyadi (2009), it is rich in calcium. In addition to containing mineral, it also contains sugar (its sugar content varies from 1.7 to 2.6 percent), and protein (from 0.07 to 0.55 percent). In Indonesia it is used as a drink and for producing *nata de coco*. Considering that it contains mineral (Silva and Bamurtuarachi, 2009), it can be potentially developed as an isotonic drink.

It is the special qualities of coconut water which has inspired the researcher to conduct a research in the use of coconut water interacted with skim milk continued with fermentation process. Coconut water is interacted with milk with reference to the dry material of the expected yoghurt product. Finally, this study also gives answers to whether milk is falsified with coconut water and informs what to do as the solution.

Specifically, this study aims at developing or diversifying the yoghurt product as the result of fermented milk combined with coconut water. The indicator of the quality of the product produced is evaluated by employing quality the physiological-chemistry approach and the lactate acid bacterial content with the reference to the Indonesian National Standard (Anonym, 1995).

MATERIALS AND METHODS

Materials

The materials used in this study were skim milk and coconut water with starter chasework bought at Tiara Dewata supermarket, Denpasar. For chemical analysis, the materials needed were 70% alcohol, aquades, NaOH O, 1 N, NA, MRS and bacteriological peptone. the tools needed were an oven, a stove, pH meter, a weight, a burette, a measuring glass, a beaker glass, thermometer, an autoclave, a reaction tube, a petri dish, a sterilization room and "quebec colony counter".

Methods

The amount of skim milk and the amount of coconut water which would be combined were determined by referring to the dry material of the yoghurt expected to produce. The dry material of yoghurt expected was 10%, making the treatment of the combination of skim milk and coconut water used around 10% ±2.5%. The suspension amount of skim milk and coconut water made was 300 cc. Therefore, the research method applied in this study was termed as *Rancangan Acak Lengkap* (RAL) with three treatments; they are A (the interaction of milk and coconut water for a 7.5% dry material of yoghurt; B (the interaction of milk and coconut water for a 10% dry material of yoghurt); and C (the interaction of milk and coconut water with a 12.5% dry material of yoghurt).

The producing procedure of yoghurt refers to that introduced by Rukmana (2001). The milk was pasteurized at 85^0C for fifteen minutes before it was lowered to 43^0 C. After that, a 2-3% starter was added. Later, 300 cc of the skim milk and coconut water was suspended for every treatment before it was kept in a topless where it was fermented at 40^0C for 8 hours in inqubator.

The variables which were investigated in this study included the pH value (AOAC (1984), the amount of acid, thickness, lactate content (Sudarmadji *et al*., 1984), the amount of bacteria and the amount of lactate acid (Hadiwiyoto, 1994). The research was conducted at *Laboratorium Teknologi Hasil Ternak.* The data which were analyzed were the same and in the event that there had been real differences then it would have been continued with LSD test (Steel and Torrie, 1988).

RESULTS AND DISCUSSION

Yogurt is a product which may save human beings. Its values may give solution to human digestion problems. The productive bacteria of lactate acid in yogurt may serve as the agent saving those who consume it. The attempt to diversify the yogurt product is made to make it closer to the consumers; in other words, they are made to be aware that it is important to consume this fermented milk product. Such a diversification in this study is made by combining milk and coconut water as its raw materials and its values can be described in the following table.

Table 1. The Values of Yogurt as the Combination of Milk and Coconut water

Variable	Treatment		
	A	B	C
Value of pH	4.53 ± 0.04 [a]	4.73 ± 0.03 [b]	4.77 ± 0.06 [b]
The Amount of Acid (%)	0.81 ± 0.03 [a]	0.76 ± 0.03 [b]	0.69 ± 0.02 [c]
Tickness (second)	15.33 ± 1.53 [a]	20.33 ± 1.26 [b]	23.83 ± 0.76 [c]
Lactose Content (%)	2.27 ± 0.32 [a]	2.74 ± 0.07 [b]	2.52 ± 0.27 [ab]
The Amount of Bacteria (CFU/g)	$2.49 \times 10^7 \pm 0.51$ [a]	$9.52 \times 10^6 \pm 0.05$ [a]	$8.22 \times 10^6 \pm 0.06$ [a]
The Amount of LAB (CFU/g)	$5.89 \times 10^6 \pm 0.25$ [a]	$4.17 \times 10^6 \pm 0.28$ [a]	$3.98 \times 10^6 \pm 0.24$ [a]

The lactose content is one of the indicators in the quality test of yogurt. The attempt made to biodegrade the milk lactose in the fermentation process is the central point in the production of this fermented milk (Miwada *et al*., 2006). The results of the study show that statistically formula B has the highest lactose content ($P<0.05$) compared to the others and formula A has the lowest lactose content. The high lactose content in formula B indicates that the condition provided by this formula tends to be not optimal. In other words, the biodegradation of the milk lactose is not maximal. As already identified that the milk lactose will become lactate acid during the process of fermentation and will result in particular degree of equality and finally the milk protein get thick and the product will be semi thick as a particular characteristic of the yoghurt product.

The yogurt thickness is an essential indicator for macroscopically indentifying the quality of the yoghurt produced. Such a thickness results from the milk protein which gets degraded and, through the process, will become fermented milk (yoghurt). Logically, the degree of the thickness of a solution will be high if its solidity is higher. In formula A less skim milk is used (22.5 g of 300 cc of the amount of milk suspended with coconut water) and the product produced is the yoghurt which is less thick ($P<0.05$) than that produced in formula B (30 g of 300 cc of the amount of the milk suspended with coconut water) and in formula C (37.5 g of 300 cc of the amount of milk suspended with coconut water). The increased portion of the skim milk used will increase the thickness of the milk suspended with coconut water. This condition will be maintained during the fermentation process and when the quality of yoghurt is tested. As far as this study is concerned, the calculated degree of thickness is supposed to be dominantly influenced by the difference in how much skim milk is used, meaning resulting from the influence of its solidity. But the thickness of yoghurt, as one of its specific characteristic, resulting from the performance of lactate acid (Miwada *et al*., 2006), is getting neglected. However, the combination of skim milk and coconut water is the formula which is potentially developed by taking the other indicators indicating the standardization of yoghurt into account.

The results of the study show that the combination of skim milk and coconut water, as the raw materials, which is continued with milk fermentation, results in a real different product ($P<0.05$). Such a difference is shown the amount of acid the yoghurt product in formula A is higher than that in formula B and C. Total acidity of the yogurt on the formula of A is closest to the ideal of total acid i.e. yogurt ranged from 0.85 to 0.95% (Widodo, 2002). In this study, the combination of these raw materials is based on the dry matter of the yoghurt product produced. According to the Indonesian National Standard of yoghurt (Anon, 1995), it is stated that the ideal dry material of yogurt is 10%. The combination of 22.5 g of skim milk with 277.5 cc of coconut water produces the highest degree of sourness, and the more use of skim milk in formula A decreases the similarity degree of the product. What is interesting, as far as this study is concerned, is that the less use of skim milk decreases the sourness degree of the yogurt product. Economically, this

means a decrease in the proportion of the milk used. The combination of skim milk and coconut water is ideally effective for producing milk whose degree of product similarity is close to the applicable standard. Furthermore, no full addition of milk is needed to produce such a similarity. Apart from that, the nutritional content of coconut water is supposed to provide yoghurt with more perfect values. The reason is that it has been potentially used as and oriented toward an isotonic drink (Hariyadi, 2009; Silva and Bamunuarachchi, 2009).

The other evidence of the combination of milk and coconut water, as the raw materials, is that it has produced fermented suspension of milk using productive lactate acid bacteria with the lowest value of pH in formula A ($P<0.05$) compared to the other two treatments. Value of pH in formula A yogurt product was closest approximates to the ideal pH value of yogurt that is 4.5 (Widodo, 2002). Formula B and formula C are not proved to show real differences. In this study, 300 cc of milk suspension and coconut water with the standard dry matter of yogurt is made. The high value of pH of the yoghurt product in formula B and formula C has highly correlated with the rise in the number of microbes found out. This is proved by formula B and formula C in which the number of microbes produced tend to be higher although statistically they do not show real differences. The use of the same starter has been responsible for the differences which are not really calculated in formula A, formula B and formula C. Besides, it is assumed that the different formulas of the three treatments do not contribute to the performance of the bacteria in the metabolism process. As already known that this study is provided with the same treatments, particularly from the process of pre-fermentation to the process of fermentation and the method introduced by Rukmana (2001) is referred to. The coconut water dominantly used in formula A or the skim milk dominantly used in formula C does not lead to the differences in performance of the bacteria, which grows normally and ideally.

It has been macroscopically observed that the total number of the bacteria calculated from the three formulas is slightly different from the total number of lactate acid calculated. Table 1 show that there is a tendency to more dominantly use coconut water than milk to produce higher population of lactate acids, although, statistically, such dominance is not really identified. There must be something which is more productive if more coconut water is used in the suspension of milk and coconut water. The dominance of the bacteria of lactate acid in yoghurt indicates the degree of the product hygiene and at the same time contributes to the increased number of its population in the intestine of those who consume yoghurt (Mckenley, 2005). In addition, the quality of the yoghurt produced will be more nutritional as it contains good nutrition and mineral (Silva and Bamunuarachchi, 2009) which is currently being developed as an isotonic drink (Hariyadi, 2009).

The use of skim milk interacted with coconut water with a 7.5% of dry material (formula A) results in better yoghurt. Viewed from the pH value and the dominant amount of lactate acid bacteria formula A is the closest to the standard. This is also indicated by the product quality test. In addition, the amount of fermented lactate is higher in formula A, although viewed from the degree of similarity, the three products are still higher than the standard. Economically, the production cost is lower as the milk used is less.

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EFFICACY OF RIPE PAPAYA SEEDS POWDER AGAINST *ASCARIS SUUM* IN PIGS

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ABSTRACT

Ascariasis in pigs is still a big problem for farmers in developing countries. Drugs used to cure the disease are available. However, long-term use of such drug often lead to worm resistant to the drugs. Herbal drugs have been shown to be effective against worms in animals and human. One of such herbal drug is ripe papaya seeds which have a strong ovacidal effect *in vitro*. The use of such drug to cure adult worms have however not been reported. A study was therefore conducted to investigate the efficacy of ripe papaya seeds against *Ascaris suum* in pigs. Pigs with the body weight of 15-25 kg were infected with A*scaris suum*. They were then treated with papaya seeds powder for three days at the dose of 3 gram per kg body weight. As a control ascaris infected pigs were treated with albendzol (a common antihelmintic drugs). The result showed that the number of worms in papaya seeds treated pigs were significantly reduced as compared to untreated pigs. The efficacy of papaya seeds powder in reducing the number of worms were not significantly different to alabendazol.

Key Words: Herb ripe papaya seed powder, worm *A. suum*

INTRODUCTION

Worm infection in pigs in the tropic is still a big problem for farmers. The prolonged use of modern medicine often leads to worm resistant to the drugs. Herbal drugs such as papaya seeds have shown to be effective against *Ascaridia gallii* and *Heterkhis galinae* in chicken with the efficacy of 100%. Other studies also showed that the efficacy of papaya seeds at the dose of 1.2 g per body weight against *Haemophylus nana* were also 100%. The efficacy of papaya seeds were also reported against *A tetraptera* which reduced the number of worm by 96.4%. Papaya seeds are also used as antihemintic supplement in cattle feed as they contain alkaloid substance which destroy the *Ascaris spp*, *Enterobius vermicularis* and *Trichuris spp*. However, the efficacy of papaya mature pollen *Ascaris suum* in pigs is unknown. The aimed to the research were studied the efficacy of ripe papaya seed powders in reducing the number of worms in pigs infected with *Ascaris suum*

MATERIALS AND METHODS

Twenty four pigs weighing 10-15 kg were infected with *Ascaris suum* at the intensity of 250-2500 EPG. The pigs were randomly divided into two groups. Group II were treated orally with 3 gram per body weight of papaya seed powder for three consecutive days and group I were treated orally with 0.04 ml zodalben (12.5% albendazole) per kg body weight. The number of worms shaded from infected pigs were observed for 1 week. Afterward the pigs were killed, and the intestine were dissected to count the number of worms that remained in the intestine. The efficacy of ripe papaya seed in reducing the number of worms were calculated by the following formula.

$$\text{Percentage efficacy} = \frac{\text{The number of worms that came out after treatment}}{\text{The number of worms that came out + number of worms remaining after necropsy}} \times 100\ \%$$

RESULTS AND DISCUSSION

The efficacy of ripe papaya seeds in reducing the number of worm in pigs infected with A*scaris suum* was as high as those treated with albendazole. Both of ripe papaya seeds at the dose of 3 gram per kg body weight and 12.5% albendazole at the dose of 0.04 ml per kg body weight gave the efficacy of 100% in reducing the number of worms in pigs infected with *Ascaris suum*.. The result showed that ripe papaya seeds are very effective in curing *Ascaris suum* infection in pigs which in accordance with other studies on the efficacy of the seeds against other worms such as Oesophagustomun, Trichuris, Trichostrongilus, Aspicularis tetraptera and Hymenolepis nana. Papaya seeds appears to inhibit the glucose uptake by the worms such *Ascaridia galli* and *Heterkhis galinae*. As glucose is the vital source of energy for worms the inhibition of glucose uptake by papaya seeds will have toxic effect on worm. Ripe papaya seeds are an effective herbal cure for *Ascaris suum* in pigs when used at the dose of 3 gram per kg body weight will give a similar efficacy as compared to 12.5% albendazole at the dose of 0.04 ml per kg body weight.

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ATTEMPT TO INCREASE THE LITTER SIZE OF BALI GILTS BY INJECTING P.G. 600 AND FEEDING GLUCOSE

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ABSTRACT

Aim of this study was to increase the litter size of Bali gilts by injecting P.G. 600 and feeding glucose. A total of 40 Bali gilts and one mature Bali boar were used. In this study a Completely Randomized Design (CRD) with 2 x 2 Factorial arrangement was used. P.G. 600 was used as the first factor (H) were divided into two, i.e. without injecting P.G. 600 (H_0) and with injecting P.G. 600 (H_1), and was used Glucose as the second factor (G) and was also divided into two, i.e. without feeding glucose (G_0) and with feeding glucose (G_1). There were four treatment combinations namely H_0G_0 (Control), H_0G_1, H_1G_0, and H_1G_1 with ten replications. A total of 10 ml of P.G. 600 containing 800 i.u. FSH and 400 i.u. LH was administered to each gilt by subcutaneous injection behind the ear on the 16th day of its estrous cycle. Feeding glucose to each gilt was started from the 14th day of its estrous cycle until the time of mating by adding 250 g of glucose a day to the basal ration. All of the Bali gilts were mated on the 3rd estrous cycle at onset of the 2nd day of its estrous. The results of this study showed the average litter size of Bali gilts receiving combined treatments H_0G_0 (Control), H_0G_1, H_1G_0, and H_1G_1 were 4.90 $\pm$ 2.33, 6.30 $\pm$ 1.57, 6.20 $\pm$ 2.04, and 7.40 $\pm$ 1.71 piglets respectualy. The average litter weight at birth per-gilt were 1.573 $\pm$ 0.404, 2.888 $\pm$ 0.682, 2.465 $\pm$ 0.811, and 3.503 $\pm$ 1.118 kg, and the average birth weight per-piglet were 0.369 $\pm$ 0.128, 0.461 $\pm$ 0.044, 0.389 $\pm$ 0.042, and 0.461 $\pm$ 0.050 kg, respectively. Statistical analysis showed the effect of injecting P.G. 600 (H_1G_0) increased significantly ($P < 0.05$) the litter size and the litter weight at birth per-gilt of Bali gilts, however, the birth weight per-piglet were not affected. Both effects of feeding glucose (H_0G_1) and treatment combination of injecting P.G. 600 and feeding glucose (H_1G_1) increased significantly ($P < 0.05$) the litter size, litter weight at birth per-gilt and birth weight per-piglet of Bali gilts. There were no interaction effects observed between treatments to the all variables recorded on this study.

Key Words: P.G. 600, glucose, litter size, Bali gilt.

INTRODUCTION

The science of reproduction could be assumed as a knife with double-edged blade. On one side, it is used to reduce the people population through family planning program in densely populated country, like in Indonesia. On the other hand, in the field of animal husbandry, it is used to increase the reproductive performance of farm animals. Among those farm animals, pig has played an importance role and can not be neglected in contributing meat or animal protein for human being. Pig as well known as a polytocous animal and polyestrous throughout the year. The duration of estrous in pigs is in the range of 30 – 60 hours, the ovulation occurred between 36 – 40 hours after onset of estrous, whereas length of estrous cycle is 21 days and duration of gestation is within 111 – 119 days (Hunter, 1982). According to Tan Hok Seng (1957), Bali pig is a crossbred between native Bali pig (*Sus vittatus*) and South China pig (*Sus vittatus*) breed. According to Devendra and Fuller (1979) Bali pig is one of tropical pigs crossbred in Indonesia with low litter size. They recorded that in a survey conducted throughout Bali involving 7,685 sows, the average litter size of Bali sows was found to be 6,2 piglets. According to Anderson (1980), there is a positive relation between ovulation rate and litter size, whereas on certain limit and conditions, higher ovulation rate will produce more litter size and *vice versa* (e.g. ovulation rates continue to increase with subsequent gestation followed by increase litter size until reaches maximal levels by the fourth or fifth parity). Anderson (1980) also stated the number of piglets farrowed increase between the first and fourth litters, but by the eighth litter the number of live births

declines and the number of stillborn increases. Injection of gonadotrophic hormones could be used to increase the ovulation rate (Hunter, 1964). On the other hand, flushing is also could be used to increase the ovulation rate (Zimmerman *et al*., 1958; 1960). Based on above theories, this study want to observed the effect of gonadotrophic hormones by using P.G. 600 injection and flushing in the form of glucose, respectively, and the combined treatments to increase the litter size of Bali gilts.

MATERIALS AND METHODS

Animals

A total of 40 Bali gilts and one mature Bali boar were used on this study.

Ration

All Bali gilts were given a basal ration consisted of 45 % pounded and cooked banana (*Musa paradisiaca*) stem, 30 % rice bran, 20 % forage (*Ipomoea reptans* + *Ipomoea batatas*), 4 % rice crust, and 1 % mineral 10. The basal ration was given to the Bali gilts *ad libitum* twice a day.

Management

Each Bali gilt was raised in an individual concrete pen completed with feed and water troughs. The pens, feed and water troughs were cleaned daily.

Design of Experiment

Completely Randomized Design (CRD) with 2 x 2 Factorial Arangement was used on this study (Battacharrya and Johnson, 1977). P.G. 600 as the first factor (H) was divided into two, i.e. without injecting P.G. 600 (H_0) and with injecting P.G. 600 (H_1). Glucose as the second factor (G) was also divided into two, i.e. without feeding glucose (G_0) and with feeding glucose (G_1). Thus, there were four kind of treatment combinations, i.e. H_0G_0 (Control), H_0G_1, H_1G_0, and H_1G_1 with ten replications, respectively. Injecting P.G. 600 as many as 10 ml containing of 800 i.u. FSH and 400 i.u. LH to each gilt was administered by subcutaneous injection behind the ear on the 16th day of its estrous cycle. Feeding glucose to each gilt was started from 14th day of its estrous cycle until time of mating by adding 250 g of pure glucose a day to the basal ration. All of the Bali gilts were mated on 3rd of its estrous cycle at onset of the 2nd day of its estrous. The variables recorded on this study were litter size per gilt, litter weight at birth per gilt and birth weight per piglet of Bali gilts.

RESULTS AND DISCUSSION

The results of this study is presented in Table 1.

Table 1. Effects of injecting P.G. 600 and feeding glucose on litter size per gilt, litter weight at birth per gilt and birth weight per piglet of Bali gilts.

Treatments	Average		
	Litter Size (piglets)	Litter Weight at Birth per Gilt (kg)	Birth Weight per Piglet (kg)
H_0G_0	4.90 ± 2.33 [a]	1.573 ± 0.404 [a]	0.369 ± 0.128 [a]
H_0G_1	6.30 ± 1.57 [b]	2.888 ± 0.682 [b]	0.461 ± 0.044 [b]
H_1G_0	6.20 ± 2.04 [b]	2.465 ± 0.811 [b]	0.389 ± 0.042 [a]
H_1G_1	7.40 ± 1.71 [b]	3.503 ± 1.118 [b]	0.461 ± 0.050 [b]

[ab] values with different superscripts within the same column are differ significantly ($P < 0.05$).

The results of this study showed that injecting P.G. 600 and feeding glucose singly or in combination increased significantly ($P < 0.05$) the litter size of Bali gilts. Treatment of injecting P.G. 600 increased significantly ($P < 0.05$) the litter weight at birth per gilt but it did not effect the birth weight per piglet, however, treatment of feeding glucose and treatment combination of injecting P.G. 600 and feeding glucose increased significantly ($P < 0.05$) the litter weight at birth per gilt and birth weight per piglet of Bali gilts. There were no interaction effects between treatments to the all variables recorded on this study.

The result of this study was supported by Pope *et al*. (1968) recorded that the litter size increased from 8.3 piglets in control to 10.3 piglets in the treated gilts injected with gonadotrophins using 1,200 i.u. of PMS. A similar result was also recorded by Longenecker and Day (1968) recorded that superovulated sows injected with 1,200 i.u. of PMS given on the day of weaning the piglets farrowed an average of 2.0 piglets more per litter. On the other hand, Baker *et al*. (1970) recorded in gilts that injecting 1,000 i.u. of PMS resulted a significant increase in litter size, i.e., 9.8 piglets in the treated gilts *versus* 8.3 piglets in control. Combined follicle stimulating hormone (FSH) and lutenizing hormone (LH) or pregnant mare's serum gonadotrophin (PMS) and human chorionic gonadotrophin (HCG) are hormones preparation usually used for inducing superovulation of eggs or ova in pigs (Gibson *et al*., 1963; Hunter, 1964, 1982; Britt and Roche, 1980). Longenecker *et al*. (1965) recorded that treatment with a single of 1,200 i.u. PMS on the day the piglets were weaned induced superovulation and increased fecundity in sows. Day *et al*. (1967) also recorded a statistically significant increase in ovulation rate was obtained in gilts injected with 1,200 i.u. PMS. FSH and PMS has a similar function, i.e., to induce growth and maturation of ovarian follicles and stimulate its production of oestrogens, whereas LH and HCG has another similar function, i.e., to induce ovulation of follicles with release of the eggs or ova (Hunter, 1982). According to Britt and Roche (1980) the injection of PMS alone or in combination with HCG has been employed to induce superovulation where the response obtained in related to the reproductive state of the female and the dose and timing of gonadotrophic hormone treatment. They declared, when combinations of PMS and HCG is given in gilts, ovulation occurs about 110 to 120 hours later, compared to about 40 hours after HCG when the two gonadotrophins are given separetely. Furthermore they explained, inseminations after the combination treatment should be given about 96 hours after the treatment, compared to 24 hours after HCG when the double treatment is used. All doses of PMS between 500 i.u. and 1,500 i.u. produce superovulation but the response is much greater in the gilts receiving the 1,500 i.u. dose of PMS (Hunter, 1964).

According to Bazer *et a*l. (1967), the gilts fed restricted feeding (1.81 kg a day) flushed for a 14 day period prior to breeding by *ad libitum* feeding increased the litter size from 8.3 piglets in control to 10.9 piglets in the treated gilts. On the other hand, Lodge and Hardy (1968) recorded that one day flushing by doubling the feed intake on the first day of estrous resulted a significant increase in litter size, i.e., 10.8 piglets in the treated gilts *versus* 8,95 piglets in control. Commonly, the convensional flushing persisted for 5 to 15 days before mating and ovulation response seems to result from increased energy intake the period of major follicular development (Zimmerman *et al*., 1960). The addition of pure energy sources (glucose and fat) to a basal diet during a day-14 period to ovulation was reported by Zimmerman *et al.* (1958; 1960) to significantly increased ovulation rate in pigs. Glucose is one of pure energy of feedstuff with high energy content. Glucose influence indirectly, whereas gonadotrophins influence directly the ovary of the gilts in inducing the growth of follicles, maturation and ovulation of the eggs or ova. In the body, one molecule of glucose produce energy as many as 288.8 Calories (Harper *et al*., 1979). They clarified, glucose could be absorbed directly by the intestine and stored as glycogen in the body.

The increase of the litter weight at birth per gilt and birth weight per piglet of Bali gilts flushed with glucose may be caused by the effect of energy intake addition from

glucose in its ration. However, of course this explanation needs further study. It was concluded that:

1. Injecting P.G. 600 increased the litter size and litter weight at birth per gilt of Bali gilts, however, the birth weight per piglet were not affected.
2. Feeding glucose increased the litter size per gilt, litter weight at birth per gilt and birth weight per piglet of Bali gilts.
3. Combination of injecting P.G. 600 and feeding glucose also increased the litter size per gilt, litter weight at birth per gilt and birth weight per piglet of Bali gilts compared to control.
4. There were no interaction effects between injecting P.G. 600 and feeding glucose to the litter size per gilt, litter weight at birth per gilt and birth weight per piglet of Bali gilts.

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STUDY THE EFFECT OF *ASEM* (*Tamarindus indica L.*) AND *KATUK* (*Sauropus androgynus*) LEAF EXTRACT IN DRINKING WATER FOR DECREASING PLASM CHOLESTEROL AND ABDOMINAL FAT OF DUCK

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ABSTRACT

This experiment was carried out to study the effect of of *asem* (T*amarindus indica L.*) and *katuk* (*Sauropus androgynus*) leaf extract in drinking water for decreasing plasm cholesterol and abdominal fat of duck age 4 - 12 weeks in Tabanan, Bali. The experiment used Completely Randomized Design (CRD) wit three treatment and four replication. Each replication consist of four duck with homogenous body weight. The diets were formulated to 2900 kcak ME/kg and 16% crude protein. The treatment were drinking water without *asem* and *katuk* leaf extract as control treatment (A), drinking water with *asem* leaf extract (B), drinking water with *katuk* leaf extract (C). All diets were in mash form. Diets and drinking water were offered *ad libitum*. Result of the experiment showed that the duck which offered *asem* (B) and *katuk* (C) leaf extract in drinking water were not affected (P>0.05) on diets and water consumption, but decreased significantly different (P<0.05) on final body weight, body weight gain, carcass weight, and feed efficiency compare with control treatment (A). Abdominal fat and plasm cholesterol total, both on treatment B and C decreased were significantly different (P<0.05) than control treatment. It can be concluded that the duck were offered asem and katuk leaf extract in drinking water increased body weight gain and feed efficeincy, but decreased abdominal fat and plasm cholesterol total of duck age 4 – 12 weeks.

Key Words: *Asem*, *Katuk* leaf extract, cholesterol, abdominal-fat and duck

INTRODUCTION

Nowadays, consumers prefer to consume livestock product which low in fat, because the estimation of high fat content in livestock products could cause the increasing of atherosclerosis illness if it is consumed. According to Santoso (2000), that high accumulation of fat in abdomen and viscera will reduce the profit and increase waste problems.

Study about the using of plant to improve the quality of livestock production is very important, because will add biodiversity of plant resources and as a basic of economic botany and also other applied botanic (Soekarman and Riswan, 1992). According to Santoso (1993), that natural content of plant is secondary organic matter which is yielded through secondary reaction of primer organic matter (carbohydrate, fat and protein).

Asem (*Tamarindus indica L.*) leafs known as a traditional medicine and spice, also very useful medically. *Asem* leafs contain phytochemical that has faal function. Result of the pre research proved that phytochemical content in *asem* leaf are *flavonoid, saponin, steol and quinon* (Kriswiyanti et al., 1997). K*atuk* (*Sauropus androgynus*) leafs as the same as asem leaf is a traditional medicine plant that contain high nutrition, as antibacterial and contain *beta carotene* as an active matter of carcass colour. Phytochemical that contain in leaf are *saponin, flavono*id, and *tannin* (Santoso, 2000). According to Karyadi (1997), *isoflavonoid* that similar with estrogen could pursue the decreasing of bones mass (*osteomalasis*), decrease blood cholesterol level and increase HDL level, while *saponin* is useful as anticancer, antimicrobial and also decrease blood cholesterol level.

Result of the pre research that carried out by Santoso (2000) indicated that offering of 3% *katuk* leaf to chicken feed could decrease the fat accumulation, decrease the chicken meat smell, and also could depress the amount of *Salmonella sp* and *Escherichia coli*.

Some researches in human and animal have proven that phytochemical combination matter in human body or animal has particular function which is very useful. The combination results enzymes that useful to stop toxin (detoxification), pursue cholesterol forming, increase hormonal metabolism, antibacterial, antioxidant, regulate blood sugar level, and anticancer (Karyadi, 1997).

Based on the above description, its likely very important to study the effect of adding *asem* and *katuk* leaf extract through drinking water to blood lipid profile and the amount of duck abdomen fat.

MATERIALS AND METODS

Place and Periode of Experiment

The experiment was carried out at Dajan Peken village, Tabanan regency, Province of Bali and Laboratory of Chemical Nutrition and Livestock Feed, Faculty of Animal Husbandry, Udayana University. The experiment was carried out for eight weeks.

Cage and Duck

The cagewere used battery colony which made from bamboo with 100 cm length, 60 cm width and 40 cm height, every cage is completed with feeding and drinking equipment.

The experiment used duck with four weeks age and homogenous body weight.

Diets and Drinking Water

Diets given is concentrate ration (mash form) and consist of yellow corn, rice bran, coconut meal, fish meal, coconut oil, and mineral. The ration formulated with 2900 kcal/kg metabolic energy and 16% crude protein. Source of drinking water is come from local PDAM.

Asem and *Katuk* Leaf Extract

Asem (*Tamarindus indica L.*) and *katuk* (*Sauropus androgynus*) leaf used were local *asem* and *katuk* which old enough. *Asem* and *katuk* leaf were mixed in water to get the extract with ratio 300 g leaf in one liter drinking water.

Experimental Design

Completely Randomize Design (CDR) was used in this experiment with three treatments and four replicates. Each replicate used four duck (four weeks age). The three treatments were:

A = ducks which given drinking water without *asem* and *katuk* leaf extract as control treatment

B = ducks which given *asem* leaf extract through drinking water for three weeks countinously

C= duck which given *katuk* leaf through drinking water for three weeks countinously

Ration and drinking water were given *ad libitum* and drinking water was changed everyday to avoid from desease. Slaughtering of duck was done at the end period of the experiment according to USDA (1977), the duck was slaughtered at vena jugularis which located between head bone and first phalanx of neck bone.

Table 1. Feed Composition Ration for Duck Age 4 – 12 Weeks

Feed Composition (%)	Treatments		
	A	B	C
Yellow corn	60.83	60.83	60.83
Coconut meal	10.26	10.26	10.26
Rice bran	15.25	15.25	15.25
Soybean	1.28	1.28	1.28
Fish meal	11.24	11.24	11.24
Coconut oil	0.67	0.67	0.67
NaCl	0.17	0.17	0.17
Premix	0.30	0.30	0.30
Asem leaf extract	-	-	+
Katuk leaf extract	-	+	-
Total	100	100	100

Table 2. Nutrient Composition of Ration for Duck Age 4 – 12 Weeks[1)]

Nutrient	Unit	Treatments			Standard[2)]
		A	B	C	
Metabolic energy	kcal/kg	2900	2900	2900	2900
Crude protein	%	7.06	7.06	7.06	16
Ether extract	%	4.54	4.54	4.54	5 – 8[3)]
Crude fiber	%	1.04	1.04	1.04	3 – 8[3)]
Ca	%	1.04	1.04	10.04	0.60
P- available	%	0.59	0.59	0.59	0.35
Arginin	%	1.23	1.23	1.23	1.00
Methionine + Sistin	%	0.68	0.68	0.68	0.60
Lysin	%	1.12	1.12	1.12	0.80

Notes:

[1)] Based on calculating according to Scott et al. (1982) table composition
[2)] NRC standard (1984)
[3)] Morrison standard (1961)

Variables Observed

Variable observed were diets and drinking water consumption, body weight gain, blood lipid profile, carcass weight, and body fatty (pad fat, mecenteric fat, duck stomach fat and abdominal fat).

Statistical Analysis

The data were analyzed with analysis of variance, and further analysis was continued using Duncan test (Steel and Torrie, 1989).

RESULTS AND DISCUSSION

Offering of *asem* and *katuk* leaf extract through drinking water (300 g leaf in one lier water) were not significantly different ($P>0.05$) to diets and drinking water consumption. This is showed that phytochemial molecule content in *asem* and *katuk* leaf weren't able to increase the ration consumption. Theoritically, ration consumption is very affected by energy metabolic content in ration. Wahyu (1978) reported that metabolic energy content in ration is the main factor that affecting consumption level on fowls. But, it tended that diets consumption was increased after offering of *asem* and *katuk* leaf extract through drinking water. Growth factor will affect amount of diets consumption. It is logic, because increasing of growth will follow the increasing of nutrient requirenment, especially energy. The increasing of duck growth, accumulatively will increase nutrient requirement and the logic consequences, the duck will increase the diets consumption and other nutrient.

Table 3. The effect of offering asem and katuk leaf through drinking water on performance of duck age 4 – 12 weeks

Variables	Treatments[1)]			SEM[2)]
	A	B	C	
Ration consumption (g/h/8 weeks)	7707.89a[3)]	7887.81a	7950.20a	79.072
Drinking water consumption (l/h/8 weeks)	24.172a	26.092a	25.971a	1.032
Lysine consumption (g/h)	86.33a	88.34a	89.04a	1.047
Final weight (g/h)	1284.08b	1379.48a	1369.84a	16.802
Body weight gain (g/h/8 weeks)	882.92b	981.07a	970.72a	16.092
Feed Converstion Ratio (FCR)	8.73a	8.04b	8.19b	0.109

Final weight and body weight gain of duck increased with the offering of *asem* and *katuk* leaf extract through drinking water. This is because phytocemical molecule in *asem* and *katuk* leaf could increase the the efficiency of nutrient using, especially protein. According to Sugahara and Kubo (1992), protein consumption and lysine amino acid could increase the energy retention as protein and decrease the energy retention as fat in the body. This opinion is supported by Sibbald and Wolynetz (1986), that energy retention as protein is increase, besides energy retention as body fat is decrease with the increasing of lysne amino acid concentration in the body that impact by the increasing of protein consumption or lysine amino acid.

Table 4. The effect of offering asem and katuk leaf through drinking water on carcass, abdominal fat, and total cholesterol plasm of duck age 4 – 12 weeks

Variables	Treatments[1)]			SEM[2)]
	A	B	C	
Slaughtering weight (g)	1286.39b[3)]	1380.06a	1370.92a	15.981
Carcass weight (g)	776.46b	865.57a	855.18a	15.096
Carcass percentage (%)	60.36b	62.72a	62.38a	0.604
Carcass physically composition (%)				
• Meat	41.69b	42.90a	42.97a	0.205
• Bone	26.62a	26.59a	26.49a	0.318
• Subcutan fat + skin	31.64a	30.51b	30.54b	0.104
Abdominal fat (% live weight)	2.41a	2.03b	2.09b	0.046
Plasm cholesterol (mg/dl)	184.93a	163.80b	167.06b	4.095
Plasm triglyceride (mg/dl)	158.07a	132.73b	135.92b	4.720

Notes:

1) A (ducks which given drinking water without asem and katuk leaf axtract), B (ducks which given asem leaf extract through drinking water), and C (duck which given katuk leaf through drinking water)
2) Standard Error of Treatments Means
3) Value with the same alphabet in the same line means not significant different (P>0.05)

The amount of abdoment fat and plasm cholesterol level decreased because the offering of *asem* and *katuk* leaf extract through drinking water. The decreasing of amount of abdoment fat and plasm cholesterol level because the effect of the increasing of protein and lysine amino acid consumption. Al-Batshan and Hussein (1999) reported that the increasing significantly of protein consumption will increase carcass weight, carcass percentage, breast meat percentage and significantly decrease the abdominal fat. The decreasing of fat accumulation could be caused by *saponin* content in asem and katuk leaf which bind endogemous bile cholesterol. *Saponin* could decrease lipid and blood cholesterol level by pursue endogenous bile cholesterol absorbtion. Besides, *saponin* could pursue pancreas enzyme activity and metabolit product, also could form unsoluble molecule complex with Zn (Ferket and Middelton, 1999). Wibowo (1990) reported that the activity of pthytochemical molecule (*scordinin*) to both of leaf extract is the same as *oksido-reductase* enzyme. *Scordini* function as growth supporting enzyme is effective in

duck body. This is proven on mouse and rabbit, whereas *scordinin* be able to depress lipid and blood cholesterol level on male rabbit. Endogenous bile salt is verry needed for emulsing of fat which will eat, so can be digested by lipase enzyme (Siregar et al., 1982).

Some result of experiment about the using of *katuk* leaf, beside having high nutrient, also content of antibacterial (Anon, 1995 in Santoso, 2000). The offering of 3% old leaf *katuk* in ration could decrease the fat accumulation, and could increase the feed efficiency of ration without decrease the chicken weight. The offering of *katuk* leaf extract amount of 4.5 g/liter drinking water could decrease the fat accumulation in the body, increase the feed efficiency of ration, and decrease the amount of *Salmonella sp*. and *E. coli* on chicken meat (Santoso, 2000).

CONCLUSION

Based on result of the experiment, it can be concluded that the offering of asem and katuk leaf extract through drinking water (300 g in one liter drinking water) could increase the body weihgt gain and decrease the amount of abdoment fat and plasm total cholesterol of duck age 4 – 12 weeks.

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SEROLOGICAL AND MOLECULAR DIAGNOSIS OF *Toxoplasma gondii* USING GRA1 ANTIGEN AND THE TACHYZOITE AND BRADYZOITE SEQUENCE SPECIFIC STAGE OF SAG1 AND BAG1 IN VILLAGE CHICKEN

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ABSTRACT

Toxoplasmosis is a zoonotic disease caused by Toxoplasma gondii. Cases of toxoplasmosis in animals have been reported to be 50-80% and in humans 40-85% (Subekti et al.,2005). Due to this high prevalence, the diagnosis of toxoplasma is needed. We have tried an ELISA test based on Gra1 antigen and developed a primer sets to detect the Tachyzoite and Bradyzoite Sequence Specific Stage of SAG1 and BAG1 from village chicken. Gra1 is a part of Toxoplasma gondii protein Gra. Gra1 is an antigen protein which is important as the principal components vacuoles in tachyzoite and cyst wall bradyzoite (Lebrun et al., 2007). The prevalence of toxoplasmosis in village chicken is necessary to find out the spread of the Toxoplasma gondii in the environment. Chicken can be infected with the oocysts from the ground. **Purpose**: - To determine the prevalence of Toxoplasma gondii in village chicken - To amplify specific DNA sequences of Toxoplasma gondii

MATERIALS AND METHODS

200 chicken sera and heart samples, the recombinant protein of Toxoplasma gondii Gra1 (provided by Prof Tunas Artama of UGM), Elisa Equipment, PCR Reaction Mix (Invitrogen). Primer sets were designed using web-base Primer 3 program and the sequence was

SAG1 primers (F: 5'-CACCTGTAGGAAGCTGTAGTCACTG-3 ';
R:5'-TCACTGTGACCATACAACTCTGTG-3').
BAG1 primers (F: 5'-AGGAGAGAAGACCTCGAAAGAAG-3 '
R: 5'-TGAACGCTAGGTTTCTGGATACG-3′

ELISA was carried out using alkaline phosphatase and read in ELISA reader. Genomic DNA was isolated using Pure-Link DNA isolation Kit (Invitrogen) and amplified with platinum taq polymerase (Invitrogen) and visualized under ultraviolet light after staining with ethidium bromide.

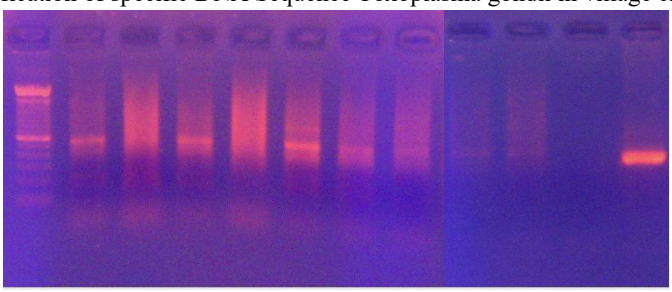
RESULTS

Prevalence of Toxoplasma gondii infection in village chicken was 66.5% (133/200).

Tabel 1. Percentage of *Toxoplasma gondii* infection in chicken from 9 districts in Bali

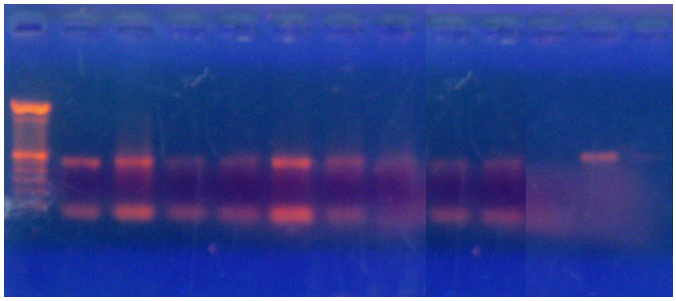
Provided village chicken	Total Number	Positive	Percentage
Denpasar (Dps)	24	14	58,3
Badung (Bdg)	24	16	66,6
Tabanan (Tbn)	20	6	30,0
Klungkung (Klk)	21	17	80,9
Bangli (Bgl)	21	7	33,3
Jembrana (Jbr)	22	9	40,9
Gianyar (Gyr)	20	20	100,0
Buleleng (Bll)	21	19	90,4
Karangasem (KA)	27	25	92,5
Total	200	133	66,5

Amplification of specific DNA Sequence Toxoplasma gondii in village chicken



M 1 2 3 4 5 6 7 8 9 10 K

Figure 1. Amplification Sequence stage specific **BAG1** *Toxoplasma gondii* M: Marker, 1 Denpasar Chicken, 2. Tabanan chicken, 3. Bangli Chicken, 4. Klungkung Chicken, 5. Buleleng chicken, 6. Karangasem chicken, 7. Badung Chicken, 8. Gianyar chicken, 9. Jembrana chicken, 10. Negative Control, *K.Tachyzoite*



M 1 2 3 4 5 6 7 8 9 10 K

Figure 2. Amplifiction Sequence Stage Specific **SAG1** *Toxoplasma gondii*. M: Marker, 1. Karangasem Chicken, 2. Tabanan Chicken, 3. Bangli chicken, 4. Klungkung Chicken, 5. Buleleng chicken, 6. Karangasem chicken, 7. Badung Chicken, 8. Gianyar chicken, 9. Jembrana Chicken, 10. Negative Control, *K.Tachyzoite*

CONCLUCION

Antigen Gra1 *Toxoplasma gondii* has been successfully applaid to determine the Toxoplasma gondii antibodi in village chicken. Primer sets developed to detect sequence stage-specific tachyzoite and Bradyzoite (SAG1 and BAG1) has successfully amplified specific DNA of Toxoplasma gondii from village chicken.

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OPTIMIZING VITAMIN-MINERAL SUPPLEMENTATION IN KING GRASS-BASED RATIONS TO MAXIMIZE RUMEN MICROBIAL PROTEIN SYNTHESIS AND ITS RELATIONSHIP WITH PRODUCTIVITY OF BALI CATTLE

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ABSTRACT

Bali cattle have a great potency to supply of national meat demand which increase progressively every year. The main constrain in Bali cattle farming is the deficiency trace minerals on native grass to result in low of Bali cattle productivity. The present study was done to determine the optimum vitamin-mineral supplementation in King grass-based rations to maximize rumen microbial protein synthesis and its relationship with productivity of Bali cattle steer. Randomized Complete Block Design used in this study consisted of four treatments and five groups based on differences in live weight cattle. Treatment consists of: S0 = concentrate as much as 5 kg + King grass given *ad libitum*, S1, S2, and S3 = S0 successively added 0.1%, 0.2% and 0.3% vitamin-mineral in concentrate. Variables observed are nutrients intake, rumen microbial protein synthesis, deposition of nutrients, live weight gain of the animals, and feed efficiencies. The data obtained were analyzed by analysis of variance, and regression analysis used to predict the optimal level of supplementation. Results showed that vitamin-mineral supplementation significantly ($P < 0.05$) effected to all of the observed variables. Supplementation levels of 0.2 to 0.3% can reduce the consumption of nutrients, but supplementation levels of 0.1 to 0.3% can increase rumen microbial protein synthesis, ruminal N-NH_3, total ruminal VFA (volatile fatty acids) deposition of nutrients, efficiency use of the ration, and can increase a live weight gain of Bali cattle steer up to 14% (0.58 vs. 0.66 kg / day) than cattle without supplements. The research concluded that vitamin-mineral supplementation 0.1 to 0.3% in ration based on King grass can increase microbial protein synthesis in the rumen and live weigh gain of Bali cattle steer, and there is a clear relationship between microbial protein synthesis (X) with live weight gain of cattle (Y) which follows the equation: $Y = 0.002X - 0.002$, $R^2 = 0.73$ *. Based on regression analysis, retrieved optimum level of vitamin-minerals supplementation in concentrate is 0.18% which can produce maximum of rumen microbial protein synthesis and live weight gain of Bali cattle fed King grass-based rations.

Keywords: Bali cattle, microbial protein, supplementation, vitamin-mineral

INTRODUCTION

Bali cattle is an Indonesian native germplasm should be preserved and developed optimally in order to improve the welfare of society and the nation of Indonesia. Approximately 55% farmers in most parts of Indonesia Bali cattle breed because of easily maintained and has many advantages.

Bali cattle belonging to ruminants which have rumen. Rumen microbes as biological machines in ruminants have a large role on the productivity of livestock. Rumen is composed of a number of bacteria, protozoa and fungi contained in the ruminant rumen general and Bali cattle in particular. One of the important role of rumen microbes are digesting the nutrients contained in food such as carbohydrates, fats, and proteins with enzymes involved produced. Results from digestion are utilized by beef cattle for growth and production. In addition to exploiting the results of the digestion of nutrients by the animal, cattle can also take advantage of microbial protein for growth. Numerous studies indicate that rumen microbial protein production correlated positively with the productivity of Bali cattle (Partama et al., 2007a, Partama et al., 2007b). This proves the importance of optimizing bioprocess to improve rumen microbial protein synthesis that significantly affect the growth of cattle.

Maximizing rumen microbial protein production in Bali cattle can still be improved to achieve maximum growth of Bali cattle. Ways that are commonly used is to provide nutrient content of diets with adequate and balanced. Adequacy of nutrients such as proteins, carbohydrates, fats, vitamins and minerals crucial rumen microbial protein production. However, the results showed that the mineral and vitamin content of animal feed ingredients in the tropics in general and Indonesia in particular is low (Kaunang, 2004). It demands made of mineral and vitamin supplementation in ruminant rations.

Supplementation of vitamins and minerals in the diet has been shown to increase the productivity of Bali cattle (Partama et al., 2003; Partama, 2006). Vitamins and minerals can stimulate productivity of microbial protein synthesis and optimization of the rumen digestive functions of fiber (Wanapati and Sommart, 1992, Partama et al., 2007a; Partama et al., 2007b), and high animal productivity is a reflection of the high microbial protein synthesis also (Firkin et al., 2006; Mullik, 2007, Partama et al, 2007a; Partama et al., 2007b). Thus, the success of rumen microbial growth spurred a great effect on the fulfillment of amino acids for ruminants. Mixture of minerals and vitamins that not only supplemented to meet the needs of microbes, but also to meet the needs of livestock.

In this regard, the research is feasible to determine the optimum vitamin-mineral supplementation in King grass-based rations to maximize rumen microbial protein synthesis and its relationship with productivity of Bali cattle steer.

MATERIALS AND METHODS

This study consisted of a series of field and laboratory experiments. Field trials conducted in the Village Serongga, district of Gianyar, Gianyar regency. Laboratory tests were conducted at the Lab. Nutrition and Food Livestock, Fapet - Udayana University, and Lab. Analytical Udayana. Experiments conducted during six months, starting from the preparation up to the observation or measurement activities in the field.

The experiment was conducted in individual cages. Cage is designed to meet the maintenance requirements of fattening Bali cattle. Required 20 individual cages to accommodate 20 Bali cattle steers with an average live weight of 319 kg or with the range of 279-367 kg. Cattle were randomly placed in individual cages with a capacity of one larvae per cage and given ration treatment according to the experimental design used.

Ration treatments consisted of King grass and concentrates supplemented with pignox (commercial product as a source of vitamins and minerals). There are four treatments in concentrate rations for four treatments ie S0, S1, S2, and S3. Concentrate on S0 is without adding pignox, while concentrate on the S1, S2, and S3 supplemented with as many consecutive pignox 0.1%, 0.2% and 0.3% (Table 1).

Table 1. Nutrient content of diets material experiment.

Nutrient	Concentrates				King grass
	S0	S1	S2	S3	
Dry matter (%)	87.64	87.64	87.64	87.64	24.80
Organic matter(%)	64.13	64.13	64.13	64.13	71.84
Crude protein (%)	12.13	12.13	12.13	12.13	5.01
Crude fiber (%)	7.76	7.76	7.76	7.76	27.20
Energy (GE, Mcal/kg)	3.22	3.22	3.22	3.22	3.39
Sulfur (S, ppm)	685.50	694.09	702.68	711.28	-
Zinc (Zn, ppm)	45.09	65.09	85.09	105.09	26.12

Description: S0, S1, S2 and S3 = vitamin-mineral supplementation in concentrate respectively 0%, 0.1%, 0.2% and 0.3%.

Provision of rations conducted two times a day in the morning at 9:00 and lunch at 14.00 pm. Given 5 kg of concentrate per cattle per day, divided into the two times of the morning and afternoon, as mentioned above. King grass given unlimited (ad libitum). The concentrate is given first and followed the King grass on every provision of both morning and afternoon. Similarly, water was supplied ad libitum provided separately with the feeding.

This research use randomized block design with four treatments ration S0, S1, S2, and S3 and five groups based on the weight of livestock. Thus there are 20 male Bali cattle in this experiment. One unit is the first experiment male Bali cattle were randomly placed in individual cages in accordance with the experimental design. Dietary treatments are: S0 = 5 kg concentrate + King grass was given ad libitum; S1 = S0 + 0.1% pignox in concentrate; S2 = S0 + 0.2% pignox in concentrate; and S3 = S0 + 0.3% pignox in concentrate.

Variables observed were dry matter and organic matter intake, protein and crude fiber intake, rumen N-NH_3, total rumen VFA (volatile fatty acids), deposition of nutrients through the conversion of cattle live weight gain with body composition (Bartle et al., 1983), levels of urinary allantoin to calculate rumen microbial protein production (International Atomic Energy Agency, 1999), live weight gain of cattle, and feed efficiencies (FCR = feed conversion ratio).

The data obtained were analyzed by variance. When a significant effect on treatment response variables, followed by orthogonal contrast test at level 5%. Regression analysis was used to determine the optimal vitamin-mineral supplementation in King grass-based rations to obtain the maximum of rumen microbial protein synthesis and its relationship with live weight gain of Bali cattle steers (Steel and Torrie, 1986).

RESULTS AND DISCUSSION

Vitamin-mineral supplementation significantly ($P < 0.05$) effect on feed and nutrients intake, rumen N-NH_3, total rumen VFA, deposition of nutrients, rumen microbial protein synthesis, FCR and live weight gain of Bali cattle steers (Table 2).

Table 2. Nutrient intake, rumen microbial synthesis, ruminal N-NH_3, total ruminal VFA, nutrients deposition, live weight gain and feed efficiencies of Bali cattle steers fed King grass-based rations with vitamin-mineral supplementation.

Variables	Supplementation Treatment			
	S0	S1	S2	S3
Dry matter intake (kg/d)	6.65^{b}	6.58^{b}	6.29^{a}	6.18^{a}
Organic matter intake (kg/d)	4.45^{b}	4.40^{b}	4.21^{a}	4.12^{a}
Crude protein intake (g/kg$W^{0,75}$/d)	7.92^{b}	7.87^{b}	7.44^{a}	7.32^{a}
Crude fiber intake (g/kg$W^{0,75}$/d)	12.18^{b}	11.84^{b}	11.39^{a}	10.83^{a}
Ruminal N-NH_3 (mM)	12.30^{a}	15.50^{b}	12.74^{a}	11.92^{a}
Total ruminal VFA (mM)	166.33^{a}	181.75^{b}	155.01^{a}	198.06^{b}
Microbial Protein Synthesis (g/d)	202.24^{a}	232.24^{c}	225.67^{b}	221.46^{b}
Protein deposition (g/kg$W^{0,75}$/d)	1.18^{a}	1.31^{b}	1.30^{b}	1.19^{a}
Fat deposition (g/kg$W^{0,75}$/d)	2.60^{a}	3.00^{b}	2.90^{b}	2.70^{a}
Feed Conversion Ratio (FCR)	11.48^{b}	10.06^{a}	9.68^{a}	10.18^{a}
Live weight gain (kg/d)	0.58^{a}	0.66^{b}	0.65^{b}	0.61^{a}

Description: The variable is similar to the number with superscript not the same, significantly different at $P < 0.05$ when compared with orthogonal contrast test; S0, S1, S2 and S3 = vitamin-mineral supplementation in concentrate respectively 0%, 0.1%, 0.2% and 0.3%, $W^{0,75}$ = metabolic weight of cattle; d = day; VFA = volatile fatty acids.

In principle, feed intake in animals is to meet the nutrient and energy needs. Livestock will stop eating when energy needs are met or stomach has been filled by the feed nutrient needs exceed the capacity, although not yet fulfilled. Level of feed intake is influenced by physiological status of livestock, feed quality and palatability. To obtain the optimal level of consumption required ration formulations that match the needs of livestock rations containing adequate and balanced nutrient.

Ration is attempted in this study meets the nutrient needs of ration ingredients consisting of 5 kg of concentrate, grass king was given ad libitum or given an average of 15 kg per cattle per day, so that these rations containing dry matter (DM) 40.51%, crude protein (CP) 10.31%, and energy (GE) 3.27 Mcal/kg, equivalent to 56.29% levels of TDN (Total Digestible Nutrients). However, the results of this experiment showed that feed intake and nutrient levels such as dry matter, organic matter, crude protein, and energy decreased when given vitamin-mineral supplementation in concentrate, especially the level of supplementation of 0.2 to 0.3% caused a decrease in consumption statistically significant. This decline in consumption caused by an imbalance of nutrients in diet, especially minerals. This causes the excess mineral supplementation, especially Zn in cattle's digestive tract that can cause metabolic disturbances are loss of appetite, reduce the accumulation of Fe and Cu in the liver and increase spending on S in the feces (Jouany, 1991). Thus, this will suppress metabolism disorders feed intake and nutrients. Decrease in consumption level has not yet led to nutrient-deficient cattle and this can be proved by the accretion of a cattle live weight gain during the experiment, although feed intake decreased with increasing levels of vitamin-mineral supplementation (Table 2).

The high nutrient deposition and live weight gain in cattle with vitamin-mineral supplementation of 0.1% due to the sufficient and balanced nutrients in the ration. N-NH_3 concentration sufficient (15,50 mM) to offset the relatively high concentration of VFA (181,75 mM) would strongly support the efficiency of rumen microbial protein synthesis. N-NH_3 is the result of protein degradation, while the VFA degradation products from carbohydrates in the rumen. The balance between these two components is an ideal prerequisite for the optimization of microbial protein synthesis. Stern et al (2006) states that the rumen bacteria can use protein and carbohydrates as energy sources. Carbohydrate is the main energy source for bacteria, and can also be used as a carbon skeleton that combines with ammonia (NH_3) to rumen microbial protein synthesis.

There is a clear relationship between levels of vitamin-mineral supplementation with rumen microbial protein synthesis following regression equation: $Y = 204.1 + 307.7 X - 855.4 X^2$, with a coefficient of determination (R^2) = 0.501 *, with the understanding of X = level of supplementation vitamin-mineral in percent (%), and Y = microbial protein synthesis in g / day. Regression equations indicate that there are levels of vitamin-mineral supplementation is 0.18% optimum causing maximal microbial protein synthesis amounted to 231.77 g per day.

The results showed that the apparent link between rumen microbial protein synthesis with live weight gain of Bali cattle fed King grass-based rations with vitamin-mineral supplementation. Relationship following the regression equation: $Y = 0.002X – 0.002$, with coefficients of determination (R^2) = 0.73 *, with the understanding of X = microbial protein synthesis (g/day) and Y = live weight gain of cattle (kg/day). This regression equation means that any increase in rumen microbial protein synthesis for 1 g will be followed by a live weight gain of cattle of 2 g per day.

CONCLUSION

The results of this study can be summarized as follows: (1) Vitamin-mineral supplementation by 0.1 - 0.3% in concentrate may increase the efficiency of utilization of feed, rumen microbial protein synthesis, deposition of nutrients, and can increase live weight gain in Bali cattle fed King grass-based rations; (2) Retrieved optimum levels of

vitamin-mineral supplementation in concentrate is 0.18% which can produce maximum of microbial protein synthesis and live weight gain of Bali cattle; (3) There is a real relationship between rumen microbial protein synthesis (X) and live weight gain of Bali cattle (Y) fed King grass-based rations with vitamin-mineral supplementation according to the regression equation: $Y = 0.002X - 0.002$, $R^2 = 0.73$* which means that any increase in rumen microbial protein synthesis for 1 g will be followed by a live weight gain of Bali cattle of 2 g per day.

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PRODUCTION OF FUSARIC ACID AND EXTRACELLULAR ENZYMES OF *FUSARIUM OXYSPORUM* F.SP. *VANILLAE* EXPOSED TO THE EXTRACT OF *AGLAOPHENIA* SP., A MARINE ANIMAL

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ABSTRACT

The purpose of this research was to study the effect of *Aglaophenia* axtract on the production of fusaric acid and two extracellular enzymes activities (cellulose and pectinase) of *Fusarium* which exposed to *Aglaophenia* extract. The experiment were carried out in vitro and the treatments of 0.2, 0.1, 0.05, 0.025, 0.0125, 0.00625, and 0% (w/v) of *Aglaophenia* extract. The results exhibited that the higher concentration of the extract given, the higher the production of fusaric acid, however, both enzyme activities were lower. Cellulase activity was depressed on 0,0016% of extract (with activity 908.86 µg/mL/minute) and was significantly lower than control (1946.74 µg/mL/minute). While the pectinase activity affected at the concentration of extract higher than 0.025%. Pectinase activity at those concentration was 389.98 µg/mL/minute, whereas in the control was 3930.89 µg/mL/minute. This proves that cellulase was more sensitive than pectinase in responding *Aglaophenia* extract.

Keywords: Fusaric acid, cellulase, pectinase, *Fusarium oxysporum*, *Aglaophenia* sp.

INTRODUCTION

Once a pathogen infected to plant cell, the pathogen use its pathogenecity factors to overcome the barriers of infection. The barriers are cuticule, cell wall, and chemically self defences. The main barier is cell wall because of its complex structure and the pathogen must degrades the complex using apropriate enzymes. Therefore, the enzymes are very important as infection factor of a pathogen. So how to decrease its activity is one of the main goal of any fungicides application.

Polygalacturonase and cellulase are enzymes that being able to degrade pectin and selulosa of cell wall components respectively (Dickinson, 2003). The two enzymes were produce preceded the others when one pathogen penetrate into plant cell (Mahalingam *et al.*, 1999). Polygalacturonase is an endo-polygalacturonase degraded polygalacturonane ring randomly and resulted to short chain oligogalacturonane. While exo-polygalacturonase degrade the molecule terminally, resulted monomer molecule such as galacturonic acid (Bateman and Basham, 1976).

Production of pectolityc and cellulolityc enzymes as main agent of patogenecity was identified on some species of *Pythium* including *P. aphanedermatum* (Sutton *et al*., 2006). Polygalacturonase secretion is one of the key factors for infection processes on plant host (Clausen and Reen, 1996), even the differences on total enzymes excretion correlate significantly to its virulent (Wei-Chen *et al*., 1998; Ohazurike and Arinze, 1999; Owen-Going *et al.,* 2004). Many species of *Fusarium* are known to be able to produce cellulase and pectinase along infection processes. While Ugwuanyi and Obeta (1997) stated that *F.oxysporum* was able to produce pectin degrading enzymes such as hydrolase, lyase, and pectin esterase.

Cellulases are one group of enzymes that degrading cellulose into glucose. The enzymes group including endo-B-1,4-glucanase, exo-1,4-glucanase (celobiohydrolase), and B-1,4-glucosidase. The two types of exoglucanase are the enzymes that degrade glucose unit from non reduction terminale of cellulose chains (Onuh and Ohazurike, 2008). Pectinolityc enzymes have important role in maceration process of cell wall to

degrade pectin on middle lamella of primary cell wall into separated cell (Onuh and Ohazurike, 2008). The hydrolysis is started by pectin esterification of pectin methyl esterase become methanol and polygalacturonic acid. The enzyme activity providing away for other enzymes activity. The activity of the next enzymes are divided into two enzymes based on its mode of action, those are: (1) endo- cutting mode, those are the enzymes cut pectin randomly into olygomer, for example, endo polygalacturonase that hydrolysis glycocide bond, (2) exo cutting mode, degrade from polymer terminal into separated monomer/dimer, for example exopectate lyase that degrade polygalacturonan into olygigalacturonide with B-elimination (Reignault et al., 2008).

The purpose of this research was to know the activity of two main enzymes functioned on *Fusarium* pathogenecity influenced by *Aglaophenia* sp. extract.

MATERIALS AND METHODS

The research was carried out in Laboratory of Biotechnology Faculty of Agriculture Udayana University. Marine animal *Aglaophenia* sp. was collected at Tukad Abu Karangasem. The research was started on October 2008 until February 2009.

Strain of fungi and marine biota samples

Fusarium (*Fusarium oxysporum* f.sp. *vanillae, Fov*) was isolated and identified in lab of Agricultural Biotechnology, Udayana University from infected vanilla plant collected from farmer garden in Tabanan, Bali. Single spore isolation was applied to the pathogen in order to obtain single strain of *Fusarium*. Marine biotas samples were collected at intertidal zone and in the depth of 1-7 metres of sea water of Tukad Abu beaches of Karangasem Regency, Bali Province.

Medium and culture conditions

The medium used to isolate *Fusarium* was Matuo medium (per litre: 1.00 g K_2PO_4, 0.50 g KCl, 0.50 g $MgSO_4.7H_2O$, 0.01 g Fe-Na-EDTA, 2.00 g L-Asparagine, 20.00 g D-Galactose, and 1.00 litre aquadest) sterilized at 121°C for 20 minutes, then added antimicrobe substances i.e.: 1.00 g PCNB 75% WP, 0.50 g Oxgall, 1.00 g $Na_2B_2O_7$, 0.30 g Streptomycin sulphate; and then shaked well) (Matuo, 1972). Culture medium for *Fusarium* bioassay were potato dextrose agar (PDA, Nissui) and potato dextrose broth (PDB, Difco) with a little modifications with pH 4.5.

Determination of mycelial protein

Experiment to determine mycelial protein was done on PDA medium using Bradford method (Dunn, 1992). Bradford method counting soluble protein of the cell. One hundred gram of mycelia (fresh materials) obtained from the experiment (done as experiment to measure colony diameter, above description) were ground on mortar with liquid nitrogen medium. The ground mycelia was added by 0.5 ml buffer (0.1 M Tris-HCl pH 8.0 and 1 mM EDTA), centrifuged on 12,000 rpm for 10 minutes, then its supernatans were diluted by the same buffer (1:5). Twenty microlitres samples were added by 1 ml 1/5 Bradford reagent, vortexed, and incubated for 30 minutes before its absorbant spectrophotometricized at A595 nm. Standard curve was determined by BSA on the same condition.

Enzyme extract and protein analysis.

Thirty mililitre PDB (*potato dextrose broth*) pH 4.5 in the 250 mL Erlenmeyer tube poured with *Aglaophenia* sp. extract until its concentration were 0.2, 0.1, 0.05, 0.025, 0.0125, 0,00625, and 0% (w/v). Into each tube was inoculated with Θ5 mm fungi colony plug of *Fov* 7 days culture. The culture was incubated at 28°C and shaked 125 rpm. After

incubation time, the culture was sieved with Whatmann paper number 2 and its filtrate considered as enzyme extract. The mycelia were used for protein analysis.

Activity of cellulase enzyme

Cellulase enzyme activity was determined using DNS method (Wu *et al.,* 2008). Recation mixture was consisted of 0.1 mL enzyme extract 1.9 mL 1% (w/v) CMC-Na (*Carboxymethylcellulose*) in phosphate buffer, incubated at 50ºC for 20 minutes. 1.5 mL DNS was added into the mixtureture and then vortexed well. The mixtureture then boiled 100ºC for 10 minutes than cooled. The volume was adjusted into 25 mL with destiled water. The reduction sugar spectrum was determined in 520 nm wave length. One unit of enzyme activity was amount of reduction sugar produced by 1 mg enzyme per minute. Glucose (Sigma, St. Louise, Mo., USA) was used for established standard curve.

Activity of pectinase enzyme

Pectinase enzyme activity especially polygalacturonase was determined using DNS method (Wu *et al.,* 2008). Reaction mixtureture was consisted of 2 mL of enzyme extract, 2 mL solution of 0.4% (w/v) pectin (*poly-D-galacturonic acid ethyl ester*, Sigma Aldrich), diluted in 0.2 M buffer ethyl acetic pH 4.4, and shaked well. The mixtureture reacted at 45ºC in water bath for 30 minutes, added with 1.5 mL DNS (*3,5-dinitrosalicylic acid*) then boiled for 5 minutes. The volume was adjusted to 25 mL with destiled water. The reduction sugar spectrum was determined in 520 nm wave length. One unit of enzyme activity was calculated by amount of *ß*-galacturonic acid produced by 1 mg enzyme per minute. Standard curve established from 0.1% (w/v) *ß*-galacturonic acid (Sigma, St. Louise, Mo., USA).

DNS Reagent

Quatitative determination of reduction sugar was done using *3,5-dinitrosalicylic acid* (DNS). A reaction between DNS and reduction sugar resulting *3-amino-5-nitro salicylic acid* with yellow color and its concentration can be determined by spectrophotometer. DNS Reagent formulated by: DNS 0.2 g, phenol 0.04 g, Na_2SO_4 0.01 g, potassium sodium (+)-tartrate tetrahydrate (*Rochelle salt*) 4 g; diluted in 100 mL water. Standard curve was costructed by: 0.5 mL sugar solution with concentration of 0-800 µg/mL), 1.5 mL DNS reagent boiled for 5 minutes, cooled and then added water until volume of 25 mL. Its absorbant was quantified by spektrophotometer on 540 nm wave length.

Protein quantification

Lowry method

One part of mycelia from above preparation was dried in oven at 80ºC until its weight fixed, and then powdered in mortar. Twenty five mg of hypha powder were added with 1 mL 2 N natrium hydroxide, pH 7, shaked and boiled 80ºC for 10 minutes. The mixture then, centrifugated 5000 rpm for 10 minutes and the supernatan was determined absorbants spectrophotometrically in wave length of 750 nm. Standard curve was costructed by Bovin serum albumin (BSA) in concentration of 0-100 µg/100 µL natrium hydroxide.

Bradford method

The mycelia at amount of 100 g in liquid nitrogen was directly ground and then added was 0.5 mL buffer (0.1 M Tris-HCl pH 8.0 and 1 mM EDTA) centrifugated at 12,000 rpm for 10 minutes and then its supernatans were diluted was the same buffer (1:5). As much as 20 µL of the samples were added by 1 mL 1/5 reagent Bradford, vortexed. The absorbants was spectrophotometrically determined at 595 nm after 30

minutes incubation. Standard curve was constructed by Bovin serum albumin at the test condition.

Fusaric acid determination

Fusaric acid determination was done using assay method according to Wu *et al.* (2008) with concentration extract of 0.2, 0.1, 0.05, 0.025, 0.013, 0.0063, and 0% (w/v). Its absorbant was determined at 268 nm of wave length and the standard curve was provided by fusaric acid (Sigma, St. Louise, Mo., USA). DNS method (Wu *et al.,* 2008) was used to determine the enzymes activities (cellulase and pectinase). Glucose and 0.1% (w/v) *ß*-galacturonic acid (Sigma, St. Louise, Mo., USA) were used to established the standard curve for each enzyme.

RESULTS AND DISCUSSION

Effect of the extract against fusaric acid of *Fov*

The higher extract concentration were given, the higher the production of fusaric acid (Table 1). The production at 0.05% (MIC value) was 188.52 µg/mL (129% much higher than control). One factor considered as a trigger to the increase is chemical stress (Lindsey and Yeoman, 1983) that was the existing extract.

Table 1. Fusaric acid production of *Fov* exposed to the *Aglaophenia* extract

Extract concentration (%)	Production of fusaric acid (µg/mL)		The increase of fusaric acid production based on control (%)
0	82.23	A	0
0.0063	99.51	A	21
0.0125	161.99	B	97
0.025	166.18	B	102
0.05	188.52	C	129
0.1	241.92	D	194
0.2	0.00	E	-

Effect of the extract against extracellular enzymes of Fov

Generally the extract was able to depress both extracellular enzymes studied, the higher concentration of the extract given, both enzymes activities were lower. Cellulase more sensitive than pectinase, that was cellulose activities was depressed at lowest concentration, while pectinase was just depressed at 0.025% of extract (Table 2). Figure 1 exhibited regression curve of activities of the both enzymes against extract concentration and its correlation were 80% and 91% respectively. Those proved that the crude extract was significantly suppressed both extracellular enzymes of Fov.

Table 2. The influence of the *Aglaophenia* extract against activity of extracellular enzymes

Extract concentration (%)	Activities of enzymes (µg/mL/minutes)			
	Cellulase		Pectinase	
0	1946.74	a	3930.89	A
0.0016	1908.86	b	3914.23	A
0.0031	1885.38	b	3908.17	A
0.0063	1817.20	c	3891.50	A
0.0125	1806.59	c	3806.65	A
0.025	183.87	d	389.98	B
0.05	166.44	d	361.20	B
0.1	164.92	d	358.17	b
0.2	0.00	e	0.00	e

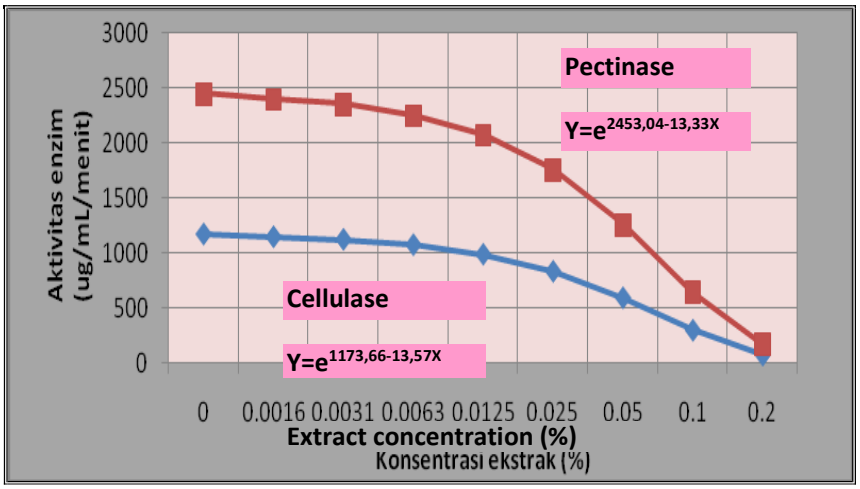

Figure 1. Relationship between extract concentration of *Aglaophenia* sp. against activity of cellulase and pectinase enzymes of *Fov*

Because of the two enzymes had important role on patogenisity of the pathogen (deVries and Visser, 2001, Dickinson, 2003), therefore the influence of the extract would decreasing the *Fov* penetration on vanilla plant.

Influence of the extract against protein of mycelia

Aglaophenian extract was significantly decrease protein content of *Fov*. When the higher concentration of the extract were given, the protein content was lower on both methods (Table 3).

Table 3. Influence of the extract against mycelium protein content of *Fov*

Extract concentration (%)	Content of protein mycelium (µg/g)			
	Lowry method		Bradford method	
0	208.71	a	208.60	a
0.0063	205.37	a	187.52	a
0.0125	195.76	b	170.24	a
0.025	179.95	c	163.88	a
0.05	159.88	d	142.79	b
0.1	127.79	e	110.00	c
0.2	93.02	f	62.95	d

Based on Lowry method, protein content of mycelia was influenced by extract concentration of 0.0125%, while on Bradford method the depression was just begun at concentration of 0.05%. The suppression was supported by the finding of Sudana (1997) that the secunder metabolite as phenolic substance (a common substance produced by antagonistic/antibiotic organisme) could suppress nucleic acid and protein synthesis of *Ceratocystis paradoxa*. The suppression was caused by suppression of glucose-6-fosfat dehydrogenase and succinic dehydrogenase due to decrease on respiration. According to Bruinenberg (cited by Sudana, 1997) the two enzymes had important role in respiration process.

The increase of extract concentration given causing the decrease of protein mycelium content. Total protein on high extract concentration (0.1% and 0.2%) were

lower than other lower extract concentration. This figure was showed by its protein bands on those concentration were thinner than the others. The suppression of protein produced caused by the low ability of mycelia to grow and forming colony as indicated by former research.

When higher extract concentration were given, the higher the production of fusaric acid. The production at 0.05% (MIC value) was 188.52 ug/mL (129% higher than control). One factor considered as a trigger to the increase is chemical that was the existing extract. The increase of extract concentration given, caused decreasing of the two enzyme activity. The cellulase activity has been suppressed on 0.0016% of extract and significantly lower compared to control. While the pectinase activity was just influenced on higher concentration that is 0.025%. The enzyme activity was 389.98µg/mL/minute, while on control was 3930.89µg/mL/minute. Those data indicated that cellulase was more sensitive than pectinase against extract given.

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DEVELOPMENT AND UTILIZATION OF SOMATIC EMBRYOGENESIS IN TROPICAL TREES: AVOCADO, LITCHI AND LONGAN

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ABSTRACT

In studies to develop *de novo* plant regeneration system from tissue cultures of tropical fruit trees, plant regeneration systems via somatic embryiogenesis of avocado (*Persea americana* Mill.), litchi (*Litchi chinensis* Sonn.) and longan (*Dimocarpus longan* Lour) have been developed. Steps to obtain plants from tissue cultures of the three species were: (1) explant sterilization and preparation, (2) induction of embryogenic cultures, (3) culture maintenance, (4) somatic embryo development and enlargement, (5) somatic embryo germination, and (6) plantlet acclimatization dan transfer *ex vitro*. Through these studies the optimal conditions for somatic embryogenesis have been described for select cultivars of the fruit species. These included the use of MS or B_5 medium components, 2,4-D or NAA auxins, BA or zeatin cytokinins additions of organic supplements (such as glutamine, casein hydrolysate), medium gelling with gellan gum or Phytagel, and incubation on the dark at 26°C, during induction of embryogenic cultures. Somatic embryo development and enlargement required addition of coconut water (10-20%) in the culture media, gelling with gellan gum or Phytagel and incubation on the dark at 26°C. Addition of GA_3 in culture media and incubation under light conditions (16 hour photoperiod) generally stimulated somatic embryo germination. The somatic embryogenesis systems have been utilized for *Agrobacterium*-mediated genetic transformation of the fruit tree species to transfer genes of agronomic importance, such as defensin antifungal protein (in avocado) and a gene for seedlessness (in litchi), as well as in cryopreservation.

Keywords: avocado, litchi, longan, tissue culture, somatic embryogenesis, genetic transformation.

INTRODUCTION

One of the prerequisites for the application of the genetic engineering approach in crop improvement is the ability to regenerate plants *de novo* from cell and tissue cultures. This is because using this approach generally the manipulation of plants has to be done at cell or tissue levels. The genetically manipulated cells or tissues are then need to be developed into whole plants to obtained genetically engineered plants. For many woody plant species including tropical fruit trees, *de novo* regeneration from cell and tissue cultures is still considered to be difficult; however, the technique has progressed rapidly in recent years.

There are two patterns of morphogenic differentiation from plant tissue and cell cultures which lead to regeneration of whole plants, these are organogenesis and somatic embryogenesis (Litz and Grey, 1992). Both patterns of morphogenic development are based on the totipotency of the plant cells. Organogenesis leading to the development of shoots or roots is of multicellular origin. In contrast, somatic embryos have usually been described as originating from proembryonic masses (PEM) of cells that develop from single cells.

Somatic embryogenesis is the process by which somatic cells differentiate into somatic embryos, which in turn can develop into whole plants. During somatic embryogenesis bipolar structure, resembling a zygotic embryo, develops from a non-zygotic cell without vascular connection with the original tissue. Somatic embryogenesis is a multi-step regeneration process starting with formation of PEM, followed by somatic embryo formation, maturation and enlargement, desiccation and plant regeneration (von

Arnold *et al*., 2002). Somatic embryogenesis may have various applications, including: (1) production of synthetic seed, (2) regeneration of genetically transformed plants, polyploid plants, or somatic hybrids, (3) cell selection, and (4) germplasm cryopreservation. The application of somatic embryogenesis to plant virus elimination, metabolite production, and in vitro mychorrhizal initiation has been investigated (Vicient and Martínez, 1998).

In our studies to develop regeneration system from tissue cultures of tropical fruit trees, plant regeneration via somatic embryogenesis in several tropical fruit tree species have been developed. This paper describes and reviews the development of somatic embryogenesis in of avocado (*Persea americana* Mill.), litchi (*Litchi chinensis* Sonn.) and longan (*Dimocarpus longan* Lour.) and its applications.

Somatic Embryogenesis in Avocado and Its Utilizations

In our study, embryogenic cultures of avocado cultivars 'Hass' and 'Suardia' were induced on semi-solid medium consisting of B5 major salts (Gamborg *et al*., 1968), MS minor salts and organic components (Murashige and Skoog, 1962), 30 g l^{-1} sucrose and 0.41 µM picloram, according to the technique previously developed by Witjaksono and Litz (1999a).

Embryogenic suspension cultures were initiated and maintained in liquid MS3:1N medium supplemented with 0.41 µM picloram at 125 rpm on a rotary shaker under low light conditions and 2-week subculture intervals (Figure 1). Somatic embryos (SEs) developed from embryogenic suspension cultures that were plated on semi-solid SE development medium containing 20% (v/v) filter-sterilized coconut water (CW) (Witjaksono and Litz, 1999b).

Mature white-opaque SEs >5 mm in diameter germinated on semi solid MS medium with 30 g l^{-1} sucrose, 4.4 µM BA, 28.9 µM GA3 and 3 mg l^{-1} gellan gum. Two to three weeks after their emergence, shoots 6-10 mm long were used as scions for micrografting according to the technique previously developed (Raharjo and Litz, 2005; Figure 2). Plant recovery was achieved by *ex vitro* side grafting. Micrografting and *ex vitro* side grafting steps were necessary because obtaining plantlets and plants directly from germination of SEs was slow and their survival was low. The grafted plants originating from tissue culture were grown under intermittent mist and 25% sunlight in a shadehouse and were then transferred to 3-gallon pots in the greenhouse.

This somatic embryogenesis system of avocado has been utilized in *Agrobacterium tumefaciens*-mediated transformation to obtain transgenic plants. Embryogenic cultures maintained in suspension cultures were genetically transformed with the plant defensin gene (pdf1.2) driven by the CaMV 35S promoter in pGPTV with uidA as a reporter gene and bar, the gene for resistance to phosphinothricin, the active compound of Basta herbicide (Raharjo *et al*., 2008). Similarly, this system has been utilized to transformed 'Suardia' avocado with SAM hydrolase gene to delay fruit ripening (Litz *et al.,* 2005). A study has also been done to use embryogenic culture for cryopreservation of avocado (Efendi and Litz, 2003).

Somatic Embryogenesis in Litchi and Its Utilizations

Plant materials used as explants in our litchi tissue culture study were leaflets in compound leaves from new vegetative flushes of mature phase 'Brewster' cultivar. The explant materials were dipped into detergent solution, rinsed with tap water, disinfested by soaking for 10 min in 500 ml of 10% commercial bleach with 5 drops of Tween-20, and rinsed 3 times with sterile water. Sterilized individual leaflets were used as explants.

Embryogenic cultures were induced in medium consisting of B5 major salts, MS minor salts and vitamins, 100 mg l^{-1} myo-insitol, 400 mg l^{-1} glutamine, 200 mg l^{-1} casein hydrolysate and 30 g l^{-1} sucrose, supplemented with 4.52 µM 2,4-dichlorophenoxyacetic acid (2.4-D), 8.88 µM benzyl aminopurine (BA), 9.30 µM kinetin or 9.12 µM zeatin, and

solidified with 3 g l^{-1} gellan gum. The medium was dispensed 50 ml per 100x20 mm Petri dish, which were sealed with Parafilm®. The cultures were incubated in total darkness at 26±1°C.

Embryogenic cultures were maintained in medium consisting of half strength MS major salts, MS minor salts and vitamins, 100 mg l^{-1} myo-insitol, 30 g l^{-1} sucrose, 4.52 µM 2,4-D, 0.91 µM zeatin and 4 g l^{-1} gellan gum. The dishes were sealed with Parafilm® and incubated in darkness at 26±1°C. Tissue was subcultured onto fresh medium of the same formulation at 4 week intervals.

For somatic embryo development, enlargement and maturation, embryogenic cultures 4 weeks after the last subculture on maintenance medium were plated on semi-solid MS medium containing 45 mg l^{-1} sucrose, 5-20% CW, 3 g l^{-1} gellan gum dispensed in 100x20 mm Petri dishes. The CW was filter-sterilized and added to the medium after autoclaving. The cultures were incubated in darkness at 26±1°C.

Mature and opaque somatic embryos ≥10 mm diameter were germinated on semisolid MS medium with 30 mg l^{-1} sucrose and 3 g l^{-1} gellan gum, incubated at 27±1°C with a 16h photoperiod (60-80 µmol sec^{-1} m^{-2}). After germination, plantlets were transferred individually into glass containers (90x55 mm) containing 60 ml semi solid MS basal medium with 30 g l^{-1} sucrose, 100 mg l^{-1} activated charcoal and 4 g l^{-1} Agargel, still under the same incubation conditions for somatic embryo germination and plantlet development were 27±1°C with a 16h photoperiod (60-80 µmol sec^{-1} m^{-2}). After 6-10 weeks, plantlets with 3-5 leaves and well-developed root were transplanted into potting media to develop into plants *ex vitro*. Figure 3 illutrates the steps in litchi somatic embryogenesis up to development of *ex vitro* plants.

This somatic embryogenesis system of litchi has been utilized in *Agrobacterium tumefaciens*-mediated transformation to obtain transgenic plants with antisense *pistilata* gene into litchi to confer seedlessness of the fruits (Padilla, personal communication).

Somatic Embryogenesis in Longan and Its Utilizations

Explant sources were unopened and very young leaflets in compound leaves from new vegetative flushes of mature phase of cultivar 'Kohala'. The media and incubation conditions for embryogenic culture induction, maintenance, SE development and enlargement, germination and plant recovery were similar to those for litchi.

Zeng *et al*. (2001) transformed embryogenic culture using *Agrobacterium rhizogenes* in which plants were recovered from the transformed somatic embryos that had hairy roots, and transformation was confirmed by Southern hybridization and PCR. Embryogenic culture of longan has been cryopreserved involving slow cooling with 'Mr. Frosty' freezing containers (Raharjo *et al*., 2004; Figure 4). Somatic embryos and plantlets were recovered after cryopreservation.

CONCLUSION

Conclusions of the studies described in this paper is as follows:

1. Somatic embryogenesis protocols for avocado, litchi and longan and have been developed. Plants have been regenerated from the somatic embryogenesis pathway.
2. Steps involved in the somatic embryogenesis system that was developed include: (a) explant sterilization and preparation, (b) induction of embryogenic cultures, (c) culture maintenance, (d) somatic embryo development and enlargement, (e) somatic embryo germination, and (f) plantlet acclimatization dan transfer *ex vitro*. In avocado more rapid regeneration of plantlets and plants required apllication of micrografting and *ex vitro* grafting.
3. For all three species, efficient somatic embryo development and enlargement required addition of coconut water (10-20%) and ommission of plant growth regulators in the culture media.

4. Addition of GA_3 in culture media and incubation under light conditions (16 hour photoperiod) generally stimulated somatic embryo germination.
5. The somatic embryogenesis systems has been utilized in *Agrobacterium*-mediated genetic transformation and cryopreservation of the tropical fruit species.

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Figure 1. Avocado embryogenic culture (*left*) and suspension culture of proembryonic masses initiated from embryogenic culture on semisolid medium (*right*)

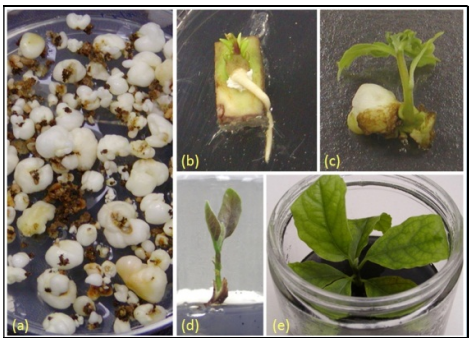

Figure 2. Somatic embryos (SEs) of avocado and regeneration of plantlets via micrografting: (a) mature and opaque SEs on SE development medium (b) *in vitro* rootstock, (c) scion from germinating SE, (d) scion flushing, (e) plantlet 6 weeks after flushing

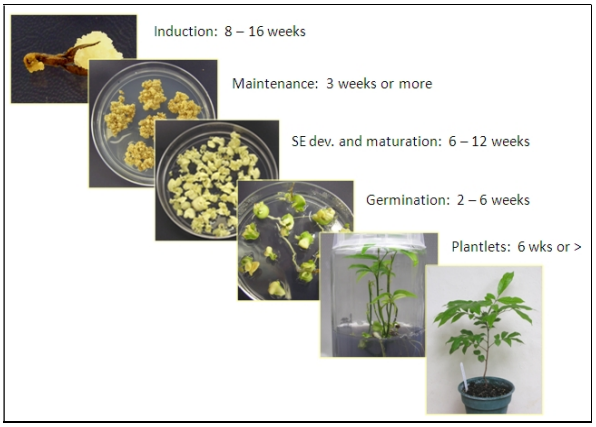

Figure 3. Steps in litchi somatic embryogenesis from indiction of embryogenic culture to development of *ex vitro* plants; similar steps also occur in longan

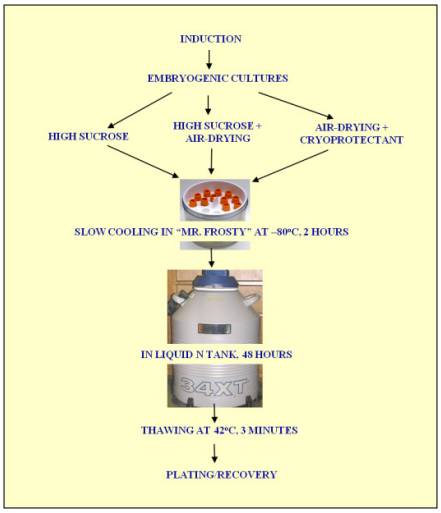

Figure 4. Steps in longan cryopreservation, involving slow cooling in Mr. Frosty containers

LEVEL OF BIOSECURITY IMPLEMENTATION ON THE POULTRY FARMS IN BALI

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ABSTRACT

Biosecurity is the most effective way to prevent poultry farms from avian influenza outbreak that affected most of broiler and layer farms. This study was conducted in six regencies in Bali province to find out production and the level of Biosecurity implementation in the three locations of broiler and layer farms. The result showed that the level of biosecurity implementation both in layer and broiler farms was almost similar. Layer farm implemented high level of biosecurity at the farm gate. Broiler farms, on the other hand was at between the farm gate and shed. And there was no difference of the level of biosecurity implemented on broiler production since most of them were contract farms. The small layer farms implement better biosecurity compare to big farms in this study, however, statistically there was no correlation between production and the level of biosecurity implementation.

INTRODUCTION

Since late 2003, bird flu out breaks has hit several Asian countries including Indonesia. In Indonesia, 33 provinces have been infected and are identified as the cause of death of several people. In addition to kill human, the influenza epidemic in birds which is called avian influenza/AI, also causing huge of losses for farmers (reduced production and income), traders (decreasing demand), consumer (protein source).

There are many ways the disease get into the farm, so, to reduce the occurrence of the disease is by blocking the entrance. Lately, the terminology which is very popular and commonly used in the response to disease outbreaks is biosecurity. The implementation in the farm includes three areas, namely at the pre entry, the point of entry and at the post entry of the farm (Jubbs and Dharma, 2008)

The implementation of biosecurity at the farm infrastructure is very difficult to implement and of course need funding. Especially with the farm conditions in Indonesia, sometimes located adjacent to the village and other village facilities. FAO (2004) categorize Indonesia's poultry industry into four sectors which include (a) the poultry sector one, is commercial poultry farm which is applied very strict biosecurity, (b) the poultry sector two is commercial farms that implement with moderate to high biosecurity, (c) the poultry sector three is commercial poultry sector own by villagers, implement low to minimum biosecurity, (d) while the village and urban backyard farm is sector four.

Many studies have been conducted on the impact of bird flu outbreaks in sector 3 poultry farms, but none of those observed how far is the biosecurity level implemented by the farms in this sector. Therefore, this research was conducted to determine the level of biosecurity implementation in the broiler and layer farms at the sector three poultry farms in Bali.

MATERIALS AND METHOD

In this study, some parts of the ACIAR AH/2006/169 project data was used, entitled cost effective biosecurity for non-commercial poultry farmers in Indonesia, with the goal is to improve the ecomic viability of broiler and layer farms through adoption of a cost and effective biosecurity. Data collected from surveys conducted in Bali in April 2009.

Location and respondents

A survey was conducted in Bali in April 2009, which consists of six regencies. The survey location was purposely chosen, based on the poultry population and AI outbreaks in the region. Respondent including broilers and layer farmers, and the number of respondent was determined using a quota sampling method (that is determined beforehand based on the budget and time available for conducting the survey). The number of respondent is summarized in Table 1.

Table 1. Number and Location of Surveyed Respondents

Business Type	Karang-asem	Bangli	Klungkung	Gianyar	Tabanan	Jembrana
Broilers	0	0	10	10	20	20
Layer	20	20	0	0	20	0
Total	20	20	10	10	40	20

Data analysis

In this study data was collected based on biosecurity risk assessment of the farm. Isolation, traffic control and sanitation are the major components of the biosecurity (WHO, 2006). Isolation refer to the confinement of live animals within a controlled environtment, while traffic control covers both human and vehicular traffics within the controlled environtment and finnaly sanitation deals with the cleanliness and disinfection of materials, people and equipment entering the controlled environtment. The risk factors associated with the biosecurity implementation on farm were analysed based on those components.

The level of biosecurity implemented by farmers can be measured in a number of entry points (pre, point and post of entry) and in number of ways including farm inputs to susceptibility of the birds. Although the risk factors can be controlled at the three point of entries, this study focused on controlling risk factor at two point of entries (at the point and post of entry) covering:

- *Level of biosecurity at the farm gate*; with regard to fence and lock, number of entrances, parking and vehicle washing, signs around perimeter, foothwash to enter the farm, shower and changing room for visitors and employes, cages used on harvest, cleaning and disinfecting cages and equipment returning from the market before reentering the farm.
- *Level of biosecurity between the farm gate and the shed*; including feed shed sealed against rodents and birds, overflow tap, feed spilt, the number of chickens and ducks wandering around the shed.
- *Level of biosecurity at the shed door*; with regard to material of the shed walls, shed locked at all time, signs at the door, concrete footbath in front of shed entrances and disinfectant, wild birds and rodents entering the shed, and things have been done to prevent entry of wild birds and rodents.

Risk factors assessed have covered three major components of biosecurity following WHO criteria, that is isolation, traffic control and sanitation. Each item of risk factor was scored based on their performance. The more close the answer to the biosecurity expectation, the higher the score achieved. The level of biosecurity then is divided into three categories: low, medium and high. Data obtained was analyzed by descriptive qualitative.

RESULTS AND DISCUSSION

Characteristics of Farmers

Similarly to the all provinces in Indonesia, the management pattern in rising chicken in Broiler and layer is different. Almost 82% broiler farms are under contract management, slightly below of the number reported by Patrick at al (2008) while the layer farms are generally independently own and managed. Contract is usually made by poultry companies to produce broilers. Farmers are guaranteed for certain price for broiler produced under the conditions agreed in the contract.

Table 2. Characteristics of Respondents Breeder in Bali Province

Description	Broiler Breeder	Layer Breeder
Management Patterns:		
Independent (person /%)	11 / 18.33	58 / 96.67
Contract (person /%)	49 / 81.67	2 / 3.33
Production Capacity (tail)	4875,00	8929,57
- Small producers (%)	60	45
- Large (%)	40	15
Age (years)	44.10	40.57
Education (years)	9.65	9.43
Experience on farm (years)	6:37	14.37

As can be seen from Table 2 that both farms are predominantly small commercial producers, the category was based on the average number of chicken managed by the farmers. Less than 5000birds are rise by broiler farms, whilst less then 9000 birds by layer farms. Approximately 60% of broiler farmers manage less than 5000 birds and 45% of layer farmers manage less than 9000 birds.

In terms of age, the average age of broiler and layer producers is almost similar 44.10 years and 40.57 years respectively. According to Ilham and Yusdja (2008) this age is still categorized as productive with education background is junior high school. This showed that the knowledge on manage the birds mainly they have got from their experienced as presented on Table 2 that both farmers have experience on manage the business more than 5 years.

Level of biosecurity at farm gate

The risk associated with the boundary and entrance is a first indicator can be seen for biosecurity implementation on farm. Results from the survey indicated that layer farm tend to have higher application on the secure boundary and fence than that broiler farm (Table 3).

Layer producer has more concerned on secure boundary fence to stop people and animal entering the farm and almost all farms entrances have lock. It seems that most of layer respondent concerned on risk factor regarding traffic control. However, they have no good application on locking the gates at all times, similarly to broiler producers. More than 60% respondent on both farms do not lock the gates at all time until permission is granted to enter.

In the term of a dedicated parking area for all vehicles outside the farm, both showed poorly performing area of biosecurity, shown by 50% and 73% of layer and broiler farms respectively do not have certain parking area outside the farm. Vehicles usually are parked on the road near the farm entrance. Meanwhile, there is only a small number of respondents who have a footbath on the farm entrance, shown by 18% of layer farms and 37 of broiler farms, however it is still found that people and animal can step over or walk around the footbath.

Table 4 presented the level of biosecurity at farm gate for both farms. It showed that layer farm implement better biosecurity measures, in terms of fence and lock. This was indicated by high number of layer farms set up this type of biosecurity. Another good biosecurity measure of layer farm is that unsold eggs did not get returned to the farm. This will decrease the spread of virus from the market.

Table 3. Risk Factor Associated with Boundary and Entrance

Risk Factor	Layer (%)		Broiler (%)	
	Yes	No	Yes	No
A secure boundary fence that is able to stop people and animal entering the farm	60	40	23	77
All farm entrances have lock	57	43	47	53
The gate is kept locked at all times until permission is granted to enter	35	65	37	63
A dedicated parking area for all vehicles outside the farm	50	50	27	73
A foothbath as you enter the farm	18	82	37	63
People and animals step over or walk around the footbath	36	64	32	68

In refer to signage to warn visitors and employees of restricted acces to certain areas into the farm, both farms perform low level of biosecurity. Only 3.33% of broiler and 6.67% of layer farms have sign, ranging from two to four in numbers.

Both broiler and layer farms put attention to the numbers of entrances on their farm, indicated by high level of of biosecurity achievement in this area (Table 4). This implies that both farms have a limited number of acces points to the farm, reducing the risk factor associated with traffic of animal or peoples on to the farms. Shower and change room common on both farm types.

Table 4. Levels of Biosecurity at Farm Gate

Risk factors	Level of biosecurity on broiler farms			Level of biosecurity on farms with laying hens		
	Low	Medium	High	Low	Medium	High
Fence and lock	27/45%	11 (18.33%	22 (36.67%)	16 (26.67%)	12 (20%)	32 (53.33%)
Number of entrance	5 (8.33%)	6 (10%)	49 (81.67%)	7 (11.67%)	8 (13.33)	45 (75%)
Parking and vehicle washing	40 (66.67%)	15 (25%)	15 (25%)	26 (43.33%)	27 (45%)	7 (11,67%)
Signs around perimeter	52 (86.67%)	6 (10%)	2 (3.33%)	56 (93.33%)	1 (1.67%)	4 (6.67%)
Unsold eggs get returned to the farm	-	-	-	12 (20%)	0	48 (80%)
Activity family living off farm enter the property	46 (76.67%)	0	14 (23.33%)	43 (71.67%)	0	17 (28.33%)
Activity non family employees living off farm enter the property	36 (60%)	0	24 (40%)	36 (60%)	0	24 (40%)
Activity visitors enter the property	56 (93.33%)	0	4 (6.67%)	52 (86.67%)	0	8 (13.33%)
Shower and change room for visitors and employees	9 (15%)	0	51 (85%)	10 (16.67%)	0	50 (83.33%)
Using own cages to sell the birds	5 (8.33%)	55 (91.67)		10 (16.67%)	50 (83.33%)	0
Cages and equipment rturning from market cleaned and disinfected before reentering the farm	44 (73.33%)		16 (26.67%)	45 (75%)		15 (25%)

This study found that despite of the low level of biosecurity implemented by broiler farm, there are two good things applied on the farm to reduce thye virus spread into the farm. Firstly, the number of entrances is limited, shown by the 83.33 percent of respondents who are considered to have a high level of biosecurity in this area. Secondly, shower and changing rooms for visitor and employees are sufficient to receive a high level biosecurity rating on 85% of broiler farms surveyed.

Table 5. The Level of Biosecurity Between The Farm Gate and The Shed

Risk factors	Level of biosecurity in broiler breeders			Level of biosecurity at the breeder laying hens		
	Low	medium	High	Low	Medium	High
Feed barn is locked against rodents and birds	28 (49.67%)		32 (53.33%)	36 (60%)		24 (40%)
Overflows tap	4 (6.67%)		56 (93.33%)	8 (13.33%)		52 (86.67%)
Feed spilt	9 (15%)		51 (85%)	37 (61.67%)		23 (38.33%)
Chickens and ducks wandering around the shed	17 (28.33%)	7 (11.67%)	36 (60%)	22 (36.67%)	3 (5%)	35 (58.33%)

Biosecurity implemented in this location should be effective to prevent pathogen which entered the farm gate get into the shed. This study showed that the possibility of the virus entering the shed is lower in the broiler farms than in the layer farms. This indicated by the level of biosecurity achieved by the broiler farms in this area (Table 5). All of the biosecurity measures in this area are implemented at high level by broiler producers.

Regarding to the feed sealed against rodents and birds, broiler farms are considered to have high level of biosecurity because this farms put their feed securely in the shed. Free ranging chicken and ducks around the shed were still commonly found around the shed on both types of farm, however in both cases the level of biosecurity achieved is considered high for the majority of farms. This implies that the number of free ranging chickens and ducks wandering the shed can be limited.

Table 5. Level of Biosecurity at The Shed Door

Risk Factors	Level of biosecurity on broiler farms			Level of biosecurity on farms with laying hens		
	Low	Medium	High	Low	Medium	High
Shed wall made of good materials	29 (48.33%)	0	31 (51.67%)	13 (21.67%)	0	47 (78.33%)
Cage locked at all times	16 (26.67%)	0	44 (73.33%)	23 (38.33%)	0	37 (61.67%)
Sign at the door	60 (100%)	0	0	56 (93.33%)	0	4 6.67%)
Foothwash at the front door containing desinfectant	23 (38.33%)	22 (36.67%)	15 (25%)	53 (88.33%)	7 (11.67%)	0 (0%)
Wild birds and rats can enter the sheds	53 (88.33%)	0 (0%)	7 (11.67%)	59 (98.33%)	0	1 (1.67%)
Things have been done to prevent entry of wild birds and rodents	-	42 (70%)	18 (30%)	-	45 (75%)	15 (25%)

Action to enhance biosecurity implementation on farm can be observed in the shed. It is very encouraging that, majority of farms of both types have the shed walls made of good material to protect the birds from virus spread (Table 5).

The data presented in Table 5 also suggest that signage on the shed doors is neglected on nearly all farms. Furthermore, provisions of a concrete footbath in front of shed entrance is negligible for layer farms.

It is clear from the survey that wild bird and rodents are able to freely enter the shed, shown by approximately 90 % of respondents showing a low biosecurity score in this area. However actions have been taken by farmers to prevent entry of wild birds and rodents by minimizing gaps between boards in the wall, building sheds off ground and occasionally use of rat bait. Interestingly, bird-proof netting is rarely used.

CONCLUSION

The results of this study suggest that a similar profile of biosecurity implementation from farm gate to the sheds exists for both broiler and layer producers. However, there was a slight different with regards to securing boundary fence and locks, where layer farm producers tended to have more consideration of these factors. On the other hand, broiler producers showed a better biosecurity performance in providing footbath in front of the shed compare to layer producers. The study provides information upon which to plan and determine the most cost effective approach to improve the implementation of biosecurity at production point. Particularly, it helps focus on decisions to those parts of the riskfactors most likely to have the virus spread, and to allow the on biosecurity enhancement to be assessed.

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THE EFFECT OF THE MOWING HEIGHT ON MOWING TORQUE AND QUALITY OF TURFGRASS TIFF WAY 146

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ABSTRACT

Rotary mower is one of mowing tools commonly used to maintain lawn or turfgrass. Mowing height and mowing torque are important factors that should be taken into account in sustaining grass quality and designing a rotary mower. The study was addressed to recognize the influence of mowing height to required mowing torque and turfgrass quality. The mowing torque was measured at mowing height of 2, 3 and 4 cm. Measurement of mowing torque in the field was done by using a specially designed turfgrass rotary mowing apparatus that representing a rotary mower mowing mechanism. The apparatus was equipped with torque measurement system. The measured average torque was used to calculate the power requirement of mowing. The needs of maximum and minimum mowing torque to mow turfgrass for all mowing height were 0.68 Nm and 0.05 Nm. The average mowing torque were 0.51 Nm, 0.24 Nm, and 0.08 Nm, at mowing height of 2, 3 and 4 cm respectively. The average power that required to mowing was 146.8 watt, 96.1 watt, and 23 watt respectively. The grass quality was evaluated in terms of color, density, yield and bundle of grass. The best quality of turfgrass surface was achieved when it was mowed at the height of 3 cm. It had density of 350 grass shoots/100 cm2, yield of 9 g/m2, and green color.

Keywords: mowing, rotary mower, turf grass.

INTRODUCTION

Mowing is one of very important factors in maintaining turfgrass to provide its better function and visual appearance. Rotary mower is commonly used in mowing the lawn and turfgrass. It works based on direct high speed impact of its blade to the turfgrass.(Stikey,1986). The rotary mower is suitable used on slanted field as well as on flat area of turf field. Well scheduled and regular mowing will very much help operator to keep the best surface quality of turfgrass (Emmons,2000).

Research on utilizing the rotary mower mechanism to find out the behavior of mowing, development of an efficient mowing blade and development of rotary mower has being done. The research has generated a push type rotary mower prototype equipped with new composition of efficient mowing blades, clippings, mowing height adjuster anf powered by an electric motor. The prototype was named as Potrum SRT-03. This mower is the third generation of former two prototypes i.e. Potrum SRT-01 and SRT-02 Suastawa,2002).

Some researches regarding development of rotary mower as well as its typical blade design were conducted. Setiadi (2000) studied the effect of blade cutting angle of rotary mower when it mowed a lawn with given Zoyzia grass. Wirza (2002) and Sanjaya (2002) studied the torque required when rotary typed mower mowed Bermuda grass lawn. This research was conducted on specially design turf bin apparatus. The torque required in mowing was significantly affected by parameters such as blade rotational speed, forward speed, blade cutting angle, and grass density. Suharyatun (2002) also tried to theoretically analyze the rotary mowing torque requirement. Based on those studies, some rotary mower prototypes with a special rotary blade design were developed, such as Potrum SRT-01, SRT-02 and the last was SRT-03. Continuous improvement is still being done to this mower in order to have the best and appropriate rotary mower.

This study was intended to find out the torque required when the Potrum SRT-03, mow a relatively strong and dense Bermuda grass at different mowing heights. The result

of the research were considered to be very useful for a better rotary mower design which gives the most efficient power requirement while maintaining the best cutting results.

MATERIALS AND METHOD

For that purpose, a 16 m x 13 m Bermuda grass Tiff way 146, field was designed and constructed to give the best uniform condition of the turfgrass. A drainage channel was made on the field to give best drainage due heavy rain. The field was constructed at Turfgrass Teaching Farm, IPB University Farm, Unit Sindang Barang. Bogor. The turfgrass field was nurtured and maintained by applying NPK Fertilizer (15:15:15), Dursban^{TM} 200EC insecticide, and Dithane M-45 fungcide.

A special mowing apparatus was also designed and constructed to represent the Potrum SRT-03 mowing mechanism. The height of turfgrass was conditioned of 5 cm when the mowing measurement was conducted. Mowing torque was measured on the mowing height of 2, 3, and 4 cm. Torque measurement also was done when the blade wqas rotating freely (not mowing) just before mowing on a flat surface. After mowing, the apparatus past on the same mowed path with rotating blade to measured the torque required due to friction between turfgrass surface ang blade lower surface. The rotational speed of the mowing apparatus blade was about 2800 rpm.

Mowing forward speed was around 0.27 m/s based on operator walking speed. Only one same operator doing the whole measurement. Mowing path length was 11 meters for one pass of measurement. At the same time, a clamp meter was also used to measured the electricity power used to operate overall measurement apparatus.

RESULTS AND DISCUSSION

Establishment of turfgrass field

Nearly four months was needed to establish the turfgrass field to be ready for the mowing experiment since the land preparation taken place. The process of establishment can be seen in Fig.1. Fertilizer was broadcasted on the field once a week and followed by watering it because Bogor was in the dry season during this work. At the time of mowing experiment, the average grass density of the field was 197 shoots/100 cm^2. The grass average moisture content during mowing was 64% (WB).

Calibration equation of torque sensor

Before measurement, calibration of the torque sensor was done. First of all, the strain amplifier was calibrated. The equation Y = 0,0022x – 0,0099 was resulted to show the relationship between the strain (με) on X-axis and voltage (volt) on Y-axis, with a coefficient of determination (R^2) Secondly, the calibration of torque sensor on the mower blade shaft was done. An equation Y = 530,23x + 11,677 was found to show the relationship of the applied torque (Nm) on X-axis and resulted strain (με) 0n Y-axis, with coefficient of determination (R^2) of 0.99. Both equations were combined and resulting an equation Y = 0.85x – 0.005 to bring relationship between the mowing torque (Y-axis) and recorded voltage during measurement (X-axis).

Mowing torque

The maximum dam minimum torque required to mow the turfgrass field at all mowing height were 0.68 Nm and 0.05 Nm respectively. Since, the average torque required to mow at the mowing height of 2, 3, and 4 cm were 0.51 Nm,0.24 Nm, and).08 Nm respectively. It clearly be seen that mowing the turfgrass field at 2 cm height was the toughest. It happened because at the that height, the blade cut mostly the stolon of the turfgrass, which is considered taugher than the grass leaf. On the order hand, the blade only cut the leaf part of the turfgrass when it was mowed at the height of 4 cm. As for

comparison, Suharyatun (2000) has measured the mowing torque of Bermuda grass in door on a turf bin test apparatus. The maximum torque recorded was 0.4 Nm.

The average torque required when the blade rotated freely (no cut and no friction) was 0.03 Nm. The average torque required when the rotating blade passed on the cut surface, at all cutting height was 0.04 Nm. These explained that there are torque needed to rotate the blade and the overcome the friction between the blade lower surface and the field surface.

Based on the measured torque and operational rotation (rpm) of the blade, the power needed for the mowing operation can be calculated. The calculation was based on the blade rotational speed at 2750 rpm. The maximum and minimum power requirement were 196.4 Watt and 14.4 Watt respectively. The average power required to mow at the height of 2, 3 and 4 cm were 146.8 Watt, 69.1 Watt and 23 Watt respectively.

By using clamp meter, roughly the electricity power needed to operate the whole measurement apparatus was measures as amount of 415 Watt. It means that there are some power was consumed for order that mowing such as lost in transmission, or due to the efficiency of the electric motor it self that used to power the apparatus. The research suggest that it still possible to develop a law power of pushed type rotary mower.

CONCLUSION

1. Maximum torque required to mow the Tiff Way 146 Bermuda grass lawn by Potrum SRT-03 mower was 0.68 Nm, gained at mowing height of 2 cm.
2. Average torque required to mow the Tiff Way 146 Bermuda grass lawn by Potrum SRT-03 mower at mowing heights of 2, 3 and 4 cm were 0.51 Nm, 0.24 Nm, and 0.08 Nm respectively.
3. Average calculated power required to mow the Tiff Way 146 Bermuda grass at mowing heights of 2, 3 and 4 cm were 146.8 Watt, 69.1 Watt and 23 Watt respectively.

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THE SUPPLEMENTATION OF VIRGIN COCONUT OIL (VCO) IN THE DIET TO DECREASED BROILER MEAT CHOLESTEROL

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ABSTRACT

The aim of this experiment is to study the supplementation of the VCO in the diets to decrease broiller meat cholesterol which has been done at Batu Bulan Village, Gianyar Regency and Animal Nutrition Laboratory. The design which was used in this experiment is a completely randomized design (CRD) with three treatments and four replicates. The three treatments were diets without VCO as a control (A), supplemented 2% VCO in the ration (B), and supplemented 4% VCO in the ration (C) respectively. Feed and water offered *ad libitum*. The variables which measured were carcass weight, carcass percentage, and meat cholesterol. The result of this experiment showed that the carcass weight, on the treatment B (supplemented of 2% VCO in the ration) and treatment C (supplemented 4%VCO in the ration) were significantly ($P < 0.05$) higher than the control, but the carcass percentage, total cholesterol, triglyceride and HDL were not significantly ($P > 0.05$) higher than the control. The LDL on the treatment C (supplemented 4%VCO in the ration) was significantly ($P < 0.05$) lower than the control. From the result of this experiment can be concluded that the supplementation of VCO from 2-4% in the diets has increased the carcass weight and decreased the broiller meat LDL.

Key words: VCO, broiller, carcass weight, meat cholesterol

INTRODUCTION

Public attention to the fat food becomes larger, especially after excessive fat consumption is known to affect health, not only to the increase in coronary heart disease but also cancer, diabetes, high blood pressure and hypercholesterolemia (Sufari, 2001). Linder (1985) reported that in developed countries the percapita meat consumption is high; the incidence of atherosclerosis is also relatively high. Even Schaerfer et al., 1995 (cited by Munisa, 2003) suggests three – quarters of eaths in American women and men associated with cardiovascular disease and cancer and the leading cause of death was coronary heart disease.

Although excessive consumption of fat is considered as one cause of coronary heart disease, we cannot consume food without fat. This is because fatty foods have an important function, namely as an energy source, the source of fat-soluble vitamins, the synthesis of certain hormones, cell membranes as well as determinants compiler texture and taste of food. In regard to this fact, the necessary step is needed to produce meat with low cholesterol levels (especially LDL) without disrupting production. One effort that can be done is by manipulating the composition of diet by using VCO (Virgin Coconut Oil).

Pure Coconut Oil (VCO) is oil produced from fresh coconut processing of raw materials without going through the process of distillation/using minimal heat without chemicals / without chemical purification processes (Alamsyah, 2005). VCO can be done by two main methods of wet milling method and the method of enzymatic/fermentation. With the help of microbial fermentation method can produce high oil yields and the possibility of damage to several compounds important to health is very low (Alimuddin and Zaraswati, 2005).

VCO which is known as a high lauric oil, can be easily absorbed by the body and quickly burned into energy for metabolism thus increasing metabolic activity, even burn LCT/long chain triglyceride (C14-C22), and not buried in the body tissues. MCT is not packed in lipoprotein and not distributed in blood, but directly transferred to the liver and converted into energy with a lower energy content of oil/fat used is 8.3 kcals/g. The result of the research on normocholesterolemic healthy men, where 30% of energy derived from fat, which showed that the addition of lauric and palmitic acid from coconut oil amounted to 5% of the energy consumption significantly increased serum total cholesterol from 166.7 to 170.0 mg/dl, lowering low density lipoprotein (LDL-C) from 105, 2 to 104.4 mg/dl, increasing High Density Lipoprotein (HDL-C) from 42.9 to 45.6 mg/dl, while the ratio decreased from 2.49 to LDL-C/HDL-C 2.39 (Alamsyah, 2005).

Utilization of VCO to decrease cholesterol levels in humans have begun to be further explored. VCO even referred as the conqueror of different diseases (Alamsyah, 2005), but the data regarding the usage of VCO on livestock especially broiler is not so much found yet, therefore further research about the effect of VCO on broiler rations to reduce cholesterol without reducing the production of beef cattle is needed. This research is expected to be a reference to produce a quality of broiler meat with low cholesterol content.

MATERIALS AND METHODS

Field research was conducted at Jl. Batuyang, Gang Pipit No. XII. 3 Batubulan, Gianyar from August 24th until September 30th, 2007, while the laboratory analysis conducted at the Laboratory of Animal Nutrition Faculty of Animal Husbandry, Udayana University Denpasar.

This study uses battery colony cage systems made of bamboo strips with a length of 1 m, width 1 m and height 2.5 m. Every each plot completed with stalls of food and drinking water.

Chicken used were strains Broiler CP 707 age 1 day (DOC) as much as 60 tails with a range of initial body weight 43.40 + 0.20 grams obtained from the PT. Tohpati Poultry, Jl WR. Supratman, Denpasar, Bali.

During the study two basal ration formulas composed which was adapted from the chicken growth phase, upon the recommendation of NRC (1984), namely 1) the phase starter rations given to chickens at the age of 0-3 weeks), and 2) Diet grower phase, given to chickens at the age of 3 weeks until the chicken is cut. Ingredients and nutrient composition of diets are shown in Tables 1 and 2. VCO / VCO is a VCO is used as a supplement that is produced by fermentationn process using Microorganism effective 10% (v/v).

Drinking water given was water with EM4 inoculated with 10 ml / liter air.dan given *ad libitum.*

This Research used Completely Randomized Design (RAL) with 3 treatments and 4 replications. Each replication utilizes 4 chickens. All 3 treatments are: A. Basal ration without supplementation VCO ;B. Basal ration supplemented with 2% VCO and C. Basal ration supplemented with 4% VCO.

Observed variables

Observed variables in this study are:

1. Carcass weight: heavy live weight minus feathers, heads, feet and viscera (USDA, 1977)
2. Carcass percentage: carcass weight divided by 100%
3. Percentage of carcass fat: fat weight of carcass weight divided by 100%.
4. Cholesterol content of meat sought is:

- Total cholesterol concentration measured using Liebermann-Burchard method

- HDL level measured using Phosphotungstic acid/magnesium chloride method
- LDL level measured using the method in Roche (1994)
- Triglycerides level measured using enzymatic calorimetry test method

Data analysis

The data obtained were analyzed by variance use of the program SPSS 13.0, in the case of significant differences (P <0.05) followed by *Least Significant Difference test* (LSD).

Table 1. Material Composition and Nutrition Content of Broiler Ration Age 0-3weeks

Material Composition	Ration Composition			Standard[1]
	A [(2)]	B	C	
Yellow Corn	47	46.06	45.12	
Coconut Cake	19	18.62	18.24	
Rice Bran	6	5.88	5.76	
Fish Meal	13	12.74	12.48	
Soy Bean	14	13.72	13.44	
Salt	0.2	0.196	0.192	
Mineral 10	0.3	0.294	0.288	
Enzim Optyzime	0.2	0.196	0.192	
Starbio	0.3	0.294	0.288	
VCO	0	2	4	
TOTAL	100	100	100	
Nutrient Contain				
ME (kkal/kg)	3209.69	3317.50	3425.30	3200
CP (%)	23.10	22.63	22.17	23
SK (%)	5.01	4.91	4.81	-
EE (%)	6.41	8.28	10.15	-
Ca (%)	1.08	1.06	1.04	1.00
P (%)	1.02	1.00	0.98	0.45

Notes: 1 = NRC. (1984); 2A = diets without (control); B = Rations + VCO 2%; C = Rations + VCO 4%

Table 2. Material Composition and Nutrition Content of Broiler Ration Age 3-7 weeks

Material Composition	Ration Composition			Standard1
	A (2)	B	C	
Yellow Corn	50	49	48	
Coconut Cake	15	14.7	14.4	
Rice Bran	14	13.72	13.44	
Fish Meal	11.5	11.27	11.04	
Soy Bean	8.5	8.33	8.16	
Salt	0.2	0.196	0.192	
Mineral 10	0.3	0.294	0.288	
Enzim Optyzime	0.2	0.196	0.192	
Starbio	0.3	0.294	0.288	
VCO	0	2	4	
Total	100	100	100	
Nutrient Contain				
ME (kkal/kg)	3201.88	3309.84	3417.81	3200
CP (%)	20.65	20.24	19.83	20
SK (%)	5.49	5.38	5.27	
EE (%)	6.81	8.67	10.54	
Ca (%)	0.97	0.95	0.93	0.9
P (%)	1.02	1.00	0.98	0.4

Notes: 1 = NRC. (1984); 2A = diets without (control); B = Rations + VCO 2%; C = Rations + VCO 4%

RESULTS AND DISCUSSION

The results are shown in Table 3 below.

Table 3. Effect of VCO supplementation in ration on carcass weight, carcass, subcutaneous fat and cholesterol of broiler meat.

Variables	Treatments			SEM (2)
	A	B	C	
Carcass weight (g)	1053,75a	1155,75b	1120,00b	16,02
Carcass percentage (%)	69,04a	70,45a	69,30a	0,49
Fat subcutan (%)	18,21a	20,03a	21,24a	0,85
Total Cholesterol (mg/100 g)	351,98a[1]	352,29a	336,54a	4,12
Triglyceride (mg/100 g)	253,71a	268,54a	253,93a	3,54
LDL (mg/100 g)	232,67b	225,36ab	209,89a	4,00
HDL (mg/100 g)	68,56a	73,22a	73,86a	1,36

Notes:
A = ration without supplemented VCO
B = VCO 2% ration supplemented
C = ration supplemented VCO 4%
1 = The same letter for the column showed no significant difference (P> 0.05)
2 = SEM = Standard Error of the Treatment Means

Table 3 shows that broiler carcass weight results in treatment A (without VCO) amounted to 1053.75%, while the carcass weight in treatment B (supplemental VCO 2%) and C (VCO supplementation of 4%) increased significantly (P <0 , 2005) respectively 9.68% and 6.29% compared with treatment A.

Statistically, the percentage of broiler carcass and subcutaneous fat in the three treatment groups (A, B and C) showed no significant difference (P> 0.05), but quantitatively on treatment B and C there was an increase.

Content of total cholesterol, triglycerides and HDL broiler meat carcasses in the three treatment groups (A, B and C) statistically was not significantly different (P> 0.05), whereas LDL levels on treatment C decreased 9.79% and statistically significantly different (P<0.05) compared with treatment A.

DISCUSSION

Results showed that supplementation of Virgin Coconut Oil (VCO) which is fermented with effective Microorganism in diet can improve carcass weight. This is because the VCO is rich in lauric acid and capric acids, where the compounds monogliserida generate a strong anti microbial properties that will be able to improve livestock health. Besides, the VCO is the source of medium chain saturated fatty acids (medium chain triglyceride / MCT) are easily digested / absorbed by the body cells as a result of its physical properties are more polar than the LCT (Long chain triglyceride) that will reduce the burden on the body for metabolic processes (Alamsyah, 2005).

Increased fat deposition that occurs as a result of increased VCO supplementation did not result in increased cholesterol levels of broiler meat (Table 3), a quantitative decrease in total cholesterol and can lead to significantly decreased levels of LDL. From the results of this study prove that the VCO does not result in negative effects to the body associated with blood cholesterol and body, especially the presence of bad cholesterol (LDL), which is causing occurrence of various diseases such as coronary heart disease, atherogenecity, cancer, and atherosclerosis. Low levels of LDL from broilers fed diets with VCO supplementation, possibly due to VCO can react directly to the thyroid and

thyroid conditions improve so that it can increase production of thyroid hormones are able to transform LDL into steroidal anti-aging (Alamsyah, 2005).

Low cholesterol meat quantitatively from treatment B and C, most likely caused by the content of phytosterol (sitosterol, compesterol, stigmasterol) in VCO which can reduce cholesterol levels. Besides VCO is used, the fermentation by Microorganisms effective so the ability to lower cholesterol levels, especially the bad cholesterol will be higher. Giving probiotics on cattle proven to reduce blood cholesterol levels and meat due to its ability inhibit the synthesis of cholesterol in the liver, that is by suppressing the activity of the enzyme 3-hydroxy-3-methyl glutaril CoA reductase.

CONCLUSION AND RECCOMMENDATION

VCO supplementation in diet on the level of 2-4% can increase carcass weight and 4% VCO supplementation can lower LDL levels of broiler meat.

In an effort to produce good quality of broiler meat, especially with low cholesterol levels, it is advisable to use VCO on the diet with higher levels of 4%.

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SEROPREVALENCE Q FEVER IN BALI CATTLE (*Bos sondaicus*) AT BALI PROVINCE BY INDIRECT IMMUNOFLOURESCENT ANTIBODY ASSAY METHOD

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ABSTRACT

Q fever is a zoonotic disease caused by Coxiella burnetii, a species of bacteria that is distributed globally. Ruminant, especially cattle may play the important role in the Transmission to human. The research of seroprevalence of Q fever in Bali's cattle were done in September 2009 in Gianyar and Badung district, Bali. A total of 150 serum and buffy coat samples were collected; 130 serum from females and 20 serum males. The indirect immunoflourescent antibody test was used to determine the seroprevalence of Q fever. The seropositive based on the dilution of serum starting from 1:16. Seropositive were observed in 30 samples (23,07%) of females and 1 male samples (5%) of male Bali's cattle. The highest titer of 1:128 was observed in 1 female with reproduction problem and 2 pregnant cattles. The results of the present study suggested that Q fever may be endemic in Bali.

Keywords: Q fever, zoonotic, prevalence, indirect immunoflourescent antibody test,

POSTER PRESENTATIONS:

AGRITECH AND FOOD

MODIFICATION OF CASSAVA STARCH WITH OXIDATION TO IMPROVE BAKING EXPANSION

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ABSTRACT

To reduce imported products dependency, need to concern the substitution of wheat to alternative carbohydrates sources, in particular cassava starch is considerable. Cassava is abundant food stuff in Indonesia. Cassava starch as main stuff in bakery industry including bread that involve baking process have not been done commonly in Indonesia. It is due to single use of cassava starch is considered not giving perfect expansion as wheat. This research aimed to identify effect of chemical modification with oxidation on cassava starch to improve baking expansion so cassava starch usage as wheat substitute can be increased. This research began with optimization of baking expansion with chemical modification using oxidizer solutions: 0.08, 0.16, 0.24, 0.32 and 0.40 % $KMnO_4$ for 15 minutes. The highest expansion of modified starch using oxidation, character comparison between modified starch and unmodified starch was done. Results indicated that modification affected significantly baking. The highest expansion value using 0.32 % $KMnO_4$ was 11.91 ml/g, which was higher than unmodified starch 7.32 ml/g, with analysis results include: amylose content of 33.19 %, pH 2.67 and paste viscosity peak of 1312 cP. Carbonyl group content of 0.126 %, carboxyl group content of 0.99 % and paste clarity of 91.57 %.

Keywords: cassava starch, oxidized starch, $KMnO_4$, baking expansion

INTRODUCTION

Food supply to meet nutrition sufficiency and balance is great problem in Indonesia where our food tenacity has been threatened due to decrease in productivity of some food. In other side, there has been dependency on imported food. To support food tenacity, an effort to use local product as import substitution is required. Cassava has potential to be developed as semi finished product, as starch, which did not contain gluten but can expand in baking process, so it has potency as substitution of wheat as main material to make loaf and others.

This research aimed to identify effect of chemical modification with oxidation and acidification on cassava starch to increase expansion in baking process, so use of cassava starch to substitution wheat can be improved.

MATERIALS AND METHODS

Materials

Main material used in this research was Gunung Agung manufactured cassava starch, produced by Sungai Budi in Lampung, obtained from its distributor in Yogyakarta. The material was treated as modified starch. Chemical material used was $KMnO_4$. Material used to chemical analysis was Pro-Analysis grade.

Methods

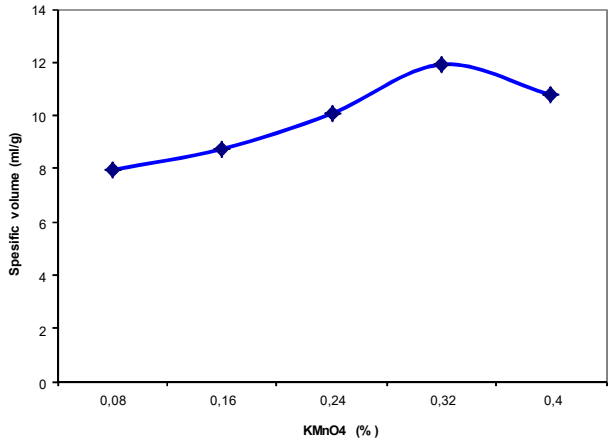
This research was begun with expansion optimization in baking with oxidation of manufactured starch with various $KMnO_4$ oxidants in different concentrations. Early, 100 g manufactured starch (dry basis) was suspended into $KMnO_4$ solution (0.08%, 0.16% to 0.4%) for 15 minutes and agitated in room temperature. Then, oxidation was stopped by adding sodium metabisulfite for 5 minutes until violet colour disappeared. To eliminate possibility of residue, washing was done three times. Reagent-free manufactured starch was dried in oven at 50^0C, grilled with mortar and sieved with 60-mesh sieve. Oxidized manufactured starch was tested on expansion level in baking process. Based on the

highest baking expansion, chemical analysis were done including pH, amylose percentage, paste viscosity, starch granule microscopic, paste clarity and carbonyl and carboxyl group contents, which then was compared with unmodified manufactured starch.

Baking expansion rate measurement is done based on the method used by Demiate *et al*. (2000) with few modifications. Manufactued-starch weighed 10 gram stirred with 30 ml aquadest is processed in the waterbath until the gelatinitation is occurred. Later, the sample is divided in 3 portions to be baked in 200 ºC temperature for 25 minutes. After the baking process, we could measure the baking expansion rate defined in specific volume (ml/g).

RESULTS AND DISCUSSIONS

Baking Expansion Value Measurement



The research indicated that oxidant concentration giving the highest baking expansion level was 0.32% $KMnO_4$ solution. Increase in expansion rate in baking process occurred along with increase in oxidant solution concentration up to 0.32% that giving baking expansion of 11.91 Ml/g higher than unmodified starch (7.32 ml/g). However, the baking expansion tended to decrease when oxidant concentration was increased. Decrease in expansion rate in baking process was expected due to excessive oxidation process in cassava starch.

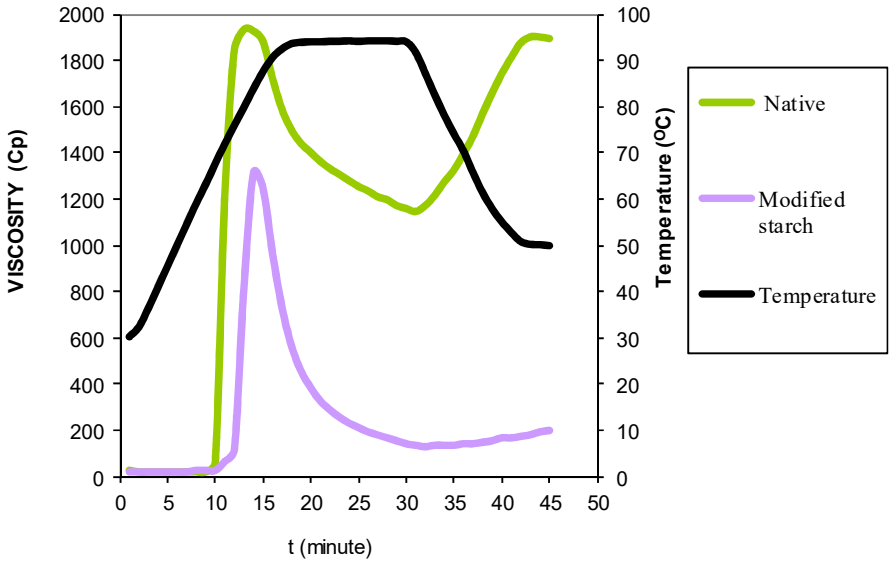
It was due to after starch oxidation that caused starch hydroxyl group oxidized forming carbonyl group and oxidized further to be carboxyl group followed with starch depolymerization that cause increase in starch solubility and made water enter easily that increasingly loose inter-molecule bond, which will increase starch granule expansion volume. Factors playing main role in cassava starch expansion in baking process were water vapor pressure due to water bond in starch, change in temperature influencing rheology and starch expansion, and process of water loss in starch tissue that depend on dough permeability. Starch oxidation process resulted in more water bond in cassava starch. More bond water lead to more water vapor formed during baking process. Then, more vapor lead to greater product expansion.

The Best Modified Starch Characteristic

Physical and Chemical Properties Native and Modified Starches

Analysis	Native	Oxidation strach (KMnO4 0,32 %)
Spesific Volume (ml/g)	7.32	11.91
pH	4.39	2.67
Amilose (%)	25.92	33.19
Carbonyl (%)	nd*	0.126
Carboxyl (%)	0.55	0.99
paste clarity (%)	60.63	91.57

***nd**= not detected



Viscoamilograms of native and modified starches with KMnO4 0,32 %

Results of analysis on modified starch in the highest baking result included amylose content of 33.19%, pH 2.67, and paste viscosity peak of 1312 cP. Observation of starch granule structure with Scanning Electron Microscopy indicated reduction of granule size compared with manufactured starch before modification, carbonyl group content of 0.126%, carbonyl group content of 0.99% and paste clarity of 91.57%.

CONCLUSIONS

Conclusion from this research was that starch modification with oxidation that have greatest expansion rate has characteristic of increasingly little starch granule size due to depolymerization, higher acidity level, lower starch paste viscosity, higher amylose content, higher carbonyl and carboxyl group content, and higher paste clarity than unmodified starch.

ACKNOWLEDGMENT

I thanks to my advisor Prof. Dr.Ir.Haryadi, MappSc. for funding my research in the completion of my master program in Gadjah Mada University and for the advice and counseling during this research.

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DETERMINATION OF THE TUBER TYPES AS A DIET FOOD OF DIABETES MELLITUS PATIENT

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ABSTRACT

The research was aimed to determine the types of tuber that was suitable to be consumed diabetes mellitus patient, based on resistant starch content and increasing of blood glucose.The method of the research undertaken in two steps, namely the characterization of tubers and determination of tuber type that was suitable for diabetes mellitus patient. This step is carried out using the bioassay and to use the completed randomized design with six treatments to include of sweet potatoes, bentul, ube, taro, taro forest, and potato tubers. The treatments were repeated ten times, in order to obtain 60 experimental units. The research results showed that there were differences among the species of tuber water content, ash, protein, fat, total sugar, reducing sugar, starch, resistant starch content and increasing in of blood glucose. The best tuber to diet consumption was sweet potato with the characteristics : moisture content of 63.59%, ash content of 1.13%, protein content of 1.05%, fat content of 1,13%, sugar total content of 3.93%, reducing sugar content of 2.82%, starch content of 20.57%, resistant starch content of 13.58% and cause increasing of blood glucose of 20.00 mg/dl.

Keyword: types of tuber, resistant starch content, increasing of blood glucose, diabetes mellitus

INTRODUCTION

One effort for the prevention of diabetes management is the proper diet and choosing the right foods (Marsono, 2002). High content of resistant starch to be one consideration in the determination of diabetes mellitus patient food (Schult et al., 2003). According to Asp and Bjork (2002), resistant starch is starch that is not substantially adsorbed in the intestine. Thereby affect the availability of calories in the body (Asp and Bjork, 2002).

Lately, tubers become the attention of nutritionists, community and government in terms of meeting the community nutrition and food security. This is due to the high carbohydrate content, easy to grow and productivity higher than that of rice (Anonymous, 2003). According Nainggolan (2006), tubers generally have the calorie and low protein content but rich in fiber. In addition, some species have tubers contain vitamin A, carotenoids, vitamin C, calcium, and fiber is better than rice or other staple food (Anonymous, 2006). Even Apriaji (2009) describes certain tubers contain the antioxidant beta-carotene, lutein, zeaxantin that can prevent cell damage caused by free radicals. Meanwhile, the content of Ca, P, Fe and K in the tubers can help prevent hypertension (Astawan, 2004). This shows the potential of tubers as food diet (low calorie), which provided for prevention of diabetes mellitus as well as for the sufferer.

The research was aimed to determine the types of tuber that was suitable to be consumed diabetes mellitus patient, based on resistant starch content and increasing of blood glucose.

MATERIALS AND METHODS

Tools and Materials

Spectrophotometer (Shimadzu UV-Visible), centrifuge (314 Ecospin, Inc. Biotron.) Vortex (37 600-mixer, Thermolyne), blood glucose test meter, coolant through,

the unit kjeltech protein, soxhlet extraction unit, the unit of analysis equipment ash content, total sugar, starch, dietary fiber and resistant starch, a mouse cage and pthers.

Material for research: sweet potato, ube, forest taro, potatoes, taro, bentul, bread, SD rats aged 3 months, a standard diet. Material for analysis: HCl, NaOH, boric acid, petroleum ether, Pb-acetate, Na-oxalate, reagent Nelson, pancreatinase, amiloglucosidase, pullunase, and others.

Implementation Research

a. Tubers characterization step

Type of tubers that were characterized are sweet potatoes, ube, forest taro, potato, butter taro and bentul. Characterization includes the fat, protein, water, ash, starch, total sugar, reducing sugar, starch and starch resistant content.

b. Tubers determination step

This experiment was using the completely randomized design with six treatments of tubers type with 10 replications. Type of tubers were a sweet potatoes, ube, forest taro, potato, taro and bentul. The experiment used the bioassay method with 60 SD rats. The steps of experiment were :

Making a standard feed

Standard feed prepared by mixing 620.69 g corn starch, casein 140g, 100g sucrose, 40 g soybean oil, 50 g of CMC, 35g mineral mix, 10 g vitamin mixture, 2.5 g and 1.8 g kholin bitartrat L- cystin. Diadon mixture by adding a little hot water if needed until the dough tough. Clay dough is then printed with a printing machine feed / pellets with a long cylindrical shape and then dried in an oven at a temperature of 50oC for 12 hours.

Preparation of experimental animals

A total of 60 SD rats aged 3 months with an average weight of 150 g placed individually in special cages which are divided into six groups, so that each group there were 10 tails. Rats placed in a clean place at room temperature with sufficient ventilation and lighting.

Giving a standards feed

Mice that had been placed in special cages next given a standard diet of each 5 g per serving with an ad libitum fed (without limitation) for 6 days.

Preparation of experimental animals treated rats for feeding

Rats were fed with standard diet for 6 days then fasted for 24 hours in a clean cage at room temperature with sufficient ventilation and lighting.

Preparation of feed treatment

The treatment of tuber cleaned by washing and peeling the skin and cut into pieces with a fistful size (weight 100 g). Tuber steamed until cooked. Furthermore, mature bulbs ready to be served as food of rats.

Measurement of fasting blood sugar levels

While the preparation of feed treatment, mice that had fasted for 24 hours, blood drawn to measure blood sugar levels when fasting blood glucose test using meters.

The food treatment

Furthermore, each rat was fed the cooked tubers according to the treatment as much as 5 g per serving with the provision of drinking on an ad libitum (without borders)

Measurement of blood sugar after a meal and determining the best bulbs

Furthermore, 30 minutes after a meal, the rats were measured her blood sugar levels with blood glucose test meters. Resulting data were analyzed diversity and continued with Duncan multiple comparison test. Tuber treatments that have a resistant starch and cause an increase in low blood sugar, was selected as food for people with diabetes mellitus.

RESULTS AND DISCUSSION

Characterization of Tubers

Characterization of several kinds of tubers, especially on their nutritional content, including moisture, ash, protein, fat, total sugar content, reducing sugar, starch and resistant starch, it appears is shown in Table 1

Table 1. Average moisture, ash, protein, fat, sugar total, reduction sugar, starch an resistant starch content of tuber types

Tuber type	Water content (%)	Ash content (%)	Protein content (%)	Fat content(%)	Sugar total (%)	Reduction sugar content (%)	Starch content (%)	Resistant starch content (%)
Sweet potato	63.59c	1.13bc	1.17d	1.13bc	3.93b	2.86b	20.57b	13.58a
Bentul	64.19bc	1.25b	1.68b	2.33a	3.02d	2.33bc	20.16b	3.09d
Ube	63.21c	1.09c	1.48c	1.81ab	4.23a	3.75a	21.67b	11.14b
Forest taro	65.13b	0.97d	1.40c	1.63b	3.53bc	2.83b	20.99b	9.46bc
Taro	60.80d	1.55a	2.75a	1.54b	3.31c	2.07c	24.66a	7.77c
Potato	68.48a	0.93d	1.59bc	0.92c	3.25c	2.73b	16.57c	11.49ab

Notes: The same letter at the back of the average value in the same column showed no significant difference in error rate (α) 5%

Table 1 shows the differences of water, ash, protein, fat, sugar total, reduction sugar, starch and resistant starch content of sweet potato, bentul, ube, forest taro, taro and potatoes. The highest water content (68.48%) found in potato tubers was lowest (60.80%) in the tubers of taro, while the highest ash content (1.55%) contained in the tubers of taro and the lowest (0.93%) there was a forest on the potato and taro. The highest protein content (2.75%) contained in the tubers of taro and the lowest (1.17%) found on sweet potato, while the highest fat content (2.33%) contained in the bentul that was not significantly different from the ube, while the lowest (0.92%) contained in the potatoes that was not significantly different from the sweet potato. The highest total sugar content (4.23%) contained in the ube while the lowest was in the bentul (3.02%). The highest reducing sugar content (3.75%) contained in the ube was the lowest (2.07%) contained in the taro that was not significantly different from the bentul. The highest starch content (24.66%) was the lowest found on taro (16.57%) contained in the potatoes. Meanwhile, there was the highest resistant starch content in sweet potato (13.58%) and the lowest found in the bentul (3.09%).

The differences of water, ash, protein, fat, sugar total, reduction sugar, starch and resistant starch content of sweet potato, bentul, ube, forest taro, taro and potato tubers occurs because each has different characteristics in terms of genetic, physiological and

environmental needs of growth. This is such opinions of Setiadi and Nurulhuda (2005), who explained that the chemical composition of tubers including nutrient content is determined by the nature of the genetic roots to absorb organic compounds (including nitrogen-containing compounds) and inorganic compounds (including minerals) as a soil nutrient. It also determined the genetic properties of compound leaves in absorbing carbon and oxygen from the surrounding air environment. Therefore, differences in the absorption of the compounds mentioned above will lead to differences in water content, ash, protein, fat, sugar total, reduction sugar, starch and resistant starch content in tubers.

Determination of Resistant Starch Type Bulbs on and Increasing of Blood Glucose

Analysis of variant showed that the type of tubers have a very significant effect on the increase in blood glucose in the body of diabetes mellitus patient. Table 2 shows the differences of blood glucose increasing from the body which consume tubers. The lowest increasing in blood glucose (20.00 mg/l) when the body consumes sweet potato which did not differ significantly with time consuming potatoes, whereas when consuming bentul highest (74.50 mg/lt).

Table 2. Average resistant starch content and an increase in blood glucose

Tuber type	Increasing of blood glucose (mg/lt)
Sweet potato	20.00d
Bentul	74.50a
Ube	65.50ab
Forest taro	36.50c
Talro	54.00b
Potato	29.50cd

Notes: Description: The same letter in the back of the average value in the same column indicate no significant difference in error rate (α) 5%.

The lowest increasing of blood glucose when consumed the sweet potato tubers than the other can be explained as follows: sweet potato containing high amylosa than other tubers, which can affect the formation of resistant starch. Amylosa has a greater ability to crystallize due to intensive hydrogen bonding making it difficult to expand or have a good gelatinization (Marsono, 1998), but easy to be retrogradated (Abia et al., 2003). This condition causes difficult to digest in the body (Meyer, 1995). This means the resistant starch of sweet potato was difficult hydrolyzed to glucose when consumed the body, resulting in the increase of blood glucose showed lower results than most other tubers consume. According to Sears and Sears (2007), foods that contain resistant starch, high if it entered the blood stream will run slower and thus do not trigger insulin makes blood glucose more stable. Based on the highest resistant starch content and the lowest increasing of blood glucose were chosen the sweet potato as food suitable for consumption of diabetes mellitus patient.

CONCLUSION

Tuber was selected as a suitable food for consumption of diabetes mellitus patient was sweet potato which has a moisture content of 63.59%, 1.13% ash, protein, 1.17%, 1.13% fat, 3.93% total sugar, reducing sugar 2.86%, 20.57% starch, starch 13.58% and caused increasing of blood glucose 20 mg/lt.

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EFFECT OF SOYBEAN PROTEIN DIET ON MUSCLE PROTEIN DEGRADATION IN ALLOXAN-INDUCED DIABETIC RATS

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ABSTRACT

A research examining the effect of soybean protein diet on muscle protein degradation in alloxan–induced diabetic rats has been conducted. The research was intended to study the ability of soybean protein in inhibiting muscle protein degradation in alloxan–induced diabetic rats. Fifty four male Wistar rats, averaging body weight of 200 grams were divided into 2 groups. The first group was injected with alloxan (100 mg/kg body weight) to induce diabetic and the other group was used as control. Each group was divided into 3 sub groups of 9 rats and were given three different diets, i.e. standard, soybean and soybean protein concentrate diets. Blood sugar and urine sugar were measured periodically every 9 days during research period, while ammonia content of inhaled air were measured before injection (day 0), 27 and 45 days after injection. At the end of experiment, femur muscle was removed to be used for determination of muscle protein content. Result indicated that soybean and soybean protein concentrates diet decreased blood sugar content of diabetic rats from 481 mg/dL to 166 mg/dL and from 366 mg/dL to 162 mg/dl, respectively. Urinary glucose level of diabetic rats fed with soybean decreased from 600 mg/dL to 166 mg/dL and so did group of diabetic rats fed with soybean protein concentrates. Ammonia was only detected in group of injected alloxan fed with standard by 18 ppb. Muscle protein content of diabetic rats fed with soybean protein concentrates was 91.73%, higher than group of diabetic rats fed with soybean and standard. This result indicated that soybean protein concentrates diet inhibited muscle protein degradation of diabetic rats beter than that of soybean and standard diets.

Keywords: diabetes mellitus, protein degradation, soybean, muscle protein

INTRODUCTION

Diabetes mellitus is by far the most important disease affecting carbohydrate metabolism, but disorder is by no means limited to this area, protein and fat metabolism are also affected. This is manifested by protein-wasting, with accelerated conversion of amino acid to glucose and increasing of muscle protein degradation. Previous study indicated that administration of carbohydrate, free amino acids, and protein hidrolysates has been shown to stimulate and inhibit protein degradation (Biolo *et al*.,1999, Volpi *et al*., 1998).

It is well known that soybean protein has beneficial effect for patient with diabetes mellitus. Zuheid-Noor *et al*., (2000) reported that soybean has hypoglycemic activity in induced diabetic rats. The aim of this research was to study the ability of soybean protein in inhibiting muscle protein degradation in alloxan–induced diabetic rats.

MATERIALS AND METHODS

This research was divided into two steps. The first step was conducted to prepare dry soybean protein concentrates and the second step was bioassay study to investigate the effect of soybean protein diet on muscle protein degradation.

Fifty four male Wistar rats, averaging body weight of 200 grams were divided into 2 groups. The first group was injected with alloxan (100 mg/kg body weight) to induce diabetic and another was used as control. Each group was divided into3 sub groups of 9 rats and were given three different diets i.e. standard, soybean and soybean protein concentrate diets (SPC). The composition of standard and experimental diets are shown in Table 1.

Blood sugar and urine sugar were measured periodically every 9 days during research period, while ammonia content of inhaled air were measured before injection (day 0), 27 and 45 days after injection. At the end of experiment, femur muscle was removed to be used for determination of muscle protein content.

Table 1. The composition of standard and experimental diets.

Ingridient	Standard diet (g/kg diet)*	Soybean diet	SPC diet
Cornstarch	620.69	547.49	604.46
Casein	140	0	0
Soybean	0	298.61	0
SPC	0	0	192.18
Sucrose	100	100	100
Soybean oil	40	0	0
CMC	50	19.95	59.28
Mineral mix.	35	19.62	29.75
Vitamin mix.	10	10	10
L-cystine	1.8	1.8	1.8
Choline bitartrate	2.5	2.5	2.5
Total	999.99	1000	1000

*Reeves *et al*., (1993)

RESULT AND DISCUSSION

A. Proximate analysis of soybean and soybean protein concentrates.

The composition of soybean and soybean protein concentrates are presented in Table 1.

Table 2. Composition of soybean and soybean protein concentrates

Composition	Soybean	Soybean protein concentrates
Carbohydrate (% db)	24.51	8.44
Protein (% db)	39.85	61.92
Fat (% db)	22.82	19.61
Water content (% wb)	7.98	7.99
Ash content (% db)	5.15	2.73

Result indicated that soybean and soybean protein concentrates contain protein by 39.85 % and 61.92 % respectively. Protein content of soybean protein concentrates was higher than soybean, because during extraction the other component were removed.

B. Ammonia Content of Inhaled air

Ammonia content of inhaled air of diabetic rats and control group are shown in Table 3.

Table 3. Ammonia content of inhaled air

Diet Treatment	Concentration of ammonia (ppb)		
	Day 0	Day 27	Day 45
Alloxan – Standard	0	0	18
Alloxan-Soybean	0	0	0
Alloxan-SPC	0	0	0
Standard	0	0	0
Soybean	0	0	0
SPC	0	0	0

Ammonia was only detected in group of injected alloxan fed with standard by 18 ppb. This result indicated that protein degradation was higher in alloxan-induced diabetic rats feed with standard diet.

C. Blood sugar and urine sugar

Blood sugar and urine sugar of diabetic rats and control group are shown in Figure 1 and Figure 2, respectively.

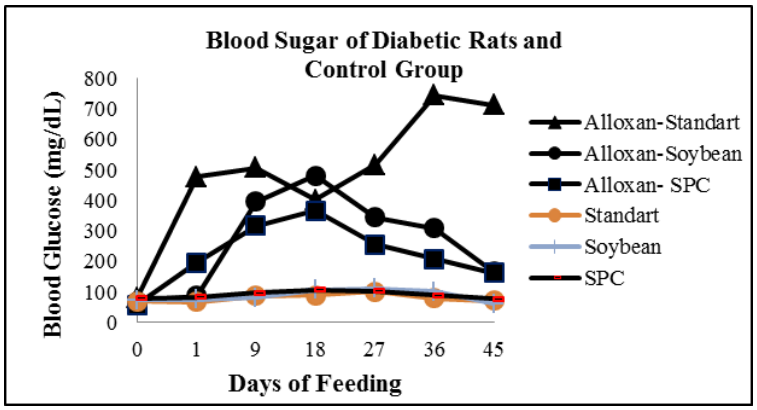

Figure 1. Blood sugar of diabetic rats and control group

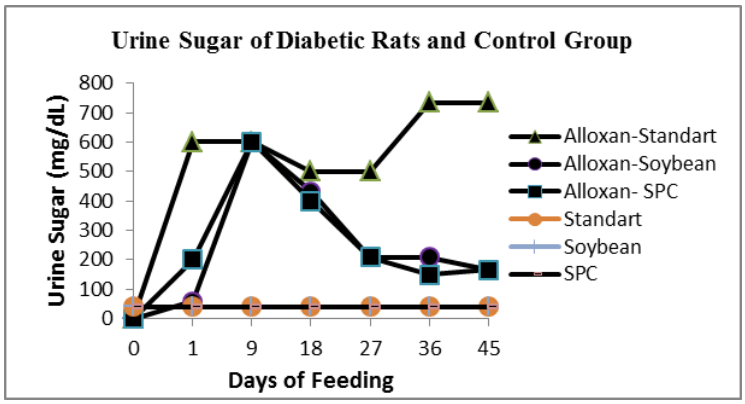

Figure 2. Urine sugar of diabetic rats and control group

Soybean and soybean protein concentrates diet decreased blood sugar content of diabetic rats from 481 mg/dL to 166 mg/dL and from 366 mg/dL to 162 mg/dl, respectively. Urinary glucose level of diabetic rats fed with soybean decreased from 600 mg/dL to 166 mg/dL and so did group of diabetic rats fed with soybean protein concentrates. This result indicated that soybean has hypoglycemic activity in alloxan-induced diabetic rats.

D. Muscle Protein Content

Muscle protein content of diabetic rats and control group are presented in Figure 3. Muscle protein content of diabetic rats fed with soybean protein concentrates was 91.73%, higher than group of diabetic rats fed with soybean and standard. This result indicated that soybean protein concentrates diet could inhibit muscle protein degradation in alloxan-induced diabetic rats.

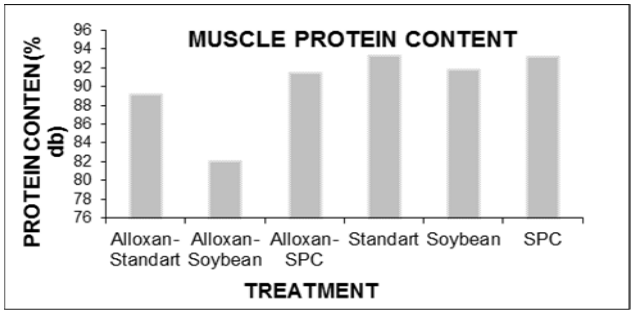

Figure 3. Muscle protein content

CONCLUSION

Result shown that soybean protein concentrates diet inhibited muscle protein degradation of diabetic rats better than those of soybean and standard diets.

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THE EFFECT OF CHLORINE CONCENTRATION ON THE VACUUM PACKED FRESH-CUT BAMBOO SHOOTS CHARACTERISTICS IN LOW TEMPERATURE STORAGE

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ABSTRACT

Bamboo shoot as fresh vegetable is not much different from other vegetables. They are perishable. The was applied Completely Randomized Design with factorial experiment pattern using chlorine concentration treatment as factor (1) consisting of 3 levels, i.e. 0, 100 and 200 ppm and storage duration as factor (2) consisting of 5 levels, each level was 0,1, 2, 3 and 4 weeks. The chlorine concentration of 200 ppm was appropriate for the vacuumed fresh-cut bamboo shoots stored in a low temperature ,i.e. giving the highest value for the brightness, for the hardness. The dominant compound found in the extract of vacuum packed fresh-cut bamboo shoot with chlorine concentration of 200 ppm stored in low temperature for 4 weeks is predicted to be acetic acid compound.

Keywords: Chlorine, Vacuum packed, Fresh-cut, Bamboo shoot, Low temperature

INTRODUCTION

Fresh bamboo shoots as vegetables have a perishable nature. Storability of fresh-cut bamboo shoots at room temperature, having only stored for 2 (two)days. Damage to shoots occurs starting from a brown discoloration, growth of fungi, slime and appears stinging smell like urine. Bamboo shoots are soaked with hipoklorin, indicating a lower microbial growth compared to controls. Bamboo shoots stored at 4 ^{0}C, can be stored over 25 days. Hopefully, soaking with chlorine and low temperature storage and vacuum packed with savings can extend the fresh-cut bamboo shoots.

MATERIAL AND METHODS

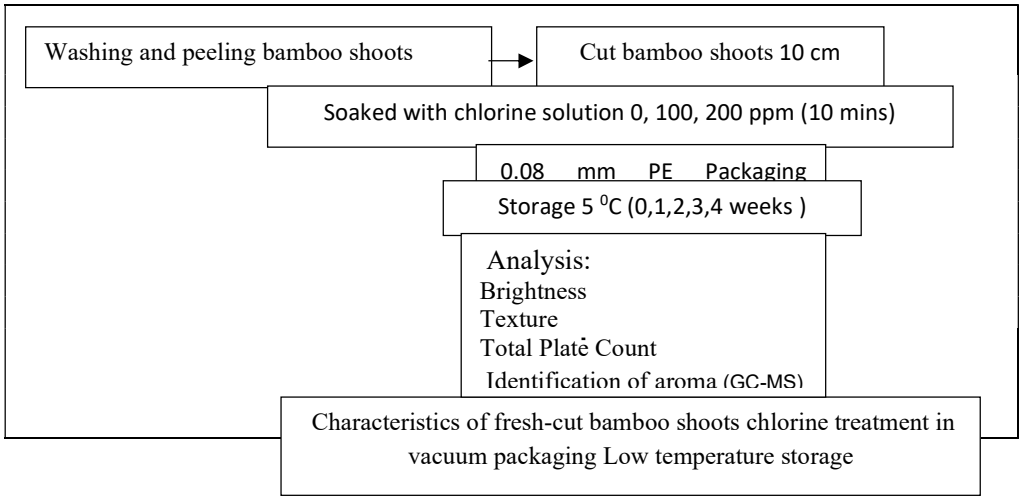
The main ingredients are bamboo shoots of bamboo species *Gigantochloa nigrociliata* Buese Kurz. Peeled and cut bamboo shoots at the end of length 10 cm, bright white color (Figure 1 a and 1 b). Then vacuum packed (Figure 2).


Fig. 1 a


Fig. 1 b


Fig. 2

This experiment using a randomized block design (RBD) factorial experimental pattern, with two factors: the concentration of chlorine (0 ppm, 100 ppm and 200 ppm) and storage time (0, 1, 2, 3, 4 weeks). The data obtained were analyzed by the method of analysis of variance (α = 1% & 5%).



RESULTS AND DISCUSSION

Brightness

Figure 4 shows the highest brightness on fresh-cut bamboo shoots in a concentration of 200 ppm chlorine vacuum packaged storage at low temperatures, due to the role of chlorine as well as sanitaiser, can suppress activity of PPO enzymes that cause changes in the brightness of the product.

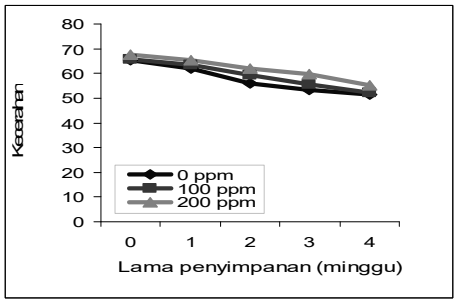


Fig. 4. Graph showing the relationship of storage duration and concentration of chlorine to the value of fresh-cut bamboo shoots brightness vacuum packaging the low temperature

Texture

Decline in value due to softening of texture on the cell wall products. One component of the cell wall is pectin, contained in fruits and vegetables in the form of an easy hydrolysed pectic substances. Pectic substances are found in the midle of the lamella cells of fruit and vegetables. The content of pectic substances in fruits and vegetables would affect the hardness (texture) of the product.

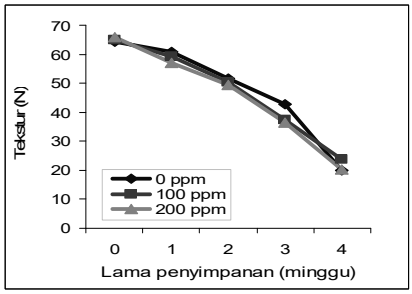


Fig. 5 Graphs the relationship of storage duration and concentration of chlorine to the value of fresh-cut bamboo shoots texture of vacuum packaging the low temperature

Total plate count (TPC)

Fig. 6 showed the highest TPC obtained on fresh-cut bamboo shoots in the immersion test 0 ppm chlorine concentration and packed vacuum that is equal to 3.64 log cfu / g, and the lowest TPC provided by the fresh-cut bamboo shoots in a soaking in 200 ppm chlorine concentration and the amount of vacuum packaged 3.21 log cfu / g. Chlorine (sodium hypochlorite) is the most powerful preservative and used as a disinfectant that will kill bacteria, viruses, mold and mildew.

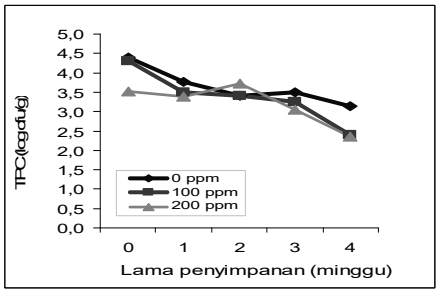


Figure 6. Graph showing the relationship of storage duration and concentration of chlorine on the TPC fresh –cut bamboo shoots low temperature vacuum packaging

Profiles compound fresh-cut bamboo shoot extracts with GC-MS

Figure 7 shows the results of gas chromatography chromatogram of the extraction of fresh bamboo shoots 92 peak chromatogram obtained. The highest peak at a retention time of 36 minute, 69 with an area of 20.12 percent is Hexadecanoic acid compounds. During the soaking process with chlorine, vacuum and storing changes in compounds that are formed.

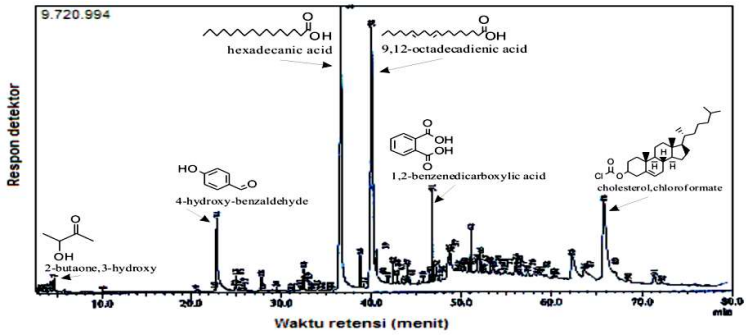


Fig 7. Extract aroma compounds profile fresh bamboo shoots.

Figure 8 shows that the extract aroma compounds profiles fresh-cut bamboo shoots in a concentration of 200 ppm chlorine vacuum packaging of low temperature storage of 4 weeks. The chromatogram obtained from the highest peak at a retention time of 2 minutes, 55 with an area of 32.87 percent which included acetic acid compounds.

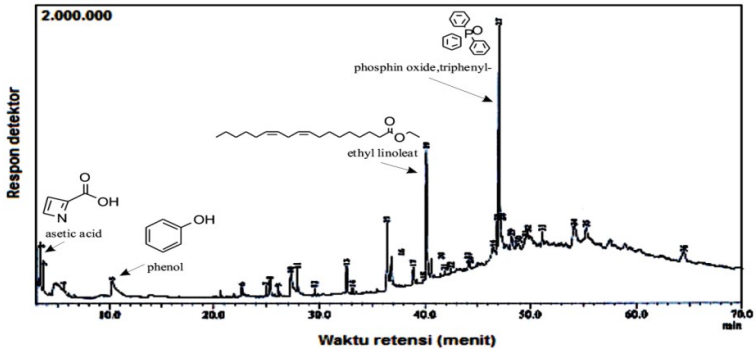


Figure 8. Extract aroma compounds profile of fresh-cut bamboo shoots in a concentration 200 ppm chlorine vacuum packaged

Differences in the number of peak chromatogram generated may be caused by physiological processes and activities of microbes. Physiological processes infresh-cut bamboo shoot is still on respiration. In the process of respiration will occur demolition macromolecules such as fats, carbohydrates and proteins Demolition of fatty acids in addition to being fat, then enter the citric acid cycle, also changed into glycerol which enter into a new process of glycolysis to the citric acid cycle.

CONCLUSION

Soaking with 200 ppm chlorine, vacuum packaging and storage at a temperature of 5^0C can preserve the brightness of color, texture and pressure decrease microbial growth.

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UV-A OXIDATION FOR CASSAVA STARCH AND ACIDIFICATION TO IMPROVE BAKING EXPANSION

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ABSTRACT

Cassava starch has low expansion ability in baking process and produce rough texture at the end result. Regarding to these, it is considerable to modify cassava starch to raise its expansion ability for baking process. One of common way to modify cassava starch is by using UV irradiation. Modification using UV were arranged in constant state along the irradiation. In this research we use UV-A. Submersion of the starch in 1% lactic acid for 30 minutes were conducted prior to the UV-A irradiation. The UV-A irradiation time varied in 7, 8, 9, 10, and 11 hours. The water content variation used are 12.5%, 20%, 30%, 40%, and 53%.The result shows that the best modification for cassava starch by using UV-A irradiation was reached after irradiation for 10 hours after the starch were submersed in 1% lactic acid for 30 minutes and the water content along the irradiation was 40%. The baking expansion ability value was 10.20 ml/g, with analysis results include: the force needed to crush the end product is 22.29 N, paste viscosity peak of 371 centipoise, from FTIR result shows that starch are having carbonil compound and carboxil compound in wave number 1651 cm-1.

Keywords : cassava starch, oxydized starch, baking expansion, UV-A

INTRODUCTION

Wheat demand in Indonesia keeps increasing from 3.4 million tons in 2005 to 3.7 million tons in 2006 (Suarni, 2009). In order to reduce importing dependency, a substitution to other source of grain instead of wheat is need to be concerned. The sunlight is identified as oxydation enhancer in specified wave-length that helps the fermentized starch to lift up while baking process. This was caused by UV light in sunlight used to dry the starch (Demiate *et al.,* 2000). Baking expansion rate of oven-dried starch is lower than ones that was dried by sunlight (Plata-Oviedo *et al*., 1998). Oxydative degradation by sunlight exposure is considered as the cause of chemical structure changes in cassava starch that leads to the expansion baking rate (Demiate *et al.*, 2000).

Through this research, a modification effort is conducted by using UV-A ray dan lactic acid submersion. The submersion is done prior to the exposure to the UV-A ray to increase the baking expansion rate. UV-A ray is used as the substitution of UV by sunlight. Lactic acid submersion with 1% concentration is used as the substitution of lactic acid in fermentation. UV A exposure is utilized by using modified UV exposing unit that is set to maintain the exposure in constant rate.

This research is held primarily to identify the maximum rate of baking expansion of cassava starch that was exposed by UV-A ray and in justified water content along the exposure. Secondly, it is considerable to identify the starch characteristic having maximum baking expansion rate.

MATERIALS AND METHODS

Materials

Prime material used for the research is manufactured cassava starch, common cassava starch, laboratory starch, and lactis acid (1%). Manufactured starch is factory-made starch using oven for drying process. Common cassava starch is made by common

people in small scale/ household industries using sun-drying and fermentation is already occurred prior to drying process while it is sedimentated. Laboratory starch is dried using cabinet dryer.

Method

Manufactured-starch modification using UV-A exposure is done to have the highest baking-expansion rate starch. Lactic acid (1%) submersion is done based on the former research done by Vatanasuchart *et al*. (2005) and Demiate *et al*. (2000), with submersion time justification. Manufacture-starch (200 g, db) is dispersed in 600 gram of 1% lactic acid solution (m/m). The submersion is done in 30 minutes by 25 ^{o}C ambience temperature prior to the UV-A exposure. Starch sample is exposed to the UV-A with 4 mm thickness in the sample plate to have optimal UV ray penetration.

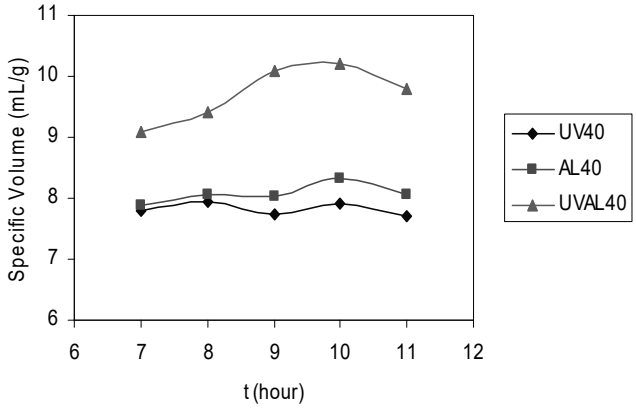
UV ray exposion is done in arranged moisture content and exposure duration. Moisture content varies at 12.5% (dry starch), 20%, 30%, 40%, and 53% (starch sediment moisture content). Moisture content arrangement is done by using cabinet dryer. The exposure duration is varied from one to eleven hours.

Baking expansion rate measurement is done based on the method used by Demiate *et al*. (2000) with few modifications. Manufactued-starch weighed 10 gram stirred with 30 ml aquadest is processed in the waterbath until the gelatinitation is occurred. Later, the sample is divided in 3 portions to be baked in 200 ^{o}C temperature for 25 minutes. After the baking process, we could measure the baking expansion rate defined in specific volume (ml/g).

RESULTS AND DISCUSSIONS

Baking Expansion Value Measurement

The results show that cassava starch exposed by UV A ray with 40% moisture content (after 1 % lactic acid submersion) is having the higher baking expansion value than ones that was not submersed in lactic acid prior to the exposure. Cassava starch exposed by UV A ray with 40% moisture content (after 1 % lactic acid submersion) is having the higher baking expansion value than ones that was submersed in 1% lactic acid solution without exposure to UV A ray. This indications leads to conclusion that lactic acid submersion and UV ray exposure have a significant effect due to cassava starch oxydation.



Notes:

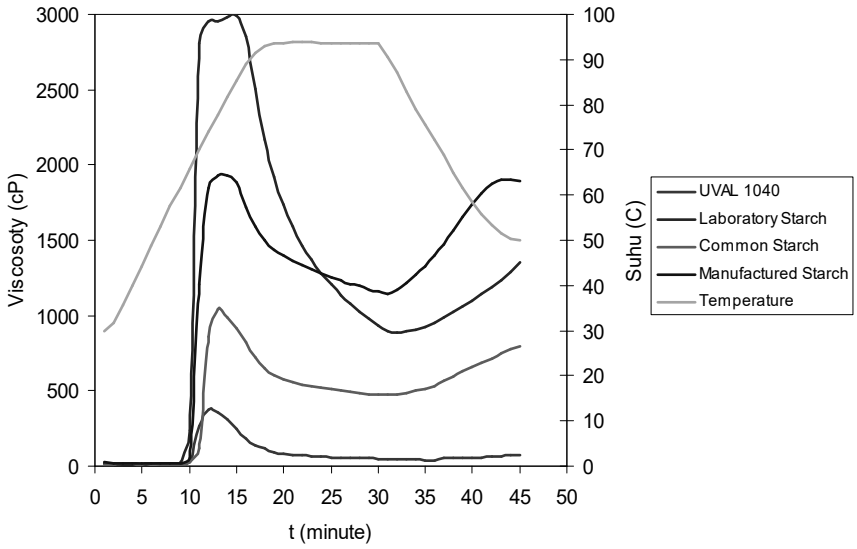
UV 40: cassava starch exposed by UV A ray with 40% moisture content (without 1 % lactic acid submersion); AL 40: cassava starch submersed in 1% lactic acid solution without exposure to UV A ray; UVAL 1040: cassava starch exposed by UV A ray with 40% moisture content (after 1 % lactic acid submersion).

The results show that the expansion capability of manufactured-starch could be improved by UV A exposure for 10 hours with 40% moisture content along the exposure (the sample already submersed in 1% lactic acid solution). We could see it in the table below that this sample is having higher baking expansion value than common starch.

The Best Modified Starch Characteristic

Cassava starch oxydation which increases the baking expansion value is affecting the cassava starch characteristic. This change is assumed as the factor that enhance the baking expansion. To identify the changes that occurred for cassava starch oxydized along UV A ray exposure, we proceed some analysis.

Starchs	Analysis			
	Hardness (N)	Amylose (%)	Viscosity (cP)	FTIR (%T)
Common starch	57,48 (a)	27,85 (a)	1043,00	32,25
Manfactred starch	60,13 (a)	25,91 (b)	1933,00	17,50
Laboratory starch	44,26 (b)	18,63 (c)	2995,00	-
UVAL 1040	22,30 (c)	29,77 (d)	371,00	16,00
UV 1040	40,00 (d)	-	-	-
AL 1040	52,43 (e)	-	-	-



Viscoamilograms of native and modified starches

CONCLUSIONS

From the results and discussions, we could pull the roots together from this research into some conclusions as follows :

1. Cassava starch samples which have the highest baking expansion value are ones that exposed by UV A for 10 hours with 40% moisture content and submersed in 1% lactic acid solution for 30 minutes prior to the UV A exposure. This sample was mentioned as UVAL 1040.
2. Baking expansion value of UVAL 1040 is 10.20 ml/gram, force needed to crush the sample product is 22.29 N, amylose content is 29.77%, and pH sample is 2.41. UVAL 1040 sample is having lower paste viscosity compared to other starch samples which has maximal paste viscosity for about 371 cP, FTIR test shows that UVAL 1040 is having carbonyl and carboxyl compound by wave length of 1651 cm^{-1} with 16% transmittance, and the observation of granular structures shows that UVAL 1040 was having size reduction if we compare it to the manufactued-starch samples before the modifications is implemented.

ACKNOWLEDGMENT

I would like to thank firstly to Prof. Ir. Haryadi, M. App. Sc whose support and counseling played a great role to this research. Secondly, I'd like to thank Dr. Ir. Nursigit Bintoro who gave counseling towards the research's improvements.

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EFFECT OF METHANOL EXTRACT OF JACKFRUIT WOOD (*ARTOCARPUS INTEGRA* MERR) ON THE GROWTH OF MICROBES DETERIORATING ARENGA PALM SAP DURING STORAGE

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ABSTRACT

Jackfruit wood is one of the plant materials which traditionally has been used as palm sap preservative by arenga palm sugar farmers. In the study of antimicrobial activity of jackfruit wood extract using disc diffusion test showed that methanol extract of jackfruit wood inhibited the growth of main spoilage bacteria found in arenga palm sap, i.e. *Leuconostoc mesenteroides* and *Lactobacillus plantarum*. The aim of this research is to find out the capability of jackfruit wood extract to slow down the growth of microbes that deteriorate palm sap during storage. The result showed, treatment of 500 ppm jackfruit wood extract could slow down the growth of lactic acid bacteria in arenga palm sap during storage, but could not inhibit the growth of yeast. The combination of jackfruit extract and lime gave hurdle effect on palm sap preservation.

Keywords: jackfruit, extract, methanol, palm sap

INTRODUCTION

Palm sugar farmer at rural area traditionally has used natural preservative from plant materials for preventing palm sap from deterioration during taping. Jack fruit wood is one of the plant material that is used frequently. Hamzah and Hasbullah (1997) repealed, the treatment of jack fruit wood of 4 g/L on palm sap during taping produced palm sap with pH of 5.82, whereas the control produced palm sap with pH of 5.00. That indicated the jack fruit wood inhibited activity of spoilage microorganism in the palm sap.

The deterioration of palm sap during taping is commonly caused by spoilage microbes activity. Spoilage microbes is commonly found in spoil palm sap are fallen within bacteria and yeast. Paparusi and Bassir (1972) and Intermediate Technology Development Group (2004) repealed, the dominant spoilage microbes found in spoilage palm sap were *Saccharomyces cerevisiae*, *Leuconostoc mesenteroides* dan *Lactobacillus plantarum*. The other microbes found were *Schizosaccharomyces pombe*, *Pichia* sp. and *Micrococcus* sp.

Antimicrobial study using disc diffusion test on methanol extract of jackfruit wood showed, the extract had antimicrobial activity against *Leu. Mesenteroides* and *L. plantarum* (Putra, 2008a). GC-MS analysis showed, the extract contained compounds, i.e. *9,19-Cyclolanost-23-en-3,25-diol* and *4,4-dimethyl Cholestan-3-one* that were presumed having antimicrobial activity. The compounds are belong to steroid derivate (Putra, 2008b).

The aim of this study was to find out the ability of jackfruit wood extract (JWE) to inhibit the growth of spoilage microorganism in arenga palm sap during storage.

MATERIALS AND METHODS

Jackfruit wood and arenga palm sap were obtained at Tabanan regency. The wood were grinded and dried using vacuum drier at 50 ^{o}C and pressure of 62 cm Hg for 4 hours. The grinded wood was sieved using 40 mesh sieve then the dried jackfruit wood powder were obtained.

Jackfruit wood extraction was carried out as follow. Twenty grams of jackfruit wood powder was extracted with 100 ml methanol by means of maceration along with magnetic stirrer stirring for 3 hours. Furthermore, the filtrate was filtered by Whatman paper No. 4, and No. 1 respectively. The filtrate was dried by *rotary vacuum evaporator* at 45 °C until all of solvent was removed, then JWE was obtained.

The study of jackfruit wood extract effect on the growth of spoilage microbes in arenga palm sap during storage was done as follow. Fifteen milliliters arenga palm sap were placed in sterile test tubes, and then the treatments (0 ppm JWE, 500 ppm JWE, and 500 ppm JWE + 2000 ppm lime) were applied. The arenga palm sap was stored at room temperature for 18 hours. During storage, lactic acid bacteria (LAB) and yeast were observed every 6 hours.

RESULTS

The growth of LAB

The effect of JWE on the growth of LAB in arenga palm sap during 18 hours storage is showed on Figure 1. During 18 hours storage, the LAB in control increased from 5.2 log cfu/ml to 6.82 log cfu/ml (31.15%), whereas in JWE treatment, the LAB increased from 5.19 log cfu/ml to 5.31 log cfu/ml (2.31%). This indicated the growth of LAB on JWE treatment was lower than the growth on control, resulting from the effect of antimicrobial activity of JWE.

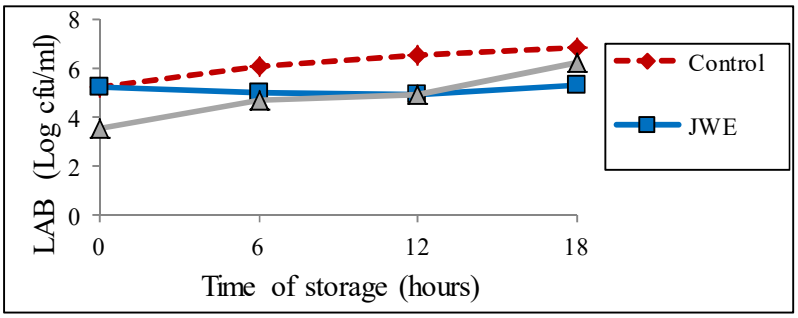

Figure 1. The growth of LAB on arenga palm sap during storage

The growth of Yeast

The effect of JWE on the growth of yeast in arenga palm sap during 18 hours storage is showed on Figure 2. The result showed, JWE treatment did not inhibit the growth of yeast in arenga palm sap. The combination of JWE and lime of 200 ppm only inhibited the yeast growth until the storage of 12 hours.

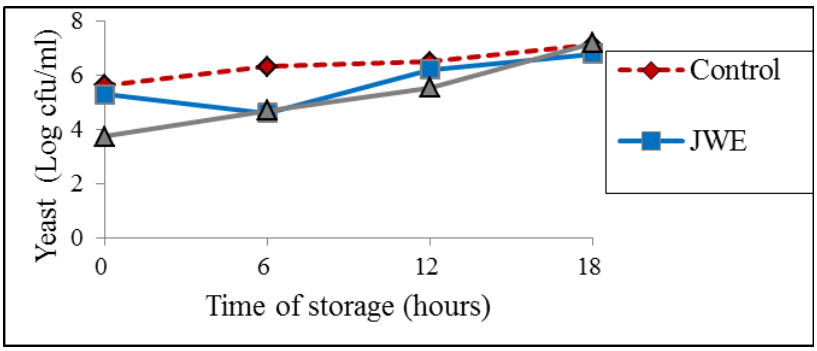

Figure 2. The growth of yeast on arenga palm sap during storage

The change of pH during storage

The profile of arenga palm sap pH during storage of 18 hours was showed on Figure 3. JWE treatment restrained arenga palm sap pH decreasing during storage. During 18 hours storage, the pH on control decreased from pH of 5.24 to pH of 4.10, whereas on JWE treatment, the pH decreased from 5.23 to 4.95. The decreasing of pH indicated increasing of organic acid that was liberated by LAB.

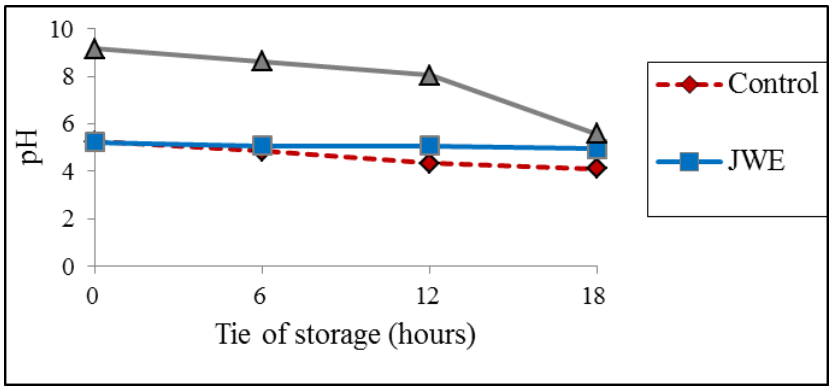

Figure 3. Profile of arenga palm sap pH during storage

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ETHANOL PRODUCTION FROM ACID HYDROLYSATE CASSAVA FLOUR WITH MIXED CULTURE *Trichoderma viride* AND *Saccharomyces cerevisiae*

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ABSTRACT

The objective of this research was to produce ethanol from acid hydrolysate cassava flour with mix cultured *Tricoderma viride* and *Saccaromyces cerevisiae*. The hydrolysis of cassava flour to glucose was conducted by 0.4 M sulfuric acid using autoclave at 121 ^{o}C, pressure at 1 atm for 10 min. The fermentation were performed in batch system for 96 hours in 30 ^{o}C. Mixed culture of *T. viride* and *S. cerevisiae* in the fermentation process of acid hydrolisate carried out in two methode that is gradually and simultaneously. The ethanol concentration 3.92 ± 0.31 % (w/v), yield 15.99 % (v/w) and fermentation effciency 49.98 % of the theoretical value was achieved using simultaneously addition of mixed culture, while gradually addition of mixed culture was produced ethanol concentration 5.35 ± 0.97 % (w/v), yield 21.82 % (v/w) and fermentation efficiency 46.557 % of the theoretical value.

Keywords: ethanol, cassava, mixed culture

INTRODUCTION

Casssava usually as a bioethanol material only use starch component, whereas, cassava not only starch content but also cellulosic content that it has potential material as a sugar sources. Most production process concerned with the conversion of starchy materials into ethanol have three steps, liquification of starch, enzymatic saccharification and fermentation of sugar to etanol. Utilization of comercial enzyme is an expensive process for the production of alchohol from starchy material.

Cellulases are a group of hydrolytic enzymes capable of degrading cellulose to the smaller glucose units. These enzymes are produced by fungi and bacteria. *Trichoderma viride* is a filamentous soil fungus known to be an effective produce cellulolytic enzyme. Starch fraction in cassava is widely used in bioethanol produce. Most often, the enzymatic hydrolysis of starch carried out with the enzyme α-amilase and amiloglucosidase. Chemical hydrolysis, especially acid hydrolysis of starch, is one of a number of technologies being depeloved as a starch conversion process to glucose. Chemical hydrolysis given advantages short residence time than enzymatic hydrolysis. In this research, cassava starch was hydrolyzed by sulpuric acid. Acid hydrolysates was fermented with mixed culture *T. viride and S. cerevisiae.* Fermentation was combined the cellulase enzymes and fermenting microbes in one vessel. This enabled a one-step process of sugar production and fermentation into ethanol. The objective of this research was to produce ethanol from acid hidrolysate cassava flour with mix cultured *Tricoderma viride* and *Saccaromyces cerevisiae*.

MATERIALS AND METHODS

Raw material

Fresh cassava was collected from Sukabumi, West Java. It was sun dried and milled to uniform size (40 mesh). *S.cerevisiae*, and *T.viride* was collected from microbiology laboratorium PAU IPB. Cassava flour was analysed to determine chemical compotition before it is used to fermentation process. Analysed that is moisture, fat, protein, carbohydrate and crude fiber contents.

Inoculum culture *T.viride* and *S.cerevisiae*

The strains *T.viride* were used in this work. Stock cultures were maintained on potato dextrose agar (PDA) and cultivation for 5 days at 30 ^{o}C. Properly sporulated cultured were used for inoculation. The strain *S.cerevisiae* were maintained on potato dextrose agar (PDA). The medium composition for cultivation was as follow: yeast extract 5 g/L, malt 5 g/L, glocose 10 g/L and peptone 5 g/L. Cultivation was conducted in 250 ml Erlenmeyer flask for 48 hours at 30 ^{o}C and agitation 125 rpm.

Cellulase Production

The composition of medium for cellulose production by *T.viride* was 14 ml $(NH_4)_2SO_4$ 10 %, 15 ml KH_2PO_4 1M, 3 ml urea 10 %, 3 ml $CaCl_2$ 10 %, 3 ml $MgSO_4$. $7H_2O$ 10 %, 2 ml Tween 80 and 1 ml trace mineral. The composition of trace mineral was 495 ml aquades; 5 ml HCl; 2.5 gram $FeSO_4$; 0.89 gram $MnCl_2$. $4H_2O$; 1.76 gr $ZnSO_4.H_2O$; 1.25 gr $Co(NO_3)_2.6H_2O$. For cellulase production, 10 % of *T.viride* spores (1,58 x 10^9/ml) were inoculated into medium and incubated at 30 ^{o}C for 7 days under aerobic condition and agitation 250 rpm. The "onggok" was used as carbon sources and added aseptically to mineral media. Cellulase production conducted in erlenmeyer flask 500 ml with 250 ml working volume. The cellulase activity was measured at the end of time fermentation process.

Hydrolysis and fermentation

Before fermentation process, cassava flour was hydrolysed to glucose by 0,4 M sulfuric acid using autoclave at 121 ^{o}C, pressure at 1 atm for 10 min. For fermentation process, the broth containing the fungal *T.viride* biomass and the cellulolytic enzyme was used to inoculate anaerobic ethanol fermentation medium. *Saccharomyces cerevisiae* were transferred from stock culture broth to 1000 ml erlemmeyer flask containing 500 ml acid hydrolisate as a substrate. The fungus and yeast culture broth were aseptically transfrred to ethanol fermentation flask with concentration 10% v/v, respectivelly. Mixed culture of *T.viride* and *S.cerevisiae* in the fermentation process of acid hydrolisate carried out in two methods that is gradually and simultaneously. Fermentation process was conducted in batch system at 30 ^{o}C for 96 hours. Sample were withdrawn after 0, 6, 12, 18, 24, 36, 48, 72, 96 hours and analysed to change of total sugar and pH substrate.

Analysis methods

The enzyme extract was centrifuged at 10,000 rpm and 4^{o}C for 10 min, and the clear of supernatant was used for enzyme assay. Filter paper activity were determined according to standard international Unit of Pure and Applied Chemistry (IUPAC) procedures (Chen et al., 2006). Filter paper activity was assaays by incubating a mixture containing a strip of Whatman no.1 filter paper (1 x 6 cm) immersed in 1ml of citrate buffer and 0,5 ml appropriatelly enzyme solution at 50 ^{o}C for 30 min. One unit of filter paper activity (FPU) is defined as amount of enzyme that forms 1 µmol glucose (reducing sugar) per minute under the assay condition. Reducing sugar was determined using the 3,5-dinitrosalicylic acid (DNS) method (Chen et al., 2006).

Total sugars was assays by the phenol-sulfuric acid method. The pH of substrate assays by pH-meter. Ethanol concentration was analysed by gas chromatography (GC) The ethanol concentration was analised at the end of time fermentation process. Fermentation and substrate effisiensi and yield was measured according to formulation: Fermentation efficiency (%) = (ethanol actual/ethanol teoritic) x 100% ; yield (% v/b) = (volume ethanol actual/ weight of cassava flour) x 100 %; Substrate efficiency (%) = (the substrate was consumed/initial substrate) x 100 %.

RESULTS AND DISCUSSION

Proximate analysis showed that the contents of cassava flour was 8.65 ± 0.10 % moisture, 2.55 ± 0.14 % ash, 6.54 ± 0.02 % fat, 1.81 ± 0.03 % proteine, 2.69 ± 0.04 % crude fiber and 62.54 ± 0.00 % starch. Crude fiber content 69.98 % hemicellulose and 13,44 cellulose. The moisture content of the substrate has a major impact on how long it can keep in the storage and still remains nutritions. Padonou *et al.* (2005) was reported that cassava flour content 0,56 % fat (wb).

Trichoderma viride was grown under aerated conditions for biomass and cellulase production. The latter group of enzymes is necessary for cellulose saccharification prior to ethanol bioconversion (Crhristakopoulus *et al.,* 1993). The production of cellulase with "onggok" as a carbon sources were examined. The result of the research showed that *T.viride* was able to produce crude enzyme cellulase with activity 4.77 ± 0.72 FPU/ml.

The result of hidrolysis cassava flour with sulfuric acid showed the concentration of total sugar and glucose was 38,93 ± 8,09 and 22,04 ± 4,31 (% w/v), respectivelly. In fermentation process, Figure 1 demonstrated the decrease in total sugar concentration and pH during gradually process. Total sugar concentration decrease from 340,29 ± 10,49 g/L to 24,81 ± 7,09 g/L after 96 h, whereas pH decrease from 5,01 ± 0,01 to 3,93 ± 0,10. Total sugar concentration rapidly decrease between 18 h and 24 h of fermentation time.

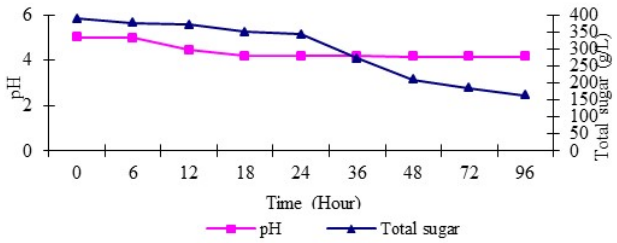

Figure 1. Pattern change of total sugar concentration and pH during gradually process.

Figure 2 demonstrated the decrease in total sugar concentration and pH during simultaneously process. Total sugar concentration decrease from 383.08 ± 3.70 g/L to 277.94 ± 17.29 g/L % after 96 h, whereas pH decrease from 5.01 ± 0.01 to 4.27 ± 0.02. Total sugar concentration rapidly decrease between 18 h and 24 h of fermentation time.

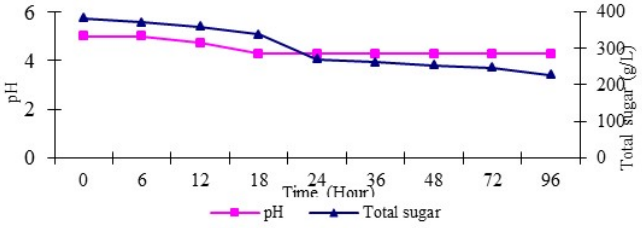

Figure 2. Pattern change of total sugar concentration and pH during simultaneously process.

During fermentation process was accured decrease total sugar concentration that it was followed by decrease pH of substrate. It indicated that the substrate accommodated the microorganism growth and produce ethanol. The decrease pH of substrate might due

to the ionitation of NH_4SO_4 that is used as nitrogen sources on fermentation process. *Saccharomyces cerevisiae* was used NH_3 as a nitrogen sources and liberated H^+ into substrate solution. Accumulation of H^+ given occasion to decrease substrate solution.

The culture *T. viride* and *S. cerevisiae* in gradually process able to produce ethanol 5.35 ± 0.98 % (w/v), whereas, the simultaneously process was able to produce ethanol 3.92 ± 0.31 % (w/v).

Table 2. Fermentation parameters of ethanol production from cassava for each treatment process

Treatment	Substrate efficiency (%)	Fermentation efficiency (%)	Yield (% v/w)
Gradually	57.83	46.56	21.82
Simultaneously	40.50	49.49	15.99

The maximum ethanol yield obtained at gradually process was 21.82 % (v/w), corresponding 46.56 % of theoretical yield. The gradually process gave higher concentration of ethanol than simultaneously process. The result of effisiensi substrate, fermentation and yield during production process for each treatment process represented at Table 2. Ethanol production by co-culture amylolytic yeast and *S. cerevisiae* in starch substrate was produced ethanol concentration 6.0 % (w/v), corresponding 93 % of theoritical yield. Suresh et al. (1998) was reported that simultaneoulsy saccharification fermentation can be conducted efficiently by using a co-culture of amilolytic *A. niger* and a non-amylolytic sugar fermenter *S. cerevisiae*.

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EVALUATION OF LYMPHOCYTE PROLIFERATION ACTIVITY OF MILLET (*Pennisetum* sp.) ON SPRAGUE DAWLEY RATS

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ABSTRACT

Millet is cereal crop that would be useful for alternative sources in food diversification programme. It has protein content more than rice and has prospect to be developed in Indonesia. Recently demand of food industry is not only nutrition concern but also for health. The result of many prior research showed that millet had bioactive compounds that function useful for health, such as to decreased degenerative diseases risk. In vitro study of millet showed that this cereal could increase lymphocyte cell proliferation. The objective of this research was to study biological potency of millet on lymphocyte cell proliferation in vivo using rats. Three groups of rats included: control, rat fed 50% and 100% rat fed of millet, sources of carbohydrate. Results of this research showed that rat fed 50%, 100% of millet showed increase proliferation activity of lymphocyte cells by 53%, 57% respectively. The conclusion of the research that millet was good for health maintenance. It increases system immunity.

Keyword: millet, proliferation lymphocyte, rats.

INTRODUCTION

Consumption of food and food derived from plant is expected to increase a long with awareness of health maintenance. It's related to recommendation by WHO on diet for prevention of cancer and other degenerative diseases. One of food from plant is use to main source of carbohydrate in Indonesia such as rice. Demand of the rice always increases every year. Indonesia has a food diversification programme. Millet has potential of alternative carbohydrate source. The result of many prior research showed that millet had bioactive compound that had potential of benefit health. Millet had phenol acid such as ferule acid, vitamin source such as A, D and mineral source such as Fe, Ca, Zn, and Mg that had potential of antioxidant sources. Millet is important grain originated from East Africa. Consumption of millet in Africa is used to important source of traditional food and In America it familiar as beer (alcohol drink).

Consumption of Millet in Indonesia is not familiar yet as food and food derived. Millet is familiar as food of bird. Kind of Millet in Indonesia is pearl millet, finger millet and foxtail millet. To improve of the millet consumption as food important information additional about potential of benefit health such as immunomodulator potency. The prior in vitro research showed millet had potential of immunomodulator. To improve of the in vitro research of immunomodulator potency of millet important in vivo research with model rats. Potential of immunomodulator of millet is used model rats can research by lymphocyte proliferation activity. Lymphocyte proliferation activity is indicator to improve quantity of lymphocyte cell (B cell. and T cell). According Zakaria et al. (2003) Lymphocyte proliferation activity is illustration of lymphocyte function and individual immunity status. Lymphocyte proliferation responds from mutagen or antigen action.

MATERIALS AND METHODS

Materials

Millet (*pinnesitum sp.*) from bird market (Bogor), standard fed of rats (IAN 1976), Sprague Dawley, aquadest water, sterill destilat water, alcohol 70%, alchohol 90%, phosphate buffer salin (PBS) (Aplichem A09649010), RPMI (Roswell Park Memorial Insitute) 1640 (Gibco, 22400-013), NaHCO3 (Sigma, S5763), NH4CL (Merk, A810845),

penicilin-streptomisin (Sigma), tryphane blue, Lipopolisakarida (LPS) *Salmonella typhimurium* (Sigma, L6366), Fetal Bovine Serum (FBS), MTT (3-(4.5-dimethylthiazol-2 yl)-2.5 diphenyl-tetrazolium bromide) (Sigma, M2128), HCL pekat, Isopropanol, gas CO_2.

Satake Grain Testing Mill, dish mill, sterill laminar hood, sentrifuse, hemasitometer, microscope, microplate weel, CO_2 incubator, Elisa reader, sterill membrane 0.22 µm

The research used model rats. There are three groups of rats included 1) control group (standard rat fed) (KO); 2) 50% rat fed of millet flour of carbohydrate source (JS); 3) 100% rat fed of millet flour of carbohydrate source (JP). Each of group is consisted seven rats, so there are 21 experiment units.

Preparing of suspension of lympocyte cells

Spleen organ of Sprague Dawley put on bottle that had consist PBS, than added 5 ml RPMI that had consist antibiotic and broke to be homogen after that put on sentrifuge tube than sentrifuge at 2500 rpm, in 10 minutes, next took pellet and added 2 ml NH4CL 0.85%, stand by in 2 minutes, than added 3 ml RPMI next sentrifuge at 2500 rpm, in 5 minutes, after that pellet washed by 5 ml RPMI two times than added 3-5 ml RPMI to got suspension of lymphocyte cell.

Amount of lymphocyte cells

Amount of lymphocyte cell on suspension of lymphocyte cell determined by tryphane blue. 50 µL suspension of lymphocyte cell put on micro plate weel than added 50 µL tryphane blue (1:1), next amount of lymphocyte cell by hemasitometer at 400 times of zoom. Amount of lymphocyte cell on living cells. Living cell has performance transparant, clear and ball shape. Living cells on suspension of lymphocyte cell as follow:

N = V/2 x F X 104 cell/ml
Notes:
N = Amount cell/ml
V/2 = Avarage amount of cell from 2 view side
F = ...factor (2)
104 = amount of cell/view area(1.0 mm x 1.0 mm x 0.1 mm)

Lymphocyte proliferation activity

Suspension of lymphocyte cell determined 2 x 106 cell/ml by added RPMI than cultured with weel microplate (96 weel). 60 µL suspension of lymphocyte cell put on weel microplate and added 30 µL mitogen (S. Thyphi) and made control by replace mitogen with RPMI. Every suspension of lymphocyte cell was made triplo after that added 10 µL FBS, then cultured in incubator at 5% CO2, 37 oC in 72 hours, four hours before finish culture, added 10 µL MTT 0.5% after finish culture added 80 µL HCL-Isopropanol 0.04 N next absorbance was acounted by microplate reader (ELISA reader) at λ 570 nm. Optical dencity (OD) value proposionaly with amount of living cell through index stimulus (IS). Lymphocyte proliferation activity determined by IS. IS acounted by formula as follows:

IS = OD treatment cell /OD control cell

Disign of research is completed radomized design. Data analysis used analysis of variance (ANOVA) (SAS release 6.12). Continue test used Duncan test. Prosedur of research could see at Figure 1,

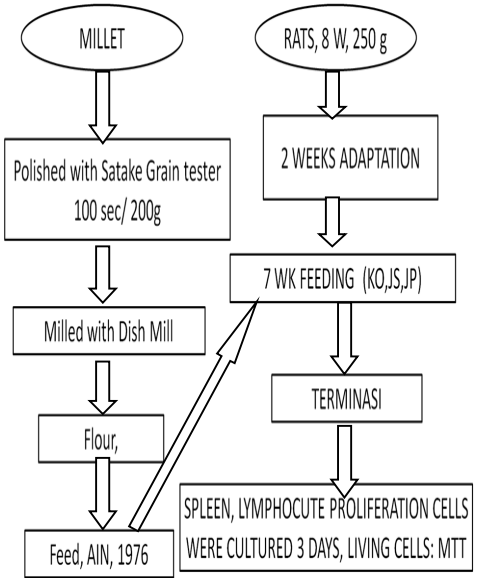

Figure 1. Procedure of research

RESULTS AND DISCUSSION

Rat Growth

Rat growth during 60 days of feeding showed to increases (Figure 2). This indicator of all rats were well. Feeding from 50% or 100% of millet no negative effect for health. Rat growth on 100% of millet could see more well than 50 % of millet and control (standard feeding), so the millet could change standard carbohydrate source on model rats.

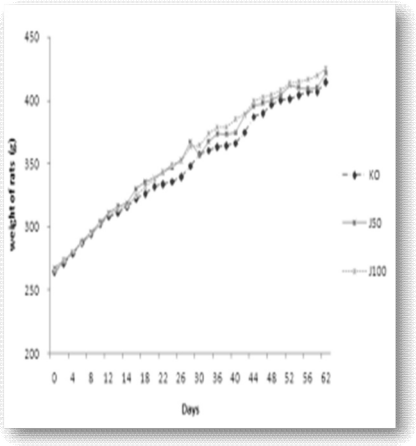

Figure 2. Rat growth during 60 days of feeding

Lymphocyte Proliferation Activity

Lymphocyte proliferation activity could see index stimulus (IS) value (Figure 3)

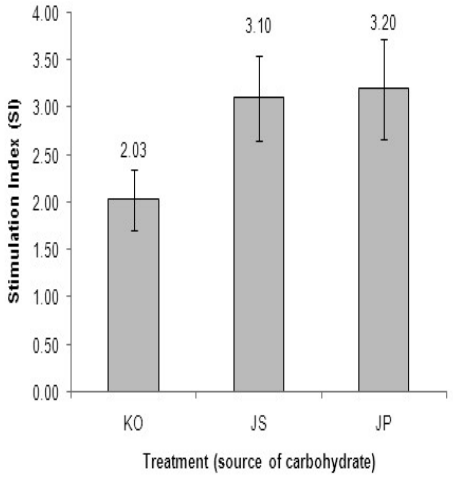

Figure 3 Index stimulus suspension of lymphocyte cells.

Index stimulus (IS) determine proliferation cell. Figure 3 showed IS of lymphocyte cell on spleen organ of Sprague Dawley rats. Lymphocyte proliferation activity on 50% and 100% of millet more low than control. The control group had the lowest IS value. It's 2.03 value. The highest IS value on 100% of millet. It's 3.20 value. The result of statistic analysis between 50% of millet with 100% millet no significant difference (P>0.5). Consumption of carbohydrate source from millet on Sprague Dawley rats could improve lymphocyte proliferation activity. Increasing of lymphocyte proliferation could trigger or created more antibody to respond antigen or mitogen. Millet had indicator on immunomodulator function.

Millet could increases IS value or lymphocyte proliferation. It had antioxidant compound such as ferulic acid, A,D vitamins, Zn and Mg mineral. Properties of antioxidant could control oxidant or free radical to became non radical. The free radical such as hydrocyl radical could break lymphocyte cells, so they could not act such as proliferation cell. IS of the control group more low than 50 % and 100% of millet. It had not antioxidant compound to protect lymphocyte proliferation activity. The best result on 100% of millet

CONCLUSION

Consumption of carbohydrate source from millet on Sprague Dawley rats (model rats) was not sign of adverse effect and had immunomodulator potency.

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OPTIMIZING THERMAL PROCESS IN PRODUCING *SIRSAK* JAM WITHOUT ADDITION OF PRESERVATIVES

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ABSTRACT

Indonesia, a tropical country, has high potency in developing tropical fruit products. Several tropical fruit-products possibly to be developed such as jam. Throughout correct and optimum processing techniques the color and aroma of fresh fruits can be retained in the jam products. The aimed of the research were to find out the effect of sterilization methods and time in producing good *sirsak* jam quality. The research consist of two factors, the first factor is sterilization methods (boiling, steaming and pressure-steaming) and the second factor is long time sterilization (15 minutes, 30 minutes, and 45 minutes). The results showed that the sterilization methods, time and their combination did not give significant differences on jam soluble solids, pH and total acidity. It was no *E. coli* detected before and after heating treatments. While, total microbial load, population of yeasts and filamentous fungi was found to be varies among treatments. However, it seemed to be their populations did not altered by the heating methods and time when jam was produced. Sensorial test on jam color performed by scoring test, preference test performed on the jam color, aroma, sweetness and sour taste showed that sterilization methods and time gave significant differences. Nevertheless their combination did not show any significant differences. It was concluded that the appropriate sterilization methods in producing sirsak jam was either by using boiling water for 30 minutes or by steaming for 15 minutes.

Keywords: jam, sirsak, sterilization, thermal process.

INTRODUCTION

Indonesia, a tropical country, has high potency in developing tropical fruit products. However, that potency not yet developed optimally. Several tropical fresh fruit-products almost can be un-useful because fresh fruits are perishable product. Sirsak is one of tropical-fruit which has specific aroma and taste. Sirsak has white flesh color, combination of sweet and sour taste, and specific aroma. Several tropical fruits possibly to be developed as some products such as jelly, jam, and canned fruit. Through out correct and optimum processing techniques the color and aroma of fresh fruits can be retained in the jam products.

Heating is one kind of preservation process on food products which is almost combine with another kind of preservation process. Effect of heat on microorganism that caused deterioration can be used for food preservative. Nutrition value, and sensorial properties common destroyed by heat, therefore thermal process must be done until the products save from microorganism and didn't change nutrition value and sensorial properties of the products.

The aimed of the research were to find out the effect of heating process, sterilization methods and time in producing good *sirsak* jam quality without any addition of preservatives and to elucidate the optimum sterilization methods (boiling, steaming and pressure-steaming) and sterilization time in producing jam.

MATERIALS AND METHODS

Materials

In this research were used *sirsak* fruit, sugar cane, and lemon juice as the materials of jam and stainless steel pan, blender, stove were used as equipment to making jam. For sterilization process were used pan, steaming pan and pressure cooker. For

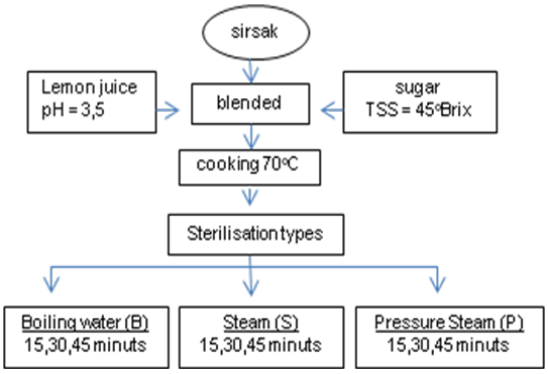
analysis were used, hand refractometer, and digital pH meter. The criteria of *sirsak* fruit in producing jam were good quality fruit like white flesh color and optimal ripped.

Methods

The research used randomized block design, consisting of two factors. The first factor was three levels of sterilization type i.e, boiling water, steaming, and pressure-steaming. The second factor was three levels of long time sterilization i.e 15 minutes, 30 minutes, and 45 minutes. This research was two times repeated.

Processes in producing jam are:

1. Flash sirsak fruit was blended by using blender to make fruit porridge
2. Lemon juice was added into blended-fruit to adjust pH until 3.5 and sugar cane was added until total soluble solid 45°Brix.
3. Mixed material was cooked at 70°C until the viscosity jam-like
4. Jam was put into the bottle and sterilized by using three type of sterilization methods for 15 min, 30 min and 45 min.



Variables determined in this research were total soluble solids (TSS) by using hand refractometer (AOAC, 1984). Total acidity (AOAC, 1970), pH by using digital pH meter, *Total Plate Count (TPC)* (Seely *et. al.* 1981), population of yeasts and filamentous fungi (Seely *et. al.* 1981), and E.coli (Seely *et. al.* 1981) was determined before and after heat process. Sensorial test on jam color performed by scoring test, preference test performed on the jam color, aroma, sweetness and sour taste (Sukarto, 1985).

RESULTS AND DISCUSSION

The result of this research shown that sterilization methods, time and their combinations did not give significant different on total soluble solids of jam. Before heating treatments, the total soluble solid is 45°Brix and after heating treatments become 47.4 °Brix – 49.7 °Brix. This fact might be caused by evaporation of water from product so that total soluble solids in product increased.

The results of the research shown that the sterilization methods, time and their combination did not give significant differences on pH and total acidity of jam. pH value correlated with total acidity in the product. In the first process pH value (pH 3.2 – 3.4) gave effect on building gel together with pectin and high concentration of sugar to make product *viscous-semi solid* (FAO, 1972).

It was no *E. coli* detected before and after heating treatments. While, total microbial load, population of yeasts and filamentous fungi was found to be varies among treatmnents. However, it seemed to be their populations did not altered by the heating methods and time when jam was produced. The data of total soluble solid pH, total acidity and microbial characteristic are shown in Table 1.

Table 1. The data of total soluble solid pH, total acidity and microbial characteristic

Treatment	TSS (°Brix)	pH	Total acidity (%)	TPC (coloni/g)
B T15	47,4	3,33	0,77	0.5×10^1
B T30	48,2	3,33	0,77	0
B T45	48,1	3,32	0,83	0
S T15	48,0	3,33	0,80	0.8×10^2
S T30	48,0	3,33	0,77	$1.0.x\ 10^1$
S T45	48,7	3,32	0,77	0
P T15	48,5	3,33	0,74	0
P T30	49,7	3,32	0,83	0
P T45	48,8	3,32	0,80	0

Sensorial test on jam color performed by scoring test showed that sterilization methods and time gave significant differences. Nevertheless their combination did not showed any significant differences. Sterilization by using boiling-water gave highest score of color followed by steaming and pressure-steaming. Long time sterilization which gave highest score of color was 15 minutes, followed by 30 minutes and 45 minutes. In this case, different in color score caused by sterilization methods and long-time sterilization difference therefore heat transfers into product also different. Increasing sterilization time induced increasing heat transfers into product so that affect the color of jam (Fig. 1).

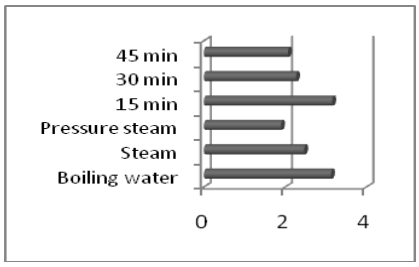
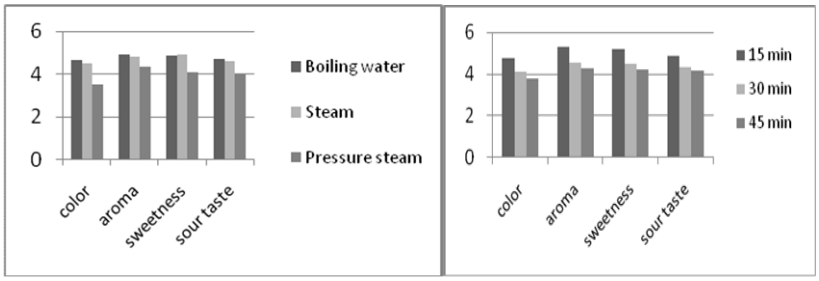


Fig.1. Sensorial test on jam color score

Preference test performed on the jam color, aroma, sweetness and sour taste showed that sterilization methods and time gave significant differences. Nevertheless their combination did not showed any significant differences. Sterilization methods, time and their combination did not give significant effects on jam texture, sweetness and sour score through out 8 months stroraging at room temperature. The data of sensorial test are shown at figures 2(a,b).



a. b.

Fig.2. Preference test on the jam color, aroma, sweetness and sour taste.

CONCLUSION

It was concluded that the appropriate sterilization methods in producing sirsak jam was either by using boiling water for 30 minutes or by steaming for 15 minutes.

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THE INFLUENCE OF COMPARISON OF PURPLE SWEET POTATO FLOUR AND WHEAT FLOUR ON CHARACTERISTICS OF PANCAKE

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ABSTRACT

The aims of this research were to find out the influence of comparison purple sweet potato flour and wheat flour toward the characteristics of pancake and to know comparison purple sweet potato flour and wheat flour produced the best characteristics of pancake. This research used randomized block design with treatment of comparison purple sweet potato flour and wheat flour was consists of six treatments, namely 100 % : 90%; 10% : 80% : 20%; 70% : 30% ; 60% : 40% and 50% : 50 % and each treatment was repeated three times. The results showed that comparison purple sweet potato flour and wheat flour influenced the characteristics of pancake. The comparison purple sweet potato flour and wheat flour 50 % : 50% yielded the best pancake with water content, ash content, starch content and anthocyanin content 23.89 %, 1.07 %; 18.18% and 20.98%, respectively.

Keywords: wheat flour, purple sweet potato flour, characteristic of pancake

INTRODUCTION

Indonesia is a country that wheat and wheat flour importer is in very high quantity recently. It's impact of the increase consumption food product which main raw material of food from wheat flour such as pancakes. The one way to decreased import wheat flour was used another raw material of food as substitution or change of wheat flour such as purple sweet potato flour. Purple sweet potato flour has potential for health benefits such as antioxidant source and dietary fiber.

The using of purple sweet potato flour as substitution of wheat flour in making of product of food such as pancake would give benefits. Purple sweet potatoes have potential to produce high yield in short period of time. They are health maintenance and performance of pancake. They have anthocyanin compound which give purple color. Color (pigment) of anthocyanin in purple sweet potato more stable than red cabbage and blueberries.

Pancake is one kind of cakes which is made by baking process. It has specific characteristic. The characteristic of pancake is depended by characteristic main raw material. Purple sweet potato in baking process often increased sweetness. To know how good was the characteristic of pancake which substitution wheat flour with purple sweet potato flour need to find out comparison of purple sweet potato flour and wheat flour. The Comparison either substitution of purple sweet potato flour and wheat flour in making of pancake not know yet. So, the aims of this research were to find out the influence of comparison of purple sweet potato flour and wheat flour on characteristic of pancake and the best characteristic of pancake.

MATERIALS AND METHODS

Raw materials and Chemicals

Raw materials were used to make pancake: wheat flour, butter, sugar, baking powder, purple sweet potatoes. Chemical materials were consisted by HCL 25%, NaOH 45%, acetic acids 3%, Luff schrool solution, sodium thiosulfat, KI 20%, H_2SO4 20%, indicator amilum, phenolphlatein, methanol, citric acid, Na-citric.

Experiment Design

The design of this research used randomized block design with treatment of comparison wheat flour and purple sweet potato flour and was consists of six treatments, namely:1) T0 (100% wheat flour : 0% purple sweet potato flour); 2) T1 (90% wheat flour :10% purple sweet potato flour); 3).T2 (80% wheat flour: 20% purple sweet potato flour); 4). T3 (70% wheat flour: 30% purple sweet potato flour); 4). T4 (60% wheat flour: 40% purple sweet potato flour) and 5).T5 (50% wheat flour: 50% purple sweet potato flour) and each treatment was repeated three times, so It had 10 experiment units. Data of experiments were analyzed by ANOVA, if the treatments were significant would continued by Duncan test (Gomez and Gomez, 1995)

Process to made purple sweet potato flour

Process made purple sweet potato flour: The first sorted of purple sweet potatoes then peeled of the skin by knife and washed by clear water. After that chopped 3 mm size, then bleaching at 90 ^{o}C temperature, 10-15 minute times. After that dried with oven at 60 ^{o}C temperature until dryness the last milled. The result of this process is purple sweet potato flour.

Process to made pancake

Process to made pancake with treatment on this research (Figure 1) and formulation pancake (Table 1).

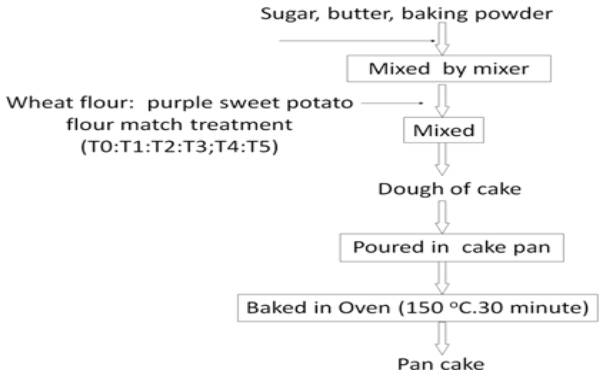

Figure 1. Process to made pancake with treatments of this research

Table 1. Formulation of pancake in 100 g raw material of wheat flour and purple sweet potato flour (g)

Materials	Treatments					
	TO	T1	T2	T3	T4	T5
Wheat flour	100	90	80	70	60	50
Purple sweet potato flour	0	10	20	30	40	50
Butter	50	50	50	50	50	50
Egg	150	150	150	150	150	150
Sugar	100	100	100	100	100	100
Baking powder	1	1	1	1	1	1

RESULTS AND DISCUSION

Water content

The result statistic analysis water content of pancake showed treatments comparison of purple sweet potato flour and wheat flour significant influence (P<0.01) on water content. The average value of water content of pancake (Table 2).

Table 2. Water content (%)

Treatments (% wheat flour : % purple sweet potato flour)	Water content (%)
TO (100 : 0)	21.41[d]
T1 (90 : 10)	21.81[cd]
T2 (80 : 20)	22.64[bc]
T3 (70 : 30)	22.99[ab]
T4 (60 : 30)	23.14[ab]
T5 (50 : 50)	23.89[a]

Table 1 showed water content of pancake from 21.41% to 23.89%. The highest of water content on comparison treatment 50% of purple sweet potato flour and 50% wheat flour. It was 23.89 % water content. Water content of pancake increased with improved addition of purple sweet potato flour. This condition was caused by water content of purple sweet potato flour more higher than water content of wheat flour. Water content of purple sweet potato was 15.71%, while water content of wheat flour was 14.50% (Anon. 1995).

Ash content

The result statistic analysis ash content of pancake showed treatments comparison of purple sweet potato flour and wheat flour significant influence (P<0.01) on ash content. The average value of ash content of pancake (Table 3).

Table 3. Ash content (%)

Treatments (% wheat flour : % purple sweet potato flour)	Ash content (%)
T0 (100 : 0)	0.76[b]
T1 (90 : 10)	0.80[b]
T2 (80 : 20)	0.89[b]
T3 (70 : 30)	1.05[a]
T4 (60 : 30)	1.06[a]
T5 (50 : 50)	1.07[a]

Table 3 showed ash content of pancake from 0.76% to 1.07%. The highest of ash content on comparison treatment 50% of purple sweet potato flour and 50% wheat flour (T5). It was 1.07% ash content. This treatment no significant influenced with T3 and T4 treatment. Ash content of pancake increased with improved addition of purple sweet potato flour. This condition was caused by ash content of purple sweet potato flour more higher than ash content of wheat flour. Ash content of purple sweet potato flour was 2.2%, while ash content of wheat flour was 0.6% (Anon 1995).

Starch content

The result statistic analysis starch content of pancake showed treatments comparison of purple sweet potato flour and wheat flour significant influence (P<0.01) on starch content. The average value of starch content of pancake (Table 4).

Table 4 showed starch content of pancake from 9.08% to 18.18%. The highest of starch content on comparison treatment 50% of purple sweet potato flour and 50% wheat flour (T5). It was 18.18% starch content. Starch content of pancake increased with

improved addition of purple sweet potato flour. This condition was caused by starch content of purple sweet potato flour higher than starch content of wheat flour. Starch content of purple sweet potato flour was 14.15%, while starch content of wheat flour was 8.67% (Anon 1995).

Table 4. Starch content (%)

Treatments (% wheat flour : % purple sweet potato flour)	Starch content (%)
T0 (100 : 0)	9.08[f]
T1 (90 : 10)	13.31[e]
T2 (80 : 20)	14.59[d]
T3 (70 : 30)	15.10[c]
T4 (60 : 30)	17.10[b]
T5 (50 : 50)	18.18[a]

Anthocyanin content

The result statistic analysis anthocyanin content of pancake showed treatments comparison of purple sweet potato flour and wheat flour significant influence ($P<0.01$) on anthocyanin content. The average value of anthochyanin content of pancake (Table 5).

Table 5. Anthocyanin content (%)

Treatments (% wheat flour : % purple sweet potato flour)	Anthocyanin content (%)
TO (100 : 0)	0.00[c]
T1 (90 : 10)	3.46[b]
T2 (80 : 20)	9.72[ab]
T3 (70 : 30)	14.95[ab]
T4 (60 : 30)	19.14[a]
T5 (50 : 50)	20.98[a]

Table 5 showed anthocyanin content of pancake from 0.00% to 20.98%. The highest of anthocyanin content on comparison treatment 50% of purple sweet potato flour and 50% wheat flour (T5). It was 20.98% anthocyanin content. This treatment no significant influenced with T2, T3 and T4 treatments. Anthocyanin content of pancake increased with improved addition of purple sweet potato flour. This condition was caused by anthocyanin content of purple sweet potato flour higher than anthocyanin content of wheat flour. Anthocyanin content of purple sweet potato flour was 110.5 mg/100 g purple sweet potato flour, while anthocyanin content of wheat flour was 0.00% (Anon 1995).

CONCLUSION

The influence of comparison of purple sweet potato flour and wheat flour showed significant on characteristic of pancake. The best result of pancake on comparison of 50% purple sweet potato flour and 50% wheat flour which characteristic: water content, ash content, starch content and anthocyanin content: 23.89 %, 1.07 %; 18.18% and 20.98%, respectively.

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EXAMINING THE RATIO OF WATER AND COW MANURE USING BIOREACTOR UAS (UPFLOW ANAEROBIC SLUDGE) TO PRODUCE BIOGAS

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ABSTRACT

This study aimed to determine the right composition of the weight ratio between water and cow manure using a bioreactor UAS (Up flow Anaerobic Sludge) and the faster way to produce biogas. Bioreactor equipped with a processes tank, a sedimentation tank, a series of gas distribution pipelines, a series of remaining process pipelines, inlet and outlet material. Temperature of biogas, pressure of biogas, and volume calculation of biogas observed during 35 days. Two treatments were set for water and cow manure, each repeated twice. First treatment was 243.75 l of water plus 243.75 l of manure (1:1). Second treatment was 121, 87 l of water plus 365, 62 l of manure (1:3). The Data was collected and analyzed using descriptive statistic and t student test. The right composition and the faster way to produce biogas was first treatment which the temperature reached about 36^{o}C on days 20, the pressures raised 1033.7 cmH_2O on days 10 and the total volume of biogas was 140.67 cm^3on days 35. From regression equation observed that the r square for temperature was 0.863 and 0.772 for treatment I and II respectively. While the r square for pressure was 0.9885 and 0.9947 for treatment I and II respectively. Both temperature and pressure were observed non significant difference. The biogas volumes were observed significantly different (136, 44 dm^3, 135, 12 dm^3 for treatment I and II respectively).

Keywords: bioreactor, biogas, cow manure, UAS.

INTRODUCTION

Whole of cow body have beneficially for farmer and even their manure also had potentially to develop. Junus, 1995 noted that cow is livestock which produce manure in greater compare with human and others, because one cow could produce 15 kg manure in a day. Utilization of cow manure to produce biogas is one of effort to keep environmental contamination in low rate (Sudarto *et. al*., 1997). A bioreactor is needed in order to produce biogas. There are many type and dimension of bioreactor, a bioreactor UAS (Up flow Anaerobic Sludge) is very convenient for farmer as simplified in design and portable. This type of bioreactor is beneficial for farmer to produce biogas for home scale.In order to support research in examination ratio of wáter and cow manure, a simple bioreactor UAS (Up flow Anaerobic Sludge) designed in capacity of 650 lt. The objectives of the study were:

1. To determine the right composition of the weight ratio between water and cow manure using a bioreactor UAS (Up flow Anaerobic Sludge) and
2. To determine the faster way to produce biogas.

MATERIALS AND METHODS

This experiment was conducted in Balai Karantina Petanian Kelas I Sanggaran Denpasar.The cow manure and water also came from the same place. A bioreactor UAS (Up flow Anaerobic Sludge) used to keep the material in fermented condition. Two treatments were set for water and cow manure, each repeated twice. First treatment was 243.75 l of water plus 243.75 l of manure (1:1). Second treatment was 121, 87 l of water plus 365, 62 l of manure (1:3).Step by step for research procedure were separation cow manure from grass, mixing water and cow manure thoroughly, input substrate to bioreactor, anaerobic fermentation process, temperature and pressure monitoring process

and statistical analysis. Temperature of biogas, pressure of biogas, volume calculation of biogas observed during 35 days. The Data were analyzed using descriptive statistic as well as student t-tests.

RESULTS AND DISCUSSION

A bioreactor UAS (Up flow Anaerobic Sludge) designed using fiber glass material to minimize linkage. Bioreactor equipped with a processes tank, a sedimentation tank, a series of gas distribution pipelines, a series of remaining process pipelines, inlet and outlet material. First and second treatments have same trend in temperature. Temperature of first treatment was 29°C before fermentation process. In day 20 have reached at 36°C and have slowly declined at 28.5°C until day 35. The regretion equation was $Y = -0.020x^2 + 0.815x + 26.48$, with R square 0.863. While for second treatment was $Y = -0.015x^2 + 0.625x + 27.81$, with R square 0.772. Sutanto (1982) in Hantoni (2000) mentioned that temperature of fermentation process will increase until reached maximum degree and after that decrease sligthly. Pressure in first and second treatment also have same trend. Increasing pressure in first treatment occured in day 10 while for second treatment occured in day 15. The regretion equation was $Y = 0.0004x^2 + 0.0083x + 1033.6$, with R square 0.9885 while for second treatment was $Y = 0.0003x^2 + 0.0082x + 1033.6$ with R square 0.9947. Both temperature and pressure were observed non significant difference at 0.05 probability level. The biogas volumes were observed significantly different at 0.05 probability level (136, 44 dm^3, 135, 12 dm^3 for treatment I and II respectively).

CONCLUSION

Generally, the right composition and the faster way to produce biogas was first treatment (1:1) which the temperature reached about 36°C on days 20, the pressures raised 1033.7 cmH_2O on days 10 and the total volume of biogas was 140.67 cm^3on days 35. Both temperature and pressure were observed non significant difference. The biogas volumes were observed significantly different at 0.05 probability level.

ACKNOWLEDGEMENTS

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TECHNOLOGY PACKAGING FOR THE TRANSPORTATION OF MANGOSTEEN

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ABSTRACT

The research aimed to design a package made of corrugated board for mangosteen packaging and to analyze the effect of packaging capacity and fruit packing pattern in the container on some quality parameters after transport, This research was conducted in three stages: measurement of physical and mechanical properties of mangosteen (stage I); planning and designing packaging (stage II); testing packaging for transportation by using the simulator (stage III). The packaging was designed with a capacity of 8 kg and 15 kg using two packing pattern, namely the fcc and Jumble (bulky). the optimal stacking of the fruit in the 8 kg capacity packaging was 8 for x direction, 4 for y direction, and 4 pieces of fruit for z direction, with the density of 62%. Therefore, gave packaging dimension of 39.4 x 21 x 21 cm. For the 15 kg container, the optimal packing pattern was 8, 6 and 5 pieces of fruit for the x, y, and z direction, respectively, with the packing density of 65.6%. Thus, gave dimension of 39.4 x 30 x 25 cm. The physical damage that occured in fruits arranged with fcc pattern was only in the form of dented rinds, while the physical damage to the fruit arranged in a Jumble pattern experienced broken calyx and dented. From data show that the rate of respiration with fcc pattern are lower than the jumble

Keywords: packaging design, fcc, jumble, transportation, mangosteen

INTRODUCTION

Facts in the field show that generally mangosteen is packed in plastic baskets of 45cm x 35cm x 15cm with a capacity of 8 kg to 10 kg in bulk. This kind of packaging and handling causes post-harvest loss due to transport by 20%. This number can be significantly reduced by designing and applying a good and effective transport packaging. The design of a good transport packaging requires suitability of packing material with the characteristics of the packaged products. Corrugated cardboard is a suitable packaging material for fruit products such as mangosteen because it has advantages such as vibration damping, pressure resistance, smooth surface and the type of packaging material received in international markets (Syerief 1989).

MATERIALS AND METHODS

The materials used are first quality, index 2 mangosteen with a diameter of 6.0 cm-6.5 cm and the designed packaging (BC flute corrugated cardboard). The instruments used are: Instron universal testing machine, vibrating table, Mettler PM - 4800 electronic balance, and carpentry tools.

The study consisted of 3 stages:
1. Measurement of physical and mechanical properties of Mangosteen
2. Packaging dimension design:

1. Counting the number of fruits (N) in the packaging

$$N = \text{Number of fruits in 1 kg (fruit/kg)} \times \text{packaging capacity (kg)} \quad \text{-----(1)}$$

Counting the number of fruit in each row/column in the packaging

$$Fv(n) = L \times \frac{M}{d}$$

$$\text{Nonsymmetric row pattern } (N) = \frac{(KA\ KB\ KC)}{2} \quad \text{----------(2)}$$

$$\text{Symmetric row pattern } (N) = \frac{(KA\ KB\ KC+1)}{2}$$

KA, KB and KC are number of fruit on the length, width and height in packaging, respectively.

2. Counting dimensions of inner packaging

$$\text{Lenght of fcc } (A) - (1.41\ KA + 0.59)a$$
$$\text{Widht of fcc } (B) = (1.41\ KB + 0.59)b \quad \text{----------(3)}$$
$$\text{Height of fcc } (C) = (1.41\ KC + 0.59)b$$

where a and b is the radius of major and minor fruit

3. Counting dimensions packaging design

$$\text{Lenght} = \text{Lenght of fcc } (A) + 8$$
$$\text{Widht} = \text{Widht of fcc } (B) + 4$$
$$\text{Height} = \text{Height of fcc } (C) + 4 \quad \text{----------(4)}$$
$$\text{Flap} = (\text{Widht of fcc}(B) + 8)/2$$

3. Transport simulation.

Transport simulation was performed using vibration table of 3.50 Hz and amplitude of 4.61 for 3 hours. Out of town or long distance road transport with truck was applied in the simulation for the equality of vibrating table with the real transport situations (Construction Testing Institute, 1986). The packaging was designed in RSC type with a capacity of 8 kg and 15 kg using two patterns of fruit packing systems, namely the fcc (face centered cubic) (Peleg, 1985) and jumble foam net Mangosteen quality parameters measured after the transport were visual physical damage, and the rate of respiration.

RESULTS AND DISCUSSION

Stage I Measurement of Physical and Mechanical Properties

A high level of uniformity in the dimensions of mangosteen fruit was found. It is shown by the low standard deviation value of the measurement data which is between 1.41 - 2.7. The Fcc pattern is a packing pattern focusing on the same amount of fruits in a packaging capacity, therefore the fruits must have dimensions that are not much different from each other

Stage II Determination of Packaging Dimensions

According to Tugimin (1986), design of a good RSC packaging has limit values for KA and KB that is the ratio of its length and width should equal to 2:1, while the KC value is limited by the height of the fruit piles inside containers that does not exceed its bioyield.

Table 1 Dimensions of the designed inner packaging

Parameters		Capasity	
		8 kg	15 kg
Number of Fruits		64	120
The amount of fruit in a row (fruit)	Lenght direction (KA)	8	8
	Width direction (KB)	4	6
	Heightdirection (KC)	4	5
Distance between the fruit (mm)	Lenght direction (Δx)	25.62	25.62
	Width direction (Δy)	26	26
	Height direction (Δz)	23.92	23.92
Dimensions of inner package (cm)	Lenght (A)	37.1	37.1
	Width (B)	19.5	28.3
	Height (C)	19.7	23.8
Volume (cm^3)	Fruit in the package	8181.2	15339.8
	Package	13126.8	23384.4
Packing density (%)		62	65.6

Table 2 Dimension of outside of the package

Packaging Capacity	Length (cm)	Width (cm)	Height (cm)
8 Kg	39.4	21	21
15 Kg	39.4	30	25

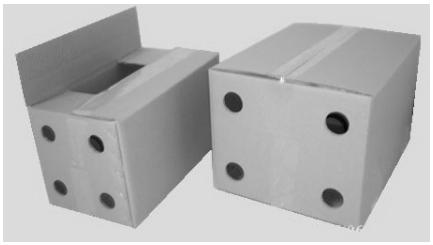

Figure 1 Packaging design results

Stage III transport simulation.

The transport simulations was carried out on a vibrating table with the average frequency of 3.50 Hz, average amplitude of 4.61 cm, and vibrated for 3 hours. Based on calculations, such condition is equal to a road journey as far 477.5 km with trucks on a frequency of 1.4 Hz and amplitude of 1.74 cm on a trip out of town**.** Sudibyo (1992) stated that during truck transportation, the products experience shocks especially in the vertical direction. Other shocks in the form of torsion and dings were ignored because they have a very small frequency. Based on these conditions, the use of vibrating table as a tool for transport simulations is appropriate because the resulting dominant turbulence of the vibration table is also in the vertical direction.

The rate of respiration

The rate of respiration of fruit is a good indication of the fruit store ability. A high rate of respiration is usually accompanied by a short-life storage (Pantastico, 1986). Respiration rate of post-transport Mangosteen are presented in Table 3.

Tabel 3 Respiration rate of each treatment.

Treatments	Respiration Rate of CO_2 (ml/kg hour)	Respiration Rate of O_2 (ml/kg jam)
FCC pattern (8 kg)	29.484	68.984
Jumble pattern (5.4 kg)	31.007	69.561
FCC pattern (15 kg)	26.302	66.737
Jumble pattern (10.2 kg)	29.032	67.519

From these data show that the rate of respiration mangosteen with fcc pattern are lower than the jumble.

Physical Damage

The physical damage that occured in fruits arranged with fcc pattern was only in the form of dented rinds, while the physical damage to the fruit arranged in a Jumble pattern experienced broken calyx and dented rind. Condition of complete calyxes is one of the quality parameters of mangosteen fruit that taken into account in international market. Therefore, the fcc packing pattern can be applied to avoid broken calyxes on mangosteen transportation.

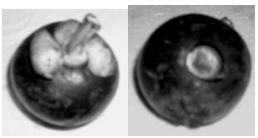

Figure 2 Physical damage on mangosteen (a) broken calyxes; (b) dented rind

CONCLUSION

1. The optimal number of fruits on the 8 kg packaging FCC Patterns was 8, 4, and 4 pieces of fruit for the long x, y, and z direction, respectively. Therefore, gave packaging dimension of 39.4 x 21 x 21 cm. For the 15 kg container FCC Patterns , the optimal packing pattern was 8, 6 and 5 pieces of fruit for the x, y, and z direction, respectively. Thus, gave dimension of 39.4 x 30 x 25 cm
2. From data show that the rate of respiration with fcc pattern are lower than the jumble

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STUDY OF WHEY POTENCY AS AN ELECTRICITY POWER SOURCE IN MFC (MICROBIAL FUEL CELL) SYSTEM USING LACTIC ACID BACTERIA

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ABSTRACT

Microbial Fuel Cell (MFC) is a system that can convert organic material into electrical energy by using microorganisms as biocatalysts. Whey, as wastewater from cheese manufacture, contains of organic material for the microorganism's growth. The purpose of this study is to assess the potency of whey and lactic acid bacteria in a single culture to produce the highest electrical voltage in the MFC system and to optimize the additional carbon sources such as molasses (1%, 2%, 3% v/v) in a chosen whey, to increase the electrical voltage. We used the soy milk whey, UHT cow milk whey, and pasteurized cow's milk whey. The bacterial cultures that we used were *Lactococcus lactis*, *Streptococcus thermophillus*, *Lactobacillus plantarum*, and *Lactobacillus acidophillus*. The MFC system is using PEM (Proton Exhange Membrane) with graphite as anode and cathode. Electrolyte solution at the cathode was using a PAC (Poly Aluminum Chloride) 10% (w/v), whereas at the anode was using whey inoculated with lactic acid bacteria. The results showed that the highest electrical voltage was provided by pasteurized cow's milk whey inoculated with *Lactococcus lactis*, i.e 608 mV (24 h). Optimization of whey and selected cultures with the addition of molasses 1% (v/v), produced the maximum electrical voltage i.e 666.7 mV (12 h.). This maximum electrical voltage was achieved when the number of bacterial cells reach 1.62 x 10^{14} cells; the enzyme activity of 0.43 µg/mL/min; reducing sugar concentration remained 3.9402 g /L; 1.8% lactic acid concentration; and pH 3.38. The maximum electrical voltage generated were 506.67 mV (14 h.) from the control; 666.67 mV (12 h.) from 1% (v/v) molasses addition; 626.67 mV (10 h.) from 2% (v/v) molasses addition; and 633.33 mV (10 h.) from 3% (v/v) molasses addition. The ANOVA test showed that the maximum electrical voltage of each treatment is significantly different. Based on these results we can conclude that the optimum voltage is given by the MFC system using 10% (v/v) *Lactococcus lactis* inoculums in pasteurized milk whey with 1% (v/v) molasses addition.

Keywords: Microbial Fuel Cell, *Lactococcus lactis*, whey, electrical voltage, molasses.

INTRODUCTION

Microbial Fuel Cell (MFC) is one of the new methods in the provision of electricity from waste organic material. MFC is a tool that uses bacteria to convert chemical energy in organic material into electrical energy. MFC reactor is usually anaerobically designed at the anode, and cathode is exposed to oxygen or other specific chemical electron acceptors [2]. The microbes in the anode will produce the electrons and protons from the oxidation of organic material, with CO_2 and biomass as a product [4].

One of organic material source that can be used in MFC is the whey. Whey is a major waste from the cheese manufacture containing 94% water, less than 1% protein, 4.5% lactose, and less than 1% fat. Because of an adequate nutrition, whey can be used as a medium for microbe's growth, especially the acidophilic microbial due to the nature of the whey which is acid. Lactic acid bacteria are examples of acidophilic microbes. The presence of sugar in the whey (mainly lactose) is the main carbon source for microbes and allows the sugars fermentation. This fermentation reaction will cause biochemical reactions and electrons release that can generate electrical currents in this MFC system. In this study, we try to review the potency of whey and lactic acid bacteria as an anode component in MFC system.

MATERIALS AND METHODS

1. Bacterial culture

The lactic acid bacteria culture used in this study were *Streptococcus thermophillus*, *Lactobacillus plantarum*, *Streptococcus thermophillus*, *Lactococcus lactis*, and *Lactobacillus acidophillus*. Meanwhile, the whey used were soy milk whey, UHT cow milk whey, and pasteurized cow's milk whey, which have different nutrients content. Before conducting the MFC system on milk whey, bacteria were activated first on the medium MRS broth. And then, a total of 10% v/v bacteria culture in MRS broth was inoculated on sterilized milk whey.

2. Bacterial and whey screening

After 24 hours, 10% v/v of bacteria was activated in whey medium. Incubation was conducted for 24 hours at room temperature. After that, the electrical voltage produced by each bacterium in whey was measured with MFC. MFC system in this study was using plastic containers. The cylindrical PEM (Proton Exchange Membrane), obtained from the MFC team of Technology School of Biological Sciences, was placed in the middle of plastic containers [1]. Graphite rods were used as cathode and anode. PAC (Poly Aluminum Chloride) 10% w/v solution was used as a catholyte solution. Anolit solution used was a medium that has been given a bacterial culture and activated. The anode and cathode were connected with multimeter.

3. *Optimization with molasses addition treatment*

The bacteria with the highest electrical voltage were then optimized by molasses addition in whey medium. The optimization was performed with the molasses addition in the pasteurization milk whey medium with a concentration of 1%, 2%, 3% v/v. The voltage, current, and electrical power parameters were observed, as well as other parameters that play a role in the biochemical process of bacteria such as pH, concentration of reducing sugars, lactic acid levels, total biomass, and enzyme activity.

4. *Measurement of lactic acid using titration method*

A total of 1 mL sample from each variety was added into 9 mL of distilled water and stirred until homogeneous. A total of 5 mL of the suspension were put into the Erlenmeyer, add 5 mL of distilled water, and 2 drops of 0.1% phenolphthalein. Solvent mixture was titrated with 0,1 N NaOH until the color changed to slightly pink and the volume was recorded.

5. Measurement of reducing sugar concentration using Somogyi-Nelson method

A total of 1 mL sample from each variety was added into 99 mL of distilled water and stirred until homogeneous. From the solution, 2 mL was taken and put into a test tube. Then 1.6 mL of Somogyi reagent I and 0.4 mL of Somogyi reagent II were added. Mixture was heated for 10 minutes. After that, the mixture was cooled on ice. Then 2 mL of Nelson reagent and 4 mL distilled water were added into the cooled solution. The solution absorbency then measured using spectrophotometer at 520 nm wavelength

6. *Measurement of total hydrolytic enzyme activity using the FDA method*

A total of 1 mL samples was taken for testing and put into centrifuge tubes. Then, 4 mL of 60 mM KH_2PO_4 buffer (pH 7.6) and 40 µL of FDA solution were added into tube. Then the tube was centrifuged with a speed of 90 rpm for 20 minutes. After that, a total of 4 mL acetone pro analyst was added then filtered using Whatmann paper no.1. The result of the filter was measured with a UV-VIS absorbance spectrophotometer at a 490 nm wavelength.

7.*The counting of bacteria numbers using FDA method*

Absorbance values were obtained from the FDA's method of measuring enzyme activity associated with the number of cells obtained by TPC (Total Plate Count).

RESULTS AND DISCUSSION

The MFC system with pasteurized milk whey, generate the highest electrical voltage when inoculated with *Lactococcus lactis* bacteria, as well as for the other types of whey (Fig. 1). The bacteria metabolisms support the release and capture of electrons that can increase the electrical voltage of the MFC system. *Lactococcus lactis*, a Gram-positive bacterium, can deliver electrons to the electrode. These bacteria metabolism produces an electron mediator ACNQ (2–amino–3–dicarboxy–1,4–naphthoquinone) [3]. The generated electrons will increase the anode electrical voltage. The results of oxidation will be reuptake by bacterial and enzymatically reduced from NADH [3].

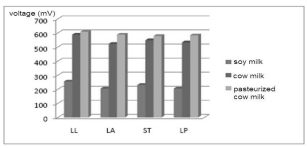


Fig. 1. The electrical voltage produced by each bacterial culture (24 h.) on each type of whey (LL = *Lactococcus lactis*, LA = *Lactobacillus acidophillus*, ST = *Streptococcus thermophillus*, LP = *Lactobacillus plantarum*)

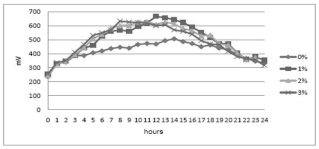


Fig. 2. The electrical voltage generated from each molasses addition treatment in pasteurized milk whey inoculated with *Lactococcus lactis*

Fig. 2 shows at the beginning of the MFC process, the electrical voltage increases until the maximum point and then decrease continuously until the anode run out of organic material source. The decrease of electrical voltage is caused by the overpotensial activation (energy activation to utilize organic matter) and Ohmic losses (higher resistance in the electrodes and the membrane) at anode [5]. The maximum electrical voltage generated were 506.67 mV (14 h) from the control; 666.67 mV (12 h) from 1% (v/v) molasses addition; 626.67 mV (10 h) from 2% (v/v) molasses addition; and 633.33 mV (10 h) from 3% (v/v) molasses addition. The optimum electrical voltage from all treatments were obtained from pasteurized milk whey with 1% (v/v) molasses addition i.e 666.67 mV (12 h). The attaining of maximum electrical voltage will be faster by adding more molasses.

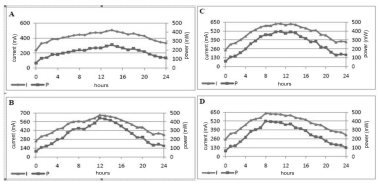
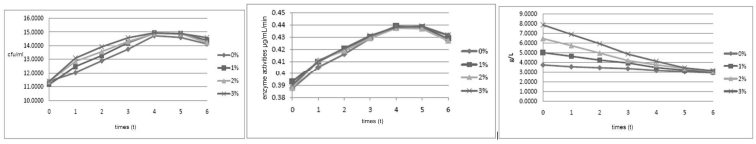


Fig. 3. Current (I) and Power (P) of electricity generated in the MFC (A) control and treatment of the addition of molasses (B) 1% (C) 2% (D) 3%

Fig. 3 shows the maximum electric currents obtained were 506.67 mA (14 h.) from control, 667 mA (12 h.) from 1% molasses addition, 630 mA (11 h.) from 2% molasses addition, and 633.33 mA (8 h.) from 3% molasses addition. The maximum electrical power obtained were 256.87 mW (14 h) from control, 444.6 mW (12 h) from 1% molasses addition, 396,97 mW (11 h.) from 2% molasses addition, and 401,13 mW (8 h.) from 3% molasses addition. Based on the data obtained, the optimum electrical current

(667 mA) and electrical power (444.6 mW) were obtained from 1% (v/v) molasses addition treatment.



(A) (B) (C)

Fig. 4. The (A) amount of cells, (B) enzyme activity, and (C) concentration of reducing sugars from each molasses addition treatment in pasteurized milk whey inoculated with *Lactococcus lactis*.

Fig. 4A shows the logarithmic phase bacterial cell growth from t_0 until t_4. The cell numbers were decreased at t_5 because of the limited number of nutrients and the increase of toxic metabolites. The average cell growth rate in the study was obtained in 1% molasses addition treatment, i.e 0.1699 cells/h. The specific average cell growth rate of control was 0.1515 cells/h; 0.1652 cells/h of 2% molasses addition; and 0.1529 cells/h of 3% molasses addition. The addition of molasses can also trigger the cell growth. It can be seen that the cells number increasing in treatment is faster than control.

Fig. 4B shows that each treatment has the same pattern of the enzyme activity increased from t_0 until t_4 and decreased at t_5 and t_6. The enzyme activity deflation was caused by the depression of cells number in culture. The enzyme activity and cells number has the same effect to electrical voltage production in the MFC system, because the determination of the cell numbers in this study was based on the amount of the enzyme activities.

Fig. 4C shows that the higher the percentage of molasses addition in the medium, the greater the reducing sugar concentration. Molasses is a solution containing carbon sources such as various kinds of sugars, mainly glucose. When 1% molasses was added, it gave 1.35 g/L difference concentration of reducing sugars at the starting process.

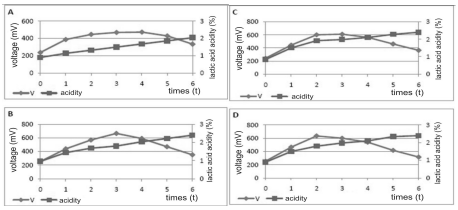


Fig. 5. Relationship between the lactic acid concentration and the electrical voltage produced in (A) control and the (B) 1% (C) 2% (D) 3% molasses addition treatment.

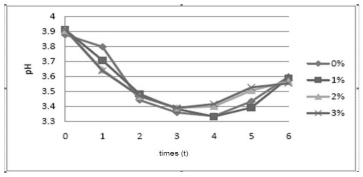


Fig. 6. The pH of each molasses addition treatment in pasteurized milk whey inoculated with *Lactococcus lactis*

Fig. 5 shows that lactic acid levels did not affect the increasing of electrical voltage directly. Lactic acid as the main product of metabolism indicated the number carried by the bacterial metabolism. The higher the lactic acid content, the greater fermentation occurred. The increasing of the fermentation amounts in microorganisms would increase its electrical voltage because of the numbers of free electrons released into anode is also increasing.

Fig. 6 shows that pH of each treatment decreased from t_1 until t_4 and increased at t_5 and t_6. This increasing pH was caused by the role of PEM and the greater number of cell death that would excrete ammonia that could raise the pH solution [6].

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OPTIMATION OF INSTANT *LEDOK* PROCESSING METHOD.

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ABSTRACT

The objective of this research was to find out the best processing method of instant *ledok*. The experiment was conducted by Completely Randomized Design, with three of raw material particle size as a treatment. The treatments were R1 (16 mesh), R2 (9 mesh) and R3 (5 mesh). The characteristics of instant *ledok* were observed such as cooking time, sensory characteristics and its nutrients contents. The results of the study showed that the best processing method was 16 mesh raw material particle size, 5 minutes cooking time and nutrients contens such as water (80.68 %), ash (0.91 %), protein (7.32 %), fat (1.81 %) and carbohydrate (9.29 %).

Keyword: instant *ledok*

INTRODUCTION

Ledok is one of traditional food from Nusa Penida, Klungkung regency which uses corn and cassava as main ingredient without using rice. Others ingredient such as long bean, read bean and *kemangi* are used as additional ingredient. Instant *ledok* is product developed from *ledok* traditional. Preparation and cooking time of traditional *ledok* take a long time. The length of cooking time of traditional *ledok* was 48 minutes, while instant *ledok* was 17.5 minutes at 100ºC (Suter,*et.al*., 2007).Comparing with cooking time of instant corn (6 minutes) and instant rice (9-11 minutes) (Joko, 2002 in Sugiyono, *et al*., 2004), the cooking time of instant *ledok* was relatively much longer because of the particle size of the main ingredient such as corn, cassava and red bean were relatively big. The aim of this study to find out the best processing method of instant *ledok.*

METHODS

Materials and Equipment

The *ledok* materials consisted of white corn, yellow cassava, red bean, peanuts, spinach, *kemangi*, *salam* leaf, onion, chilli, salt, and lime. The materials for analysis consisted of H_2SO_4, NaOH, Tablet Kjeldhal, Boric acid, HCl, alcohol, Petroleum benzene, methyl red, and methyl blue. The equipments used included cooking pan, gas stove, knife, and milling equipment. The equipment for analysis included oven, desiccators, analytic balance, Kjeldhal set and Soxhlet set.

Design Experiment

The experiment was conducted by Completely Randomized Design, with three of raw material particle size as treatments. The treatments were R1 (16 mesh), R2 (9 mesh) and R3 (5 mesh)., and the experiment was replicated twice.

The formulation of raw materials was white corn (110 g), cassava (55 g), red bean (55 g), peanuts (55 g), spinach (9,0 g), *kemangi* (3,0 g), *salam* (5,0 g), galanggal (5,0 g), water (3 x total weight of raw materials), spices (15 g) such as onion, red chilli, salt and lime with ratio 4 : 6 : 1 : 4 in fresh form.

Experimental Steps

a. Preparation of raw materials of instant *ledok*

The preparation of instant *ledok* included: 1) yellow cassava was firstly pealed, then washed and chopped into size of 0,5 x 0,5 x 0.2 cm. Then the chopped cassava was steamed at 100 ^{o}C for 35 minutes and finally after cooling the steamed cassava was dried at 70^{o}C in oven dryer; 2) Peanuts and red beans was boiled at 100^{o}C, and then dried at 70^{o}C in oven dryer. The ratio of peanuts or red bean and water used in boiling was 1:3; 3) White corn was boiled at 100^{o}C, for 37 minutes and then dried at 70^{o}C in oven dryer. The ratio of white corn and water used in boiling was 1:3; 4) Spinach, kemangi and salam leaf blanched at 85^{o}C for 5 minutes, and then continued by drying at 70^{o}C in oven dryer until dried; 5) Lengkuas was minced and then dried in oven drier at 70^{o}C until dried. Preparation for spices involved the activities as follows: firstly the raw materials of spices such as onion, red chili, salt, and lime were weighed as formulation, and then blended using mixer. Finally, the blended spices were dried into the oven drier at 70^{o}C until dried.

b. Processing of instant *ledok*

Firstly, each of the ingredients and spices was weighed following the formulation as mentioned above. Instant *ledok* was processed as follows: water was heated until boiled (100^{o}C), and then all of the ingredients and spices were filled by the boiling water and stirred continuously until ready to serve.

c. Analysis

The analysis performed into the instant *ledok* included: 1) the length of cooking time, sensory analysis (color, aroma, taste, texture, and overall preference) using Hedonik Test (Larmond, 1977) and analysis of nutrition fact (carbohydrate, protein, fat, ash, and water content) using the *Proxymate analysis* methods (Apriyantono, *et al.*, 1989).

RESULTS AND DISCUSSION

1. Cooking time

The effect of particle size on the length of cooking time are showed on Table 1. Particle size R1 and R2 result the length of cooking time less than 8 minutes, where as R3 longer than 8 minutes, if added 150 ml water/50 g total weight of raw material, and if added 200 ml water/50 g total weight of raw material the length of cooking time is 5 minutes are showed by R1, however R2 and R3 the length of cooking times longer than 8 minutes.

Table 1. The length of cooking time instant *ledok*

Particle size	The length of cooking time (minute)	
	Water 3 x Tw	Water 4 x Tw
R1	3	5
R2	7	>8
R3	> 8	> 8

R1 = 16 mesh, R2 = 9 mesh and R3 = 5 mesh. Tw = total weight of raw material

2. Sensory characteristics of instant *ledok*

The effect of processing method on the sensory characteristic of instant *ledok* was tabulated in Table 2. The processing methods were not significantly affecting the preference on color, aroma,taste,texture and over all preference of the instant *ledok.* That is mean all of the instant *ledok* acceptable with score range 4.6 – 4.7 (neutral).

Table2. The average score of sensory test instant *ledok*.

Processing methods	Color	Aroma	Taste	Texture	Overall preference
R1V3 *)	4.38 a **)	4.77 a	4.38 a	4.77 a	4.69 a
R1V4	4.62 a	4.92 a	4.31 a	4.69 a	4.77 a
R2V3	5.08 a	5.08 a	4.85 a	4.62 a	4.77 a

***)** R1 = 16 mesh V3 = 3 x Tw R2= 9 mesh V4 = 4 x Tw.

3. Nutrition content of instant *ledok*

Based on data in Table 1 and Table 2, the best processing method of instant *ledok* was the particle size 16 mesh and the length of cooking time 5 minutes. These processing method yield nutrition content of instant *ledok* was water 80,68 %, ash 0,91 %, protein 7,32 %, fat 1,81 % and carbohydrate 9,29 %. The picture of particles size and instant *ledok* can be seen on Figure 1.

Figure 1. Particles size and instant *ledok*

CONCLUSION

Based on the study on the length of the cooking time and sensory characteristics the best processing method of instant *ledok* was the particle size 16 mesh and the length of cooking time 5 minutes. These processing method yield nutrition content of instant *ledok* was water 80,68 %, ash 0,91 %, protein 7,32 %, fat 1,81 % and carbohydrate 9,29 %.

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THE INFLUENCE OF SKIM MILK POWDER CONCENTRATION ON MICROCAPSULE CHARACTERISTICS OF *SALAM* LEAF (*EUGENIA POLYANTHA* WIGHT.) FLAVOR EXTRACT

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ABSTRACT

The purposes of this research were to find out the influence of skim milk powder concentration on microcapsule characteristics of *salam* leaf flavor extract and to determine the skim milk powder concentration that produced the best microcapsule of *salam* leaf flavor extract. This research used randomized block design with treatment of skim milk powder concentration which was consists of five levels, namely 10, 15, 20, 25, and 30% and each treatment was repeated twice. The samples were observed for their objective characteristics namely: rendement, water content, solubility, and profile compounds. The results showed that the concentration of skim milk powder influenced microcapsule characteristics of *salam* leaf flavor extract. The concentration of skim milk powder of 30 % yielded the best microcapsule of *salam* leaf flavor extract with rendement, water content, and solubility of 30 %, 88,71 %, 4,96 % ($^{w}/_{w}$), 84,25 %, respectively. On the other hand, the flavor compounds on the surface of the microcapsules were decreases with increasing concentration of skim milk powder. Concentration of skim milk powder 30% were not detected of any flavor compound.

Keywords: microcapsule, flavor, skim milk, *salam* leaf (*Eugenia polyanta* Wight.)

INTRODUCTION

Research on the flavor compounds (essential oil) contained in *salam* leaf has not been widely published. In Indonesia the people, especially in Sumatra, Java, and Bali, are using *salam* leaf as a flavoring in cooking (Katzer, 2004). *Salam* leaf can also be used as medicine of cataracts, stroke, uric acid, cholesterol, diabetes, hives, and stomach inflammation (Anon., 2009a).

Wartini (2007) has made separation of flavor compounds in *salam* leaf by some methods. Flavor extract produced from all methods of distillation were liquid form and consist of terpene compounds (monoterpenes, sesquiterpenes) and non terpene compounds (aldehydes) that labile compounds against heating and chemical reaction were changes during process of separation. Otherwise, flavor compounds in liquid form are not convenient in practical use, so that it is necessary to make efforts to resolve the issue.

Microencapsulation is one way to provide stable flavor compounds in solid (powder) form. In this process, flavor compounds are made in an emulsion using a certain encapsulant and subsequently is then homogenized and dried by freeze dryer. The selection of encapsulant is a critical step because it will affect the stability of the emulsion prior drying and storage life of product after drying.

In this present study, skim milk powder was used to encapsulate the flavor compounds of *salam* leaf. Skim milk powder is protein which has an emulsifier function. The use of proteins as encapsulant has several advantages of functional properties such as solubility, viscosity, emulsification, layers or films forming, the ability to interact with water, and high binding capacity of flavor compounds (Bylaite *et al*., 2001).

Previous research, Young, *et al*., 1993a, Young *et al*., 1993b , showed that the concentration of encapsulant used to encapsulate citrus oil was in the range of 10-30% depend on the material of encapsulant used. Bylaite *et al*. (2001) used skim milk powder at a concentration of 30% in the manufacture of microcapsules caraway oil. Whey protein

isolate (WPI) with a concentration of 25% is used in the microencapsulation of ethyl caprylic (Sheu *et al*., 1995). Use of skim milk powder has never been applied to encapsulate the flavor compounds from *salam* leaf flavor extract.

The objective of this research were to find out the influence of skim milk powder concentration on the characteristics of *salam* leaf flavor extract microcapsules and to determine the skim milk powder concentration that produced the best microcapsules.

MATERIALS AND METHODS

Materials

Materials used in this study were consisted of raw material and chemicals. Raw material used was *salam* leaf with homogenous specification such a green color, length (5-15 cm) and width (3-8 cm), which was obtained from Karangasem regency. The chemicals used were: distilled water, encapsulan (skim milk powder), anhydrous MgSO4.

Experimental design

Randomized block design was used to design the laboratory experiment with treatment of skim milk powder concentration which was consists of five levels, namely 10, 15, 20, 25, and 30% and each treatment was repeated twice. The samples were observed for their objective characteristics that were rendemen, water content, solubility and profile of the flavor compounds. Objective data were analyzed by ANOVA followed by LSD test (Steel and Torrie, 1993).

Extraction of *salam* leaves

The extraction was carried out by steam distillation method. The *salam* leaves were sliced to a certain size (± 0.5 cm) and was then extracted in steam distillation apparatus. The extraction process was carried out for three hours to obtain extract of *salam* leaves and addition of anhydrous $MgSO_4$ into the extract was done to absorb the excess water.

Process of encapsulation

Microcapsules of *salam* leaves flavor extract were produced by using encapsulant of skim milk powder. The concentration of skim milk powder experimented were 10, 15, 20, 25 and 30% in destiled water. The skim milk solutions were stored in a cool room (18^{o}C) and left for 12 hours to improve hydration. The extract of *salam* leaves flavor was then blended at encapsulant solution (0.3% flavor extract in encapsulant solution) and homogenized for 20 minutes at 20 amplitudes. The emulsions formed subsequently were frozen in the freezer at temperature of -28±2^{o}C for 12 hours. The frozen emulsions were dried in a freeze dryer (temperature gauge -50^{o}C, vacuum gauge 5 mTor) for 26 hours. The products were then crushed and sieved (60 mesh size) to find the powder, and kept in a closed container. The products were stored in a refrigerator before analysis.

Variables analysis

Microcapsule powder was analyzed its characteristics, namely rendement (AOAC, 1975), water content (Sudarmadji *et al*., 1997), solubility (Yuwono, 1998), and profile of flavor compounds using gas chromatography. In this experiment was determined the flavor compound on the surface of microcapsules. One gram of microcapsules was diluted in 2 ml n-hexane and mixed thoroughly. And then the mixture was kept to separate the solution and solid phase. The solution phase of 1µl was then injected into GC (Varian 3300) with CW20M column (10% of carbowax) in length of 2 m and operational condition as follow: the injector temperature was 200^{o}C; the initial and final column temperature were 60^{o}C and 200^{o}C respectively with the gradually increasing temperature by 5^{o}C per minute; TCD detector was used with temperature of 250^{o}C; and the mobile phase was gas of nitrogen.

RESULTS AND DISCUSSION

The concentration of skim milk powder used as encapsulant affected the characteristics of microkapsule of *salam* leaves flavor extract significantly (Table 1). Increasing of skim milk powder concentration used in process of encapsulation produced microcapsules in higher rendement and better solubility, but gave dryer microcapsules.

Table 1. Characteristics of microcapsule of salam leaf extract flavor

Concentration of skim milk powder (%)	Rendement (%)	Water content (%w/w)	Solubility (%)
10	83.90 d	7.09 a	71.81 c
15	85.51 c	6.45 b	74.15 c
20	86.69 b	5.78 c	77.21 b
25	87.31 b	5.47 c	79.58 b
30	88.71 a	4.96 d	84.25 a

*) The different letters following the average values mean significant differences (P <0.05)

The concentration encapsulant of 10% and 30% resulted the lowest (83.90%) and the highest (88.71%) rendemen, respectively. The higher concentrations of skim milk powder, result the higher rendement. This was occurred because the increasing of skim milk powder concentration will increase the viscosity and total solid of the mixture before being dried. The higher viscosity of the coating core material microcapsules are formed faster, so that the core material of microcapsules immediately protected and the resulting yield will be high (Bhandari *et al*., 1992).

The concentration encapsulant of 30% and 10% gave the lowest (4.96%) and the highest (7.09%) water content results, respectively. This was occurred due to the water content of microcapsules was influenced by encapsulant solution concentration. The lower concentration means more volume of solvent used to dissolve encapsulant at the same volume and the higher concentration means less volume of solvent used to dissolve encapsulant at the same volume. The same time of drying process was done of each treatment by freeze dryer and using the same process conditions. Thus, the volume of solvent that evaporated in the drying process for each treatment is relatively the same. As a result, the lower concentration of encapsulant gives a higher water content.

The concentration encapsulant of 10% and 30% resulted the lowest (71.81%) and the highest (84.25%) solubility of microcapsules, respectively. Solubility in water of a material is influenced by the water content of the material. High water content causes the material to be difficult to spread in the water because the material tends to sticky. Consequently, the material is not able to absorb in large amount of water. The material with high water content will be damp and sticky, and the lower material will be dispersed (Yuwono *et al*., 1998).

Gas chromatography analysis was used to determine the number of flavor compounds that could be encapsulated. The higher concentration of encapsulant resulted the more effective of the encapsulation process, which means that more extract flavor compounds were entrapped in microcapsules or fewer compounds in the surface of the microcapsules. This is in accordance with the results of studies showing that there were no compounds on the surface of microcapsules of *salam* leaves flavor extract, which used skim milk powder in concentration of 15, 20, 25 and 30% in encapsulation process. While using 10% of skim milk powder showed 10 compounds on the surface of microcapsules. Profile of compounds on the surface of microcapsules of *salam* leaves flavor extract regards to the treatment of skim milk powder 10, 20 and 30% respectively are presented in Figure 1.

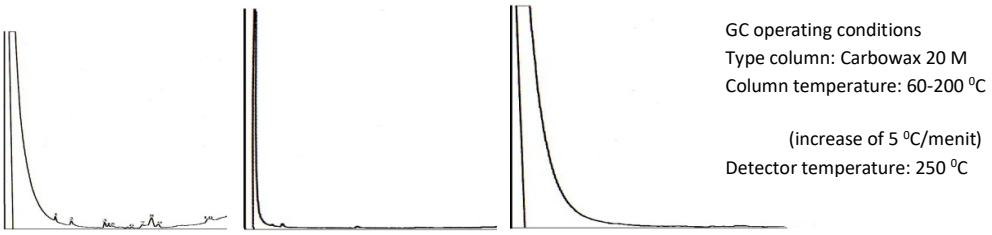

Fig.1. Profile of flavor compounds on the surface of microcapsules of *salam* leaf extract. (a)10% skim milk powder, (b) 20 % skim milk powder, and (c) 30% skim milk powder

The concentration of skim milk powder influenced microcapsule characteristics of *salam* leaves flavor extract. Flavor compounds on the surface of the microcapsules were decreases with increasing the concentration of skim milk powder. In concentration of 30% skim milk powder used as encapsulant were not detected of any flavor compounds on the surface of the microcapsules. The concentration of 30 % skim milk powder using in encapsulation process yielded the best microcapsules of *salam* leaves flavor extract with rendement, water content, and solubility of 88,71 %, 4,96 % ($^w/_w$), 84,25 %, respectively.

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THE INFLUENCE OF WHEAT FLOUR SUBSTITUTION WITH YELLOW PUMKIN (*Cucurbita moschata ex.* Poir*)* ON CAROTEN CONTENT AND CHARACTERISTIC OF SWEET BREAD

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ABSTRACT

The aim of the research were to find out the influence of wheat flour substitution with yellow pumpkin on caroten content of sweet bread and to know the substitution level that maked the best characteristic of sweet bread. The research used randomized block design with wheat flour and yellow pumpkin compare treatments. The treatments were 100 % wheat flour (control), 90 % wheat flour : 10 % yellow pumpkin, 80 % wheat flour : 20 % yellow pumpkin, 70 % wheat flour : 30 % yellow pumpkin, 60 % wheat flour : 40 % yellow pumpkin and 50 % wheat flour : 50 % yellow pumpkin. Each treatment repeated by 3 times so that obtained 18 unit experiments. The parameter of this research were total of sugar, water content, ash content, total of caroten and sensoris evaluation (overall acceptance). The data obtained was analyzed by variant analysis, and if showing the significant influence, analyzed continued by Duncan test. Result of the research showed that different level of wheat flour substitution with yellow pumpkin had significant effect on all variable. The more of wheat flour substitution with yellow pumpkin, the more total of sugar, water content, ash content and total of caroten. Total of sugar range from 1.72 % up to 2.25 %, water content range from 27.63 % up to 38.61 %, ash content range from 0.89 % up to 1.47 % and total of caroten range from 1176.32 µ g / 100g up to 4496.32 µg/100g. The treatment of 50 % wheat flour : 50 % yellow pumpkin yield sweet bread with best characteristic with product criteria were total of sugar 2.25 %, water content 38.61 %, ash content 1.47 %, total of carotene 4496.32 µ g/100g, and overall acceptance was very like.

Keywords: sweet bread, wheat flour, yellow pumpkin, caroten.

INTRODUCTION

Bread is a food made from flour through a fermentation process (Anon., 1983). One alternative to reduce the consumption of wheat flour in making sweet bread is to use the other commodities that can be obtained locally at a cheaper price, one of them is pumpkin. Pumpkin (*Cucurbita moschata,* ex. Poir) is one of many agricultural commodities, especially carotenoids and vitamins are very beneficial for health (Anon., 2005). Yellow pumkin has complete nutrient. In 100 grams of pumpkin contain 180 SI of provitamin A (carotene), 52 mg of vitamin C and some other mineral components (Sudarto, 1993). Given the large pumpkins role to improve the nutrient content. The processed of product diversification with pumpkin needs to be done to increase added value. One way to use pumpkins is substitution sweet flour in making bread. However, research must be done about the level of substitution of the right pumpkins that can be used in making sweet bread so it could get the best sweet bread characteristics. Besides the use of pumpkins is expected to improve the nutritional value, especially the carotene content in sweet bread.

MATERIALS AND METHODS

The materials used in making sweet bread was wheat flour (Cakra Kembar), yeast (fermipan), salt (Dolphin brand), sugar (Gulaku), margarine (Blue Band) , powdered milk (Dancow instant), eggs and water. While the chemicals for the purposes of analysis, namely: acetone, petroleum ether, HCl, Luff schoorl, NaOH, H2SO4, KI, Sodium thiosulfat, indicators PP, Na2SO4 and starch indicator.Tools were used in this study were electric oven, pan, dough bowls, tablespoons, timber mill. Tools for analysis of aluminum

cup, porcelain cup, tube, stick mixer, water bath, centrifugasi, eksikator, analytical scales, paper sieve, funnel, measuring cup, pipette drops, pipette volume, mortir, and spectrophotometer.

This research used randomized block design with wheat flour and yellow pumpkin compare as treatments, there were:

Lo = 100% wheat flour (control)
L1 = 90% wheat flour: 10% yellow pumkin
L2 = 80% wheat flour: 20% yellow pumkin
L3 = 70% wheat flour: 30% yellow pumkin
L4 = 60% wheat flour: 40% yellow pumkin
L5 = 50% wheat flour: 50% yellow pumkin

Each treatment was repeated three times so obtained 18 experimental units. The data were analyzed by variance and if the treatment had effect will be followed by Duncan test (Steel and Torrie, 1995). The raw material for making sweet bread the others wheat flour and pumkin were 2% of fermifan, 20% of sugar, 10% of margarine, 10% of powdered milk, 1,5% of salt and 10% of egg yellow. The process for making sweet bread were wheat flour and yellow pumkin added by starter (yeast, water, sugar), salt , margarine, powdered milk, egg yolk and water, so mixing and stirring dough until smooth, milling dough, fermentation (45 minutes), formation or molding dough and oven (150 ºC; 20 minuts) or until the bread mature.The parameters observed included: total of sugar (Luff Schoorl method in Sudarmadji and Suhardi, 1984), water content (oven method in Sudarmadji and Suhardi, 1984), ash content (heating method in Sudarmadji and Suhardi, 1984), total of carotene (spectrophotometric method (Anon, 2003) and sensory evaluation (overall acceptance) using hedonic test (Larmond, 1977).

RESULTS AND DISCUSSION

Based on objective analysis and sensory evaluation (overall acceptance) of sweet bread was obtained an average value which can be seen in Table 1.

Total of sugar

Based on analysis of variance to the total of sugar sweet bread showed that treatment with pumpkin substitution had significant effect (P <0.05) to the total of sugar produced sweet bread. Table 1 shows that the average value of total sugar sweet breads ranged from 1.72 percent up to 2.25 percent.

Table 1. Average value of analysis results of sweet bread

Treatment	Total of Sugar (%)	Water content (%)	Ash content (% bb)	Total of carotene (µg/100 g)	Overall acceptance
L_0	1.72 f	27.63 e	0.89 d	1176.32 d	4.67 d
L_1	1.80 e	32.58 d	1.10 c	1707.35 cd	4.53 d
L_2	1.83 d	34.85 c	1.21 b	2121.18 bcd	4.80 cd
L_3	2.15 c	36.06 bc	1.36 a	3030.65 abc	5.40 ab
L_4	2.17 b	37.55 ab	1.39 a	3527.76 ab	5.27 bc
L_5	2.25 a	38.61 a	1.47 a	4496.32 a	5.87 a

Notes: the average value followed by the same letters indicate no significant difference (P> 0.05).

The highest total of sugar was obtained in treatment L5 (50% wheat flour: 50% pumpkin), it was 2.2 percent. While the lowest total sugar was obtained by treatment Lo (100% wheat flour), it was 1.72 percent. The total of sugar tends to rise along with the addition of pumpkin. This is due to pumpkin also contains a moderately high carbohydrate 6.6 grams in 100 grams of material. According Winarno (2004),

carbohydrates in fruits contain monosaccharides such as glucose and fructose which has a sweet taste, therefore the addition of pumpkin caused increase of total sugar.

Water content

Results showed that treatment with pumpkin substitution had very significant effect (P <0.01) to water content of sweet bread. The average water content of sweet bread ranges between 27.63 percent up to 38.61 percent. Obtained the highest water content in treatment L5 (50% terigu: 50% pumpkin), it was 38.61 percent. While the lowest water content in treatment Lo (100% wheat flour), it was 27.63 percent. The water content tends to rise along with the addition of pumpkin. This is because the water content in pumpkin bigger than wheat flour. The water content of pumkin is 91.20 gram/100 grams (Sudarto, 1993), while the water content of wheat flour is 11, 8 gram/100 grams (Anonim., 1995).

Ash content

The result showed that pumpkin substitution had very significant effect (P <0.01) to ash content. The average value of ash content ranging from sweet breads 0.89 percent up to 1.47 %. The highest ash content was obtained by treatment L5 (50% wheat flour: 50% pumpkin), it was 1.47 %. While the lowest water content obtained by treatment L0 (100% wheat flour), it was 0.89 %. The ash content tends to rise along with the addition of pumpkin. This is because the mineral content of the pumpkin is higher than wheat flour. The pumkin contain calcium and phosphor 45 mg and 64 mg respectively (Hendrasty, 2003), while calcium and phosphor of wheat flour are 14 mg and 8 mg (Anon., 1981).

Total of Carotene

The result showed that treatment had very significant effect (P <0.01) to total carotene of sweet bread. Table 1 shows that the average value of total carotene sweet breads ranging from 1176.32 µg/100g up to 4496.32 µg/100g. The highest total carotene was obtained by treatment of L5 (50% wheat flour: 50% yellow pumkin), it was 4496.32 µg/100g. While the lowest total carotene was obtained by treatment L0 (100% wheat flour), it was 1176.32 µg/100g. The total of carotene tends to rise along with the addition of pumpkin. This is because the pumpkin contains carotene 180 SI or approximately 1000-1300 IU/100 g (Hendrasty, 2003), while wheat flour didn't contain carotene.

Overall acceptance

The result showed that treatment had very significant effect (P <0.01) to overall acceptance sweet breads. The average value given by the panelists ranged from 4.53 up to 5.87 with criteria like until very like. The highest value was obtained by treatment L5 (50% wheat flour: 50% yellow pumkin), it was 5.87.

CONCLUSION

The treatment for making sweet bread with best characteristic was 50% wheat flour : 50% yellow pumkin with product criteria were total of sugar 2.25 %, water content 38.61 %, ash content 1.47 %, total of caroten 4496.32 µ g/100g, and overall acceptance was very like.

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THE EFFECT OF SUGAR CONCENTRATION AND HEATING TEMPERATURE ON CHARACTERISTIC OF TAMARILLO (*CYPHOMANDRA BETACEA*) JAM

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ABSTRACT

The aim of the research were to know the effect of sugar concentration and heating temperature on characteristic of tamarillo jam and to know sugar concentration and heating temperature precise to produce tamarillo jam with the best characteristic.This research was undertaken by factorial experiment using randomized block design. There were two factors in this research namely : (1) sugar concentration (45%, 50%, and 55%) and (2) heating temperatures (60°C, 70°C and 80°C). There were two replications for each treatments, so that be 18 experiments unit. The data was analyzed by ANAVA and followed by different test of reality. The parameter of this research were total of anthocyanin, acidity (pH), viscosity, total of soluble solid and sensory evaluation include color, smell, texture, taste and overall acceptance. Result of the research showed that treatment of sugar concentration and heating temperature had an significant effect on all variable. Concentration of sugar 55% and heating temperature 60°C yield tamarillo *jam* with best characteristic with product criteria anthosyanin 0.30%, pH 3.61, viscosity 0.0080 g/cm.sec, total of soluble solid 62.1 °Brix and overall acceptance 5.1 (like).

Keywords: sugar, heating temperature, characteristic, tamarillo jam.

INTRODUCTION

Tamarillo fruit (*Cyphomandra betacea*) has the nutritional content and vitamins are very important for the health of human body such as anthocyanin, carotenoids, vitamins A, B6, C and E. Based on evaluated the functional aspect of tamarillo fruit, it has very good properties as a source of natural antioxidants (Kumalaningsih and Suprayogi, 2006). Tamarillo fruit rarely served as a table because it tends to sour taste. The fruit is more suitable if the serve in processed form, such as made syrup, juice or a mixture of salad or jam. With a very high acid taste of the fruit tamarillo cause a lot of people do not like to consume fruits in fresh condition so that the necessary efforts to cultivate such Tamarillo fruit used as jam. There have been no studies on the effect of sugar concentration and treatment temperature on the characteristics tamarillo jam produced, so the study entitled the influence of sugar concentration and heating temperature on the characteristics of tamarillo jams are needed to determine how best to produce jams of tamarillo fruit.

MATERIALS AND METHODS

The main material used in this study was the Tamarillo fruit (*Cyphomandra betacea*) whose skin is yellowish red or dark purple with a texture soft when pressed with fingers and relatively nice aroma. Other materials were lime and sugar. Materials used for chemical analysis is distilled water, HCl, methanol, KCl, 0.1 M citric acid solution and 0.1 M Na-citrate. The tools used in this study pH-meter digital, hand refractrometer, with a sloping glass 45^o angle, the measuring cup, glass beaker, pipette drops, a thermometer, and analytical scales etc. The experimental design in this study is factorial Randomized Block Design, which consists of two factors. The first factor was concentration of sugar which consist of three levels, which were 45%, 50%, and 55%. The second factor was heating temperature which consist of three levels, there were 60°C, 70°C and 80°C. Based on the first and second factor, we obtain nine combinations then repeated by 2

times so that obtained 18 unit experiments.The data obtained were analyzed by variance and when significant it was followed by least significant difference test.

RESULTS AND DISCUSSION

Anthocyanin content

The result showed that the sugar concentration showed no significant effect (P> 0.05), while the heating temperature showed a significant effect on the anthocyanin content of tamarillo jams. The average value of anthocyanin content of tamarillo jams can see at Table 1.

Table 1. Anthocyanin content of tamarillo jams (%).

Temperature (°C)	Concentration of sugar (%)			Average
	G_1 (45)	G_2 (50)	G_3 (55)	
T_1 (60)	0.28	0.25	0.30	0.28 a
T_2 (70)	0.28	0.23	0.29	0.26 a
T_3 (80)	0.21	0.18	0.22	0.20 b
Average	0.25 a	0.22 a	0.27 a	

Note: the average value followed by the same letters indicate no significant difference (P> 0.05).

Anthocyanin content of tamarillo jam ranged from 0.18% up to 0.30%. Table 1 shows that the anthocyanin content tends to decrease along with the addition of heating temperature. Anthocyanin is water soluble and volatile compounds, in addition to high temperatures, thus causing the pH increase at high pH is unstable and anthocyanin content would decrease. According Kumalaningsih (2006) the stability of anthocyanin is influenced by pH and heat.

Acidity (pH)

The result show that the interaction of the two treatments, the sugar concentration showed no significant effect (P>0.05) but heating temperature showed a significant effect (P<0.01) on acidity of Tamarillo jams. The average acidity value of Tamarillo jams can be seen in Table 2.

Acidity of tamarillo jams ranged from 3.59 to 3.66. Table 2 shows that the pH tends to increase along with the addition of heating temperature. Winarno (2002) states the causative agent of sour taste is H, if the concentration of hydrogen ions (acidity) increases the pH will fall.

Tabel 2. Acidity of tamarillo jams (%)

Temperatures (°C)	Concentration of sugar (%)			Average (%)
	G_1 (45)	G_2 (50)	G_3 (55)	
T_1 (60)	3.59	3.60	3.60	3.59 b
T_2 (70)	3.64	3.64	3.65	3.64 a
T_3 (80)	3.66	3.65	3.66	3.65 a
Average	3.63 a	3.63 a	3.64 a	

Note: the average value followed by the same letters indicate no significant difference (P> 0.05).

Acid is a substance soluble in water and produce hydrogen and is a weak organic acid that can release H ions when heated high temperature (Winarno *et al.,* 1989).

Viscosity

The result show that the concentration of sugar, heating temperature and its interaction have very significant effect (P <0.01) on viscosity of tamarillo jams. The average value viscosity of tamarillo jams can be see in Table 3.

Table 3. Viscosity of tamarillo jams

Temperatures (°C)	Concentration of sugar (%)		
	G_1 (45)	G_2 (50)	G_3 (55)
T_1 (60)	0.014 a a	0.009 b a	0.008 c a
T_2 (70)	0.014 a a	0.009 b a	0.007 c a
T_3 (80)	0.010 a b	0.008 b a	0.007 c a

Note: the average value followed by the same letters indicate no significant difference (P> 0.05).

Viscosity of tamarillo jams ranged 0.007 up to 0.014 g/cm.sec. Table 3 shows that the viscosity tends to decrease along with the addition of sugar concentration and heating temperature. Desrosier (1988) states that the higher the sugar content diminishes as the water retained by the gel structure, so that the gel which formed more solid. The higher the temperature of the cooking process at tamarillo increasingly experiencing water evaporation resulting in a more viscous Tamarillo jams. Due to the increased viscosity of water outside the starch granules are free to move into bond.Winarno(2002). The more viscous a fluid is needed so that the greater the force that the fluid can flow (Kusumah, 1988).

Total Soluble Solid

The result show that sugar concentration and heating temperature has significant effect (P> 0.05) on total soluble solid but its interactions no effect.The average value of total soluble solids of tamarillo jams can be see in Table 4.

Tabel 4. Total soluble solid of tamarillo jams (ºBrix)

Temperature (°C)	Concentration of sugar (%)			Average
	G_1 (45)	G_2 (50)	G_3 (55)	
T_1 (60)	48.60	56.20	62.10	55.63 b
T_2 (70)	51.80	59.60	61.80	57.73 ab
T_3 (80)	53.80	60.60	64.40	59.60 a
Rata-rata	51.40 c	58.80 b	62.76 a	

Note: the average value followed by the same letters indicate no significant difference (P> 0.05).

The total of soluble solid ranges from 48.60 up to 64.40 ºBrix. Increasing the value of total soluble solids of jams due to the high concentration of sugar was added with the help of a high temperature then the elements dissolved in the product will increase and the increasing in heating temperature resulted in many experienced evaporation of water content.

Overall Acceptance

The overall acceptance value of jams tamarillo treated sugar concentration and heating temperature has a value ranging from 4.35 (normal) up to 5.30 (like). Tamarillo jam which has the highest value to the overall acceptance by panelists were G3T1 (55% of sugar and 60 ºC of heating temperature) with a score 5.30 (like).

CONCLUSION

The treatment for making tamarillo jam with best characteristic was 55% of sugar and 60ºC of heating temperature with product criteria anthosyanin 0.30%, pH 3.61, viscosity 0.0080 g/cm.sec, total of soluble solid 62.1 °Brix and overall acceptance 5.1 (like).

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ANALYSIS COMPOUNDS AND TOXICITY TEST OF CORIANDER SEEDS (*CORIANDRUM SATIVUM* L.) ESSENTIAL OIL

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Analysis compounds and toxicity test of coriander seeds essential oil has been done. The essential oil was extracted by steam distillation, while the toxicity of the oil was tested against *Artemia salina* Leach larvae. The oil obtained was fractionated into ethanol and n-hexane. The toxic fraction was analyzed by Gas Chromatography and Mass Spectroscopy (GC-MS). The oil content was about 0.27%. The ethanol fraction of the oil was toxic with the LC_{50} of 44.67 ppm toward *Artemia salina* Leach, while the n-hexane fraction was not toxic (LC_{50} > 1000 ppm). GC-MS analysis of the toxic oil indicated that linalool was the mayor compound with the percentage of 84.58%, followed by 8.14% of terpineol, 3.32% of camphor, 2.50% of geraniol, and 0.55% of borneol.

Keywords: Coriander, essential oil, linalool, toxicity test

INTRODUCTION

Coriander (*Coriandrum sativum* L.) is one of the medicinal plants commonly used by Indonesian people as individual or together as a mixture with other plants. In addition, the coriander is also used as seasonings in a variety of foods. Part of coriander plant that can be used as a medicine are leaves, fruit, or seeds, which have analgesic properties, anti-inflammatory, carminative (can remove gas from the digestive tract), and anti diabetic (Handayani, 2000 and Ammar, 1997).

Coriander contains essential oil that is composed of 55-74% linalool (monoterpene alcohols) and 20% monoterpene hydrocarbons (Kizili, 2004). Gas chromatography and mass spectroscopy (GC-MS) were applied to analyze the compounds, linalool and monoterpene hydrocarbons derivatives provide a characteristic peak at m/z 93 (Lewinsohn, 2001). Other components of coriander are palmitic acid, linoleic acid, linolenic acid, flavonoid glycosides (quercetin, isoquercetin, and rutin) (Ammar, 1997).

Brine shrimp Lethality Test can be used as prescreening for anticancer or antitumor agent, because the result showing LC_{50} < 1000 often has high correlation with antitumor agent (Colegate, 1993 and Meyer, 1982). Preliminary toxicity test using brine shrimp *Artemia salina* Leach showed that ethanol extract was toxic with LC_{50} of 51.286 ppm.

Based on the preliminary tests, that coriander was toxic towards *Artemia salina* Leach larvae and the essential oil content of coriander, therefore it was worthwhile to investigate the essential oil composition of coriander seed (*Coriandrum sativum* L.) and the toxicity of the oil.

MATERIALS AND METHODS

Materials

Coriander seeds (*Coriandrum sativum* L) were collected from Badung market, the seeds were ground become powder. *Artemia salina* Leach egg was provided by technical training centre UNUD. Chemicals used are ethanol, n-hexane, Dimethyl sulfoxide (DMSO), calcium dichloride ($CaCl_2.2H_2O$), and sodium chloride (NaCl) pro analysis (*Merck*).

Apparatus

The apparatus used is a blender, a set of steam distillation equipment, a set of glassware, and gas chromatography and mass spectroscopy (GC-MS).

Methods

The essential oil of coriander seed was extracted by steam distillation. The oil obtained was fractionated into ethanol 70% and n-hexane. Furthermore, the toxicity of the ethanol and n-hexane extract was tested against *Artemia salina* Leach larvae. The toxic fraction was analyzed by Gas Chromatography and Mass Spectroscopy (GC-MS).

RESULTS AND DISCUSSION

The essential oil content was around 0.27%. The oil was partitioned with ethanol 70% and n-hexane. Each fraction was tested for toxicity against *Artemia salina* Leach larvae. Toxicity test results are presented in Table 1.

Table1. Toxicity Test of Essential Oils Againts *Artemia salina* Leach Larvae

Fraction	Concentration	The Number of The Dead Larvae			The Number of The Survive Larvae			LC_{50}
		1	2	3	1	2	3	
Etanol	10 ppm	3	4	3	7	6	7	44.67
	100 ppm	6	6	5	4	4	5	
	1000 ppm	9	8	8	1	2	2	
n-hexane	10 ppm	0	1	1	10	9	9	>1000
	100 ppm	1	2	2	9	8	8	
	1000 ppm	1	1	2	9	9	8	

Note: The number of larvae per concentration of 10 larvae (total larvae at three concentrations of 30 larvae)

Based on the table above, ethanol fraction was a toxic fraction with LC_{50} of 44.67 ppm. N-hexane fraction was not toxic because the LC_{50} values were greater than 1000 ppm. Therefore, the ethanol fraction was analyzed by GC-MS to identify the components contained therein. Chromatogram of essential oil from the ethanol fraction is shown in Figure 1.

From the chromatogram appeared eight peaks with retention times (tr) of 6.350, 6.591, 6.904, 7.072, 7.237, 7.808, 8.275, and 9.935 minutes. This shows that the essential oil isolated contains eight components, where there is one major peak with a retention time of 6.350 minutes (peak 1) with the percentage of 84.58%. Five main peaks were analyzed by mass spectroscopy. They were subsequently compared with the database to determine the fragmentation pattern of each peak.

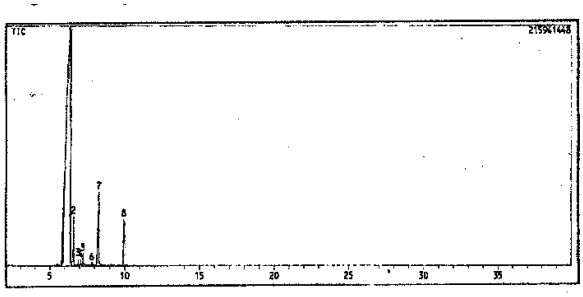


Figure 1 Chromatogram of coriander seed ethanol fraction essential oil

Mass spectrum of peak 1 is shown in Figure 2a. It is compared with the spectrum of the database (Figure 2b). The spectrum of peak 1 is in accordance with the spectrum of linalool. It can be seen that the peak and fragmentation patterns are relatively similar. Linalool has a molecular weight of 154, but the peak of linalool molecular ion (M^+) does not appear. This is because the tertiary alcohol linalool. According to the literature, molecular ion peak of primary and secondary alcohols have a small peak, while the molecular ion of the tertiary alcohol almost does not appear (Silverstein e*t al*, 1991). Peak with m/z = 93 is a typical fragmentation peak for linalool and other derivatives of monoterpenes (Lewinsohn, E. and Schalechet, F., 2001)

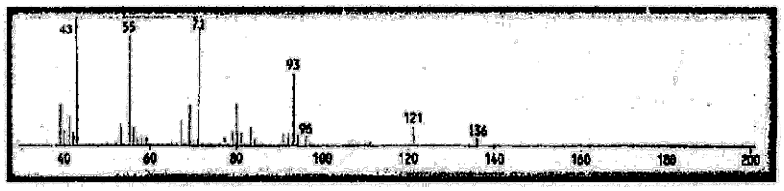


Figure 2a. Mass spectrum of peak 1 (tr 6.350 minutes)

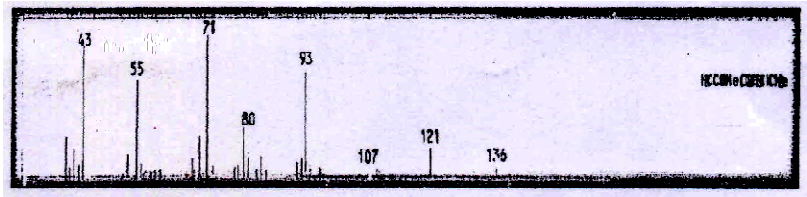
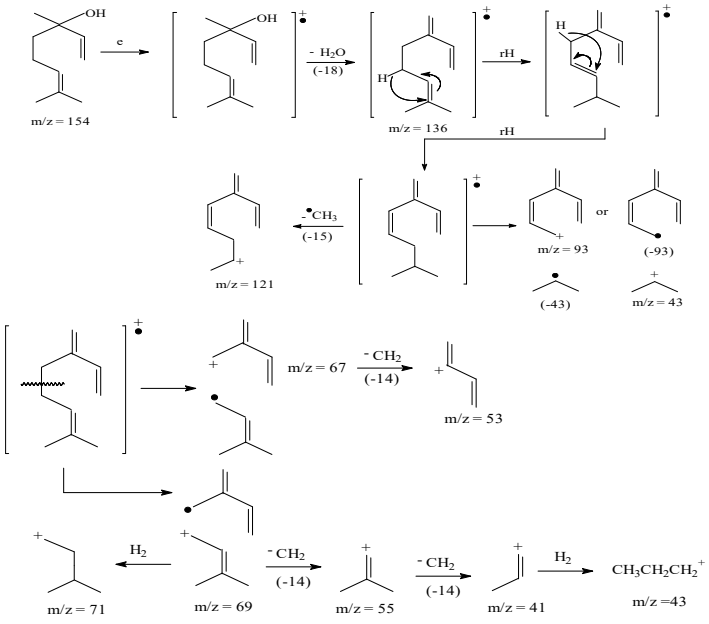
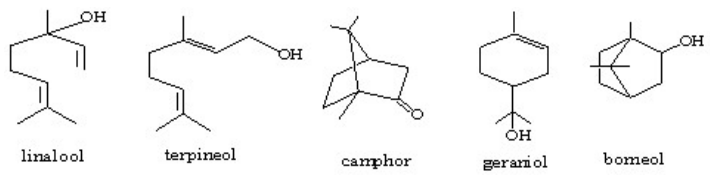


Figure 2b. Mass spectrum of linalool standard.

Fragmentation pattern of linalool estimates are as follows:



Based on mass spectroscopic data, the other components of the toxic essential oil are terpineol (8.14%), camphor (3.32%), geraniol (2.50%), and borneol (0.55%).



The toxicity of the essential oil was probably caused by linalool and geraniol. Linalool is usually used for pesticides, it has major potential for use in control programs for the cat flea *(Ctenocephalides felis)* (Hink et al, 1988). Geraniol is a natural antioxidant. It has been suggested to help prevent cancer (Anonymous, 2010).

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PROFILE BETA AND ALPHA CELLS OF PANCREATIC TISSUE ON DIABETIC RAT GIVEN TEMPE ISOFLAVONE

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ABSTRACT

The purpose of this study was to analyze the profile of beta and alpha cells in pancreatic tissue diabetic rats given tempe isoflavones. A total of 20 male white rats Spraque Dawley strain aged two months have been used in this study. Rats were divided into four treatment groups, namely (1) negative control group (K-), (2) positive group tempe isoflavone (IT), (3) positive group with diabetes mellitus (DM), and (4) group of diabetes mellitus and given tempe isoflavone (DM + IT). Treatment was given for 4 weeks. Blood glucose levels in the analyze at weeks to 0, 2, and 4. At the end of the study, rats were sacrificed by anesthesia and cervical os dislocatio. Pancreatic tissue was taken and carried out to analyze of beta and alpha cells by immunohistochemistry. The results showed that administration of isoflavone can decrease blood glucose levels in diabetic rats. Immunohistochemistry staining results showed that administration of isoflavone could inhibit the rate of beta cell damage and is able to stimulate pancreatic beta cells to produce the hormone insulin. In general, the number of alpha cells not decrease in the state of diabetes, and it's number increases at administration tempe isoflavone, although not significantly different compared with other treatments.

Keywords: isoflavone, diabetic rat, beta and alpha cells, tempe

INTRODUCTION

Diabetes mellitus is a metabolic syndrome that multifactor condition characterized by chronic hyperglycemia resulting from the inability of pancreas to produce enough insulin or the body's inability to use insulin effectively (Stumvoll *et al*. 2005). Causes of diabetes include genetic factors, obesity, free radicals, immune system, and glucose toxicity (Robertson *et al*., 2004).

The pancreas has two units namely exocrine and endocrine glands. Exocrine gland produces a number of digestive enzymes and endocrine gland or also known as islets, containing four types of cells that have the ability to produce hormones. These cells is alpha cells to synthesize of glucagon, beta cells synthesize of insulin, delta cells synthesize of somatostatin, and pancreatic polypeptide cells synthesize of polypeptide enzymes. Insulin and glucagon hormones involved in regulating carbohydrate metabolism. Insulin function lowering blood sugar, while glucagon opposite, namely raise blood sugar levels (Kim *et al*., 2007).

In accordance with the provisions of Word Health Organization (WHO) (1999), Clinically there are two major groups of diabetes mellitus (DM), that is of type 1 diabetes, also known as IDDM (insulin-dependent diabetes mellitus), and type 2 diabetes also known as NIDDM (non insulin-dependent diabetes mellitus). Type 1 DM patients, there is generally a thorough destruction of insulin-producing beta cells in approximately 90% resulting in severe insulin deficiency and the patient must get insulin regularly. In type 2 diabetes mellitus, pancreatic beta cells still produce insulin or the beta cells are not damaged, sometimes higher than normal levels, but the body develops immunity to the effect that resulted in relative insulin deficiency.

Tempe as a fermented soy food reportedly contains isoflavone compounds (genistein, daidzein, glisitein, and factor-2) is beneficial to health (Nakajima *et al*., 2005). Isoflavones have a very strong antioxidant activity (Rimbach, 2003), other than reported also that the bioactive compounds genestein isoflavones can stimulate the pancreas to increase insulin secretion in vitro (Liu *et al*. 2006).

The purpose of this study was to evaluate the profile of beta and alpha cells in pancreatic tissue on diabetic rats given tempe isoflavones.

MATERIALS AND METHODS

Preparation of animal experiments

In this study was used a total of 20 white male rats Spraque Dawley strain the average weight of 200 g. Rats were divided into four treatment groups, each treatment consisted of five rats. Rats made into diabetes mellitus (DM) was induced by a single dose of 120 mg alloxan/kg bw intraperitoneally (Kim et al. 2006). Tempe isoflavones used in doses of 10 mg/200 g bw / day. Rats were divided into four treatment groups, namely (1) negative control group (K-), (2) positive group tempe isoflavone (IT), (3) positive group with diabetes mellitus (DM), and (4) group of diabetes mellitus and given tempe isoflavone (DM + IT). Treatment was given for 4 weeks. During treatment all of rats blood glucose levels were observed at weeks 0, 2, and 4.

Analysis of blood glucose levels

The concentrations of glucose were determined by glucose oxidase biosensor method, using Blood Glucose Test Meters GlucoDr^{TM} AGM-2100 model (manufactured by allmedicus Co. Ltd. Korea).

Immunohistochemistry staining

Immunohistochemical staining of pancreas tissue preparations using two-step indirect method (Beesley, 1995). After deparafination and rehydration, tissues were incubated with H_2O_2 in methanol to eliminate endogenous peroxidase activity, then incubated in albumin bovine serum (BSA). After being washed with PBS and then incubated in primary monoclonal antibody anti-insulin or anti-glucagon at a temperature of 4°C. Then tissue was incubated in secondary antibody Dako envision Peroxidase (Dako K 1941). Antigen-antibody reaction products were visualized by using diamino benzidine (DAB). Positive results of alpha and beta cells are shown with brown color. Total alpha and beta cells counted per field of view at 400x magnification, and performed on five different fields of view randomly on each tissue preparations.

Data analysis

The experimental design used was Completely Randomized Design. The data obtained were analyzed by analysis of variance (ANOVA). Statistical differences between the treatment group were determined by the Duncan test.

RESULTS AND DISCUSSION

Results of analysis of blood glucose levels during the experiment are presented in Table 1.

Table 1. Blood glucose level (mg/dl) rats during of 4 weeks treatment

Week	Group treatments			
	K(-)	IT	DM	DM+IT
0	108.6±7.0a	107.6±8.3 a	105.8±5.2 a	104.4±6.9 a
2	107.6±5.6 a	106.6±7.7 a	323.8±34.2 b	271.0±44.5 c
4	109.2±6.3 a	104.8±7.4 a	355.0±70.9 b	248.6±21.9 c

Value of followed by different letters in the same row indicate significantly different test results ($P < 0.05$). K (-) = negative control (normal rats), IT = positive group of isoflavones, DM = diabetes mellitus group, DM + IT = group of diabetes mellitus and were given isoflavone

In Table 1, it appears the negative control group (K-) and a group of rats were given isoflavones (IT) has a blood glucose level with the same fluctuation of blood glucose levels range of between 104.8 to 109.2 mg/dl and not significantly different (P > 0.05). Conversely, groups of diabetic rats (DM) showed that blood glucose levels rise sharply until the end of the experiment with levels of 355.0 mg/dl and significantly different (P <0.05) compared with other groups. In diabetic rats, and given the isoflavone group (DM + IT), an increase in blood glucose levels can be reduced and lower than the positive group of diabetic rats and statistically significantly different (P <0.05).

Results of analysis of beta cells and alpha cells in rat pancreatic tissue during the experiment are presented in Table 2.

Table 2. The average of beta and alpha cells of pancreatic islets of rats

Treatment	Averege of number beta cells	Averege of number alpha cells
K(-)	77.26 ± 1.01^{c}	39.66 ± 0.89^{ab}
IT	79.73 ± 1.65^{d}	40.30 ± 1.05^{b}
DM	41.33 ± 1.36^{a}	38.46 ± 0.70^{a}
DM+IT	52.10 ± 0.98^{b}	39.80 ± 0.60^{ab}

Figures followed by different letters in the same column indicate significantly different test results (P <0.05). K (-) = negative control (normal rats), IT = positive group of isoflavones, DM = diabetes mellitus group, DM + IT = group given isoflavone and diabetes mellitus.

In Table 2, it appears that treatment of rats with the isoflavone group (IT) have a total of 79.73 units of beta cells as well as higher than other groups, the rats in control group (K-) with the amount of 77.26, positive group of diabetic rats and given isoflavones (DM + IT) with 52.10 total, as well as positive group diabetic rats (DM) and total 52.10, are statistically significantly different (P <0.05). Conversely, the positive group of diabetic rats and were given isoflavones (DM + IT), the number of beta cells is higher than the positive group of diabetic rats. In contrast to beta cells, alpha cells are not many decrease. Alpha cell numbers were highest in rat given the isoflavone group (IT) with of 40.30 alpha cells, then followed with the rat group DM + IT (39.8), mice control group (K-) (39.66) , and the group of diabetic rats (DM) (38.46)

Alloxan and other diabetogenic compounds have been widely used to make animal models of diabetes, because the ability of alloxan in specifics to make damage to the pancreatic beta cells (Badole *et al*. 2007) that causes reduced production of insulin resulting in diabetes type 1. The results showed that the influence is evident alloxan diabetes in rats that causes damage to the beta cell islets, although there are variations between individual rats. According Szkuldelski (2001) there is a variation of islets histopathological changes induced by alloxan. This can be influenced by the mode of administration (intravenous, sub-cutaneous, intraperitoneal) and the resistence of each individual animal.

The number of beta cells in diabetic rats given tempe isoflavones (DM + IT), still more when compared with other rats, and significantly different (P <0.05), but the number of alpha cells in all treatments did not differ much. This fact illustrates that the isoflavone content of tempe able to resist or inhibit further destruction of beta cells of pancreatic islets which are progressive from free radicals and alloxan, and the consequent effects of hyperglycemia. Inhibition of the rate of damage caused by tempe isoflavone compounds act as antioxidants. As antioxidants, isoflavones can reduce free radical activity by preventing the chain reaction. Lee *et al*. (2004) who reported that there is great potential for isoflavones to health because of its ability as an antioxidant and prevent cell damage caused by free radicals.

By immunohistochemistry there appeared to be increasing the number of beta and alpha cells in the provision of isoflavones that is visible from the detection of hormone in the nucleus and cytoplasm in both cells. This suggests that isoflavones can induce the secretion of the hormone insulin and glucagon. As disclosed by Liu *et al*. (2006), that gensistein isoflavones can stimulate basal insulin secretion in pancreatic beta cells through activation of protein kinase mechanism.

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ANTIOXIDANT ACTIVITY OF SELECTED COMMERCIAL SEAWEEDS IN BALI

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INTRODUCTION

Antioxidants are compounds that protect cells against the damaging effects of reactive oxygen species, such as singlet oxygen, superoxide, peroxyl radicals, hydroxyl radicals and peroxynitrite (El-Baky et al., 2007). Antioxidants can cancel out the cell-damaging effects of free radicals(Sauriasari, 2006; Trilaksani, 2003). Seaweeds have become a major food ingredient in products especially in Japan, Korea, and China. In Asia, seaweeds have been used for centuries in the preparation of salads, soups and also as low-calorie foods (Kato et al., 2004).

In Bali, there are three types of seaweeds that have been consumed as vegetables and food. These seaweeds local name are *Bulung Boni* (*Caulerpa* spp.), *Bulung Sangu* (*Gracilaria* spp.), and *Euchema spinosum*. People in Bali have been consumed these seaweeds for a long time a go, but until this time there are no research or publication about the antioxidant activity of these seaweed, so this research aims to know the total phenolics , and antioxidant activity of these seaweeds.

MATERIAL AND METHODS

Material research are *Caulerpa* spp., *Gracilaria* spp., and *Euchema spinosum* seaweeds that has been collected from the beaches in Bali, 98% ethanol from Bratacho chemical, Folin Ciocalteu phenol from Merck, acid from Sigma, sodium carbonate from Merck, DPPH radical (-2.2-1-picryldihydrazil diphenil radical) from Sigma. The instrument used is a spectrophotometer (Turner SP-870), vortex (thermolyne), oven, a test tube (pyrex), mycropipette 1000 µl Gilson, digital scales, aluminum foil (Klin pack) , blender (Miyako), vacuum evaporator.

1. Seaweed extraction

Dried seaweeds were grinded by a blender become powder. 100 gram seaweed powder added with 300 ml ethanol and then stirred with a magnetic stirrer for 1 hour at room temperature. Furthermore, filtered with Whatman paper No. 42 in order to obtain a filtrate. The filtrate concentrated by rotary evaporator.

2. Determination of Total Phenolic Seaweed Extracts

Analysis using the Folin-Ciocalteu phenol reagent. 50-100 µl of sample was dissolved in ethanol. Added 1 ml Folin-Ciocalteu phenol reagent then shaken slowly. 5 ml sodium carbonate 20% was added and shaken. After 20 minutes the solution was measured with a spectrophotometer at a wavelength of 750 nm. Determination of total phenol used (+) – galat acid solution .

3. Determination of antioxidant activity using DPPH (1,1-Diphenyl-2 Picrylhidrazyl)

1 ml extract sample of seaweeds added with 3 ml of DPPH solution. Measured absorbance with spectrophotometer at a wavelength of 516 nm for 20 minutes. Determination of DPPH radical activity using a standard solution curve with the concentration of galat acids respectively 0, 50, 100, 150, 200, 250, 300 ppm. Measurement of antioxidant activity by DPPH method using galat acid as the standard.

RESULTS AND DISCUSSION

The highest percentage of total phenolic content found in *Euchema spinosum* seaweed is 2.5473%, then *Bulung Boni* (*Caulerpa* spp.) 1.9216%, and the lowest in *Bulung Sangu* (*Gracilaria* spp.) 0.8970% (Figure 1). In the determination of total phenolic compounds used standard solution of galat acid. Maximum absorption galat acid obtained at a wavelength of 750 nm. Before the inspection the total phenolic content, first made calibration curve galat acid standard solution with concentrations of 0, 100, 150, 250, and 300 mg / L. Preparation of calibration curve is useful to help determine the phenol content in the samples through a regression equation of calibration curve. From examination of galat acid standard solution calibration curve obtained by regression equation Y = 0.0021 x + 0.733 and correlation coefficient (R2) = 0.9926. The R2 is close to 1 proved that it is a linear regression equation.

The highest percentages of antiradical activity found in *Bulung boni* (*Caulerpa* spp) is 28.0857%, *Bulung Sangu* (*Gracilaria* spp,) 9.6714%, and the lowest in *E. spinosum* 2.2000%

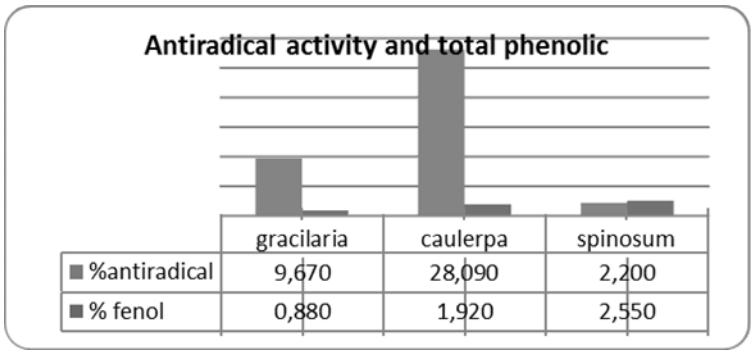

Figure 1. Percentage of total phenolic and antiradical activity of *Gracilaria* spp., *Caulerpa* spp., and *E.spinosum*

This condition indicates that antiradical activity is not affected by polyphenol compounds contained. The higher total polyphenol was not necessarily have a higher antiradical activity. In addition to containing phenolic compounds, seaweeds also contain carotenoids, that have important role as an antioxidant (Anonim, 2008; Haliwell, 2002). In seaweeds sample after added a solution of DPPH, resulting in color changes in a solution of DPPH in ethanol, which was originally concentrated violet colour to yellow. This is in accordance with Andayani *et al*. (2008), measurement of antioxidant activity of the sample performed at a wavelength of 516 nm which is the maximum wavelength of DPPH. The existence of the antioxidant activity of the samples resulted in color changes in a solution of DPPH in ethanol which was originally colored pale violet to yellow. DPPH is a stable free radical at room temperature and is often used to evaluate the antioxidant activity of some compounds or extracts of natural materials. DPPH accept an electron or hydrogen radical to form a stable molecule. Interaction of antioxidants with DPPH either electron transfer or hydrogen on the DPPH radical, would neutralize the character of the DPPH free radical. If all the electrons in DPPH free radical into pairs, then the solution color changed from dark purple to yellow light and the absorbance at a wavelength of 517 nm will be lost. This change can be measured stoichiometry corresponds to the number of electrons or hydrogen atoms are captured by molecules of DPPH due to the antioxidants (Gurav et al., 2007).

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THE UNIQUENESS OF NATA DE COCO PRODUCED BY *ACETOBACTER XYLINUM* USING SUGAR CANE MOLASSES MEDIUM

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ABSTRACT

In recent years, there is an increase demand of Nata de coco in Indonesia due to not only it's an excellent ingredient for sweet fruit cocktails, ice cream, and other recipes but also is highly regarded for its high dietary fiber. It is a food product produced by the fermentation of coconut water, which gels through the production of microbial cellulose by *Acetobacter xylinum*. This bacteria are the Gram-negative that can synthesize cellulose when grown in an undefined medium containing glucose. Cellulose is composed of the homopolymer of β-1,4-linked D-glucose. How to make Nata de coco is widely becoming known, but many attempts to produce in large quantities have failed due to various problems faced during the production process (i.e the presence of undesirable micro-organisms in the fermenting mixture, inadequate nata thickness caused by an inferior starter culture and in cases where there is no yield at all). This research aim to produce Nata de coco by using sugar cane molasses due to utilizing inexpensive carbon sources and applicable to large-scale. The experiment was done by adding combination sugar cane molasses with ammonium phosphate. Three level concentration of molasses are treated i.e. 7.5% ,10.0% and 12.5%; and ammonium phosphate was added with two level concentrations i.e. 0.1% and 0.2%. Fermenting was done in room temperature for 15 days. Glacial acetic acid is needed for achieving optimum pH ranges from 5.4 to 6.2. The result showed that sugar cane molasses was effective in improving the production rate, nata thickness and yield. The product have a unique color that look like brown sugar. A lower molasses concentration (7,5% w/v) is essential for efficient nata production. Molasses has a clear advantage over pure sugars as a carbon source from an economic viewpoint.

Keywords : *Acetobacter xylinum,* molasses, physical properties, Nata de coco

INTRODUCTION

Bacterial cellulose produced by *Acetobacter xylinum* at the air-liquid interface of coconut water is popularly known as *a nata de coco*. This unconventional product base on coconut water is native to the Philippines and was developed locally first in 1949 (Jagannath *et al*., 2008). Over the year, it has become popular in other countries like Indonesia, Japan, Korea and USA. A. xylinum uses the nutrients in the coconut water medium, forms a thin slimy, transparent layer of cellulose on the surface of the medium, which thickness with age forming a thick whitish sheet after 15-20 days. This thick sheet of cellulose is cut into cubes, washed and boiled in water before cooking in sugar syrup. Nata so formed is more than 90% water imbedded in cellulose and not only it's an excellent ingredient for sweet fruit cocktails, ice cream, and other recipes but also is highly regarded for its high dietary fiber.

Acetobacter xylinum strains are known as a very efficient producer of bacterial cellulose which, due to its unique properties, has great application potential. One of the most important problems faced during cellulose synthesis by these bacteria is generation of cellulose non-producing cells, which can appear under submerged culture conditions (Alina Krystynowicz *et al*., 2005). Bacterial cellulose demonstrates unique properties including high mechanical strength, high crystallinity, and high water retention ability, which make it an useful material in many industries, such as food, paper manufacturing, and pharmaceutical application.

The bacterium *A.xylinum* produces pure cellulose. A single cell may polymerize up to 200,000 glucose residues per second into β-1,4-glucan chains. Advantages of using a bacterial system for production of cellulose is that the bacterium grows rapidly under controlled conditions and produces cellulose from a variety of carbon sources including glucose, ethanol, sucrose and glycerol. The biosynthetic pathway for cellulose biosynthesis is very well understood in *A. xylinum*. The pathway from the substrate glucose to cellulose involves a number of reactions in which glucose is first converted to glucose-6-phosphate by enzyme glucokinase. In the second step, glucose-6-phosphate is converted to glucose-1-phosphate by enzyme phospoglucomutase. In the next step, glucose-1-phosphate is converted to UDP-glucose in presence of UTP and the enzyme UDGP-pyrophosphorylase. The UDP-glucose produced is used as a substrate by enzyme cellulose synthase (Saxena, Dandekar and Brown, 2004; Stephanie and Tetsuo, 2005).

How to make nata de coco has widely becoming known, however many attempts to produce in large quantities have failed due to various problems faced during the production process i.e. poor quality nata results from the presence of undesirable micro-organisms in the fermenting mixture, inadequate nata thickness is caused by an inferior starter culture. There are different strains of the nata organism and some strains produce thinner and softer nata than others. Moreover, some strains of nata produce a very leathery textured product which is unacceptable when processed into the sweetened products. In several cases, there is no yield at all. Beside of that, the scale of its production, processing and use is relatively small because of problems with selection of sufficiently efficient producers and cost of culture media.

Bae and Shodia (2005) reported that molasses is a potential medium in producing of bacterial cellulose by *A. xylinum*. Molasses, as a runoff syrup, is a by product of the final crystallization stage in the process of the sugar production. Because molasses is one of the most economical carbon sources, it is widely used as a substrate in microbial fermentation, e.g., in production lactic acid, polyhydroxybutyrate, ethanol and xhantan gum. However, maintaining a lower molasses concentration is essential for efficient bacterial cellulose production, this being due mainly to the complex nature of molasses. Recently, new technologies or redesigned products to meet high-value market criterion are necessary. The production of nata de coco from A. xylinum is considered as one of the most beneficial. Improved fermentation processes and using inexpensive carbon sources are required for a breakthrough toward an economical process for nata de coco production.

Base on several issues as mentioned above, this research aim to study the characteristics of nata de coco products produced by A. xylinum which using sugar cane molasses substitutes refined sugar that commonly used as a carbon sources for nata de coco production.

MATERIALS & METHODS

Materials

The material used in this research including fresh coconut water, glacial acetic acid, sugar cane molasses, culture *A. xylinimum* (nata starter) and ammonium phosphate. Fresh coconut water was obtained from local markets. Sugar cane molasses was obtained from "PG Jatiroto" which factory located at Lumajang Regency, East Java. The grade of molasses is the finest quality or called unsulphured molasses due to it is made from the juice of sun-ripened cane and the juice is clarified and concentrated, so that does not require treatment with sulfur. The chemical agents such as glacial acetic acid and ammonium phosphate were reagent grade.

Culture of *A. Xylinum* (Nata starter)

A freeze-dried culture of *A. Xylinum* was obtained from the "Puslit Bioteknologi-LIPI" that the culture was found to be pure and biochemical and morphological tests revealed that the organism conforms to the generic. The bacteria was inoculated with the medium that contains 1% glucose, 0.1% NH_4Cl, 0.115% citric acid, 0.33% Na_2HPO_4, 0.01% KCl, 0.025% $MgSO_4$. $7H_2O$, and 7.5 mg of nicotinamide per liter which both allows cellulose synthesis and can be used as a nata starter.

Formulation and procedures for Nata de coco processing

The collected fresh coconut water is filtered through a cheesecloth. For every five liter of fresh coconut water in a container is mixed with the sugar cane molasses and ammonium phosphate at particular concentration level base on each treatment combination. This research designed with six combination treatments, namely T_1 (7.5% w/v molasses and 0.1% v/v ammonium phosphate), T_2 (7.5% w/v molasses and 0.2% v/v ammonium phosphate), T_3 (10.0% w/v molasses and 0.1% v/v ammonium phosphate), T_4 (10.0% w/v molasses and 0.2% v/v ammonium phosphate), T_5 (12.5% w/v molasses and 0.1% v/v ammonium phosphate) and T_6 (12.5% w/v molasses and 0.2% v/v ammonium phosphate).

The container is covered and the mixture is boiled for 10 minutes. It is then to cool after boiling and 35 ml. of glacial acetic acid is added. The second step, 500 ml of nata starter is added to the mixture. It is subsequently transferred to big mouthed clean jars leaving ample space atop mixture and covered with clean cheese cloth. The culture is allowed to grow at room temperature for 15 days or more. Harvest is ready after 15 days or more, making sure that all conditions are aseptic so as to enable one to reuse the remaining liquid which serves as starter for succeeding preparations. The final step is dessert making. The "nata" is cut into cubes and is subjected to a series of boiling with fresh water until acidity is totally removed. One kilo of refined sugar is added for every kilo of nata and are mixed. It is brought to boiling until the "nata" cubes become transparent.

Moisture contents and thickness of nata measurement

Moisture contents were determined in triplicate using oven drying. The sample was cooled in a desiccators to room temperature and weighed. Weight loss used to calculate the percentage of moisture content in nata de coco product (AOAC, 1984).

Thickness of nata measured by using micrometer. It consists of a thimble with a rotating screw in one end and a frame at the other end. Inside the thimble, a spindle moves with the rotation of the screw. The sample to be measured is inserted into the frame, between the spindle and the anvil situated at the opposite end of the frame, and the screw is rotated until the object is fixed between the spindle and the anvil. Micrometers have a reading error of around 0.003 mm.

Taste panel evaluation

The test panel was composed of faculty members and graduate students with previous experience in evaluating the sensory parameters examined. Panel member were familiar with the evaluation form and had previously received at least on hour of instruction in its use. Panelists were seated in portioned booths with lighting in a room separate from the sample preparation room. Panelists were given water for rinsing and six samples were evaluated during each tasting session. Samples were presented in random order within a replicate and 2 replicating were completed. The sensory parameters evaluated, texture, flavor, color and overall acceptability on a 7-point of scale. The score of the panelist were subjected to analysis of variance, ANOVA and Duncan's multiple range procedure (Bowman and Cahill, 1975; Larmond, 1977).

Statistical analysis

Treatments were arranged as a factorial design and data were analyzed using Statistical Package for the Social Sciences(SPSS) procedures. When a significant main effect existed, difference between treatments was determined using Duncan' Multiple Range Test (Bowman and Cahill, 1975).

RESULTS AND DISCUSSION

Moisture contents and thickness of Nata

Analysis of variance (ANOVA) indicated there were no significant interactions between sugar cane molasses and ammonium phosphate effects on moisture contents of Nata de coco products. The mean of the moisture contents was 90.2% with a standard deviation of 2.3%. However, the moisture content values tended to show a small numerical decrease as the percentage of molasses in the mixture increased. This trend agreed with our proximate analysis of nata products shown in Table 1. As the amount of molasses in the patties increased, the moisture retained during fermenting increased. Similar results were obtained by Jagannath et al. (2009). Different processing conditions significantly ($P < 0.05$) influenced the water holding capacity of nata, while no effect of substrates was indicated. The moisture content was neither affected by processing conditions nor by various substrates.

The thickness of nata is one of the important parameter affecting quality of products. As mentioned above, how to make de coco has widely becoming known, however many attempts to produce in large quantities have failed due to various problems faced during the production process i.e. poor quality nata results from the presence of undesirable micro-organisms in the fermenting mixture, inadequate nata thickness is caused by an inferior starter culture. Analysis of variance indicated there were significant interactions between sugar cane molasses and ammonium phosphate effects on thickness of Nata de coco products. The Duncan's multiple range test indicated that there was significance in thickness of nata for most the products processed. Maximum thickness of nata was obtained at treatment 7.5% w/v molasses and 0.2% v/v ammonium phosphate concentrations. These conditions also produced good quality of nata-de-coco with a smooth surface and soft chewy texture.

Table 1-Moisture contents and thickness of Nata de coco products for various combination treatments[a]

Parameter	Treatments					
	T_1	T_2	T_3	T_4	T_5	T_6
Moisture contents (%)	91.8±2.1	90.2±1.9	89.6±3.4	91.8±2.8	90.3±1.9	89.2±2.1
Thickness (cm)	1.9±0.2[b]	2.2±0.1[c]	2.1±0.1[c]	2.2±0.2[bc]	2.3±0.3[d]	2.3±0.2[d]

[a] Values are means ± SME (n=9)

[b-d] Means in the same row bearing different superscripts are signficanly different (P<0.05)

Sensory evaluation

Sensory panel evaluation is shown in Table 2. All products were equally acceptable by the panelist. The Duncan's test showed that there were significant differences in sensory scores among the panelist. The panelist consistently rated T_2 (7.5% w/v molasses and 0.2% v/v ammonium phosphate) treatment more desirable (P<0.05) than other treatments. In all cases suggests that lower levels of sugar cane molasses might result in better sensory response. The concentration of sugar cane molasses were also scored consistently the 12.5% w/v rated as less (P<0.05) desirable than other concentration in each case. Bae and Shoda (2005) also suggest maintaining a lower

molasses is essential for sensory consideration. Overall acceptability scores indicated that nata de coco produced by treatment T_2 is the most desirable.

Table 2 Sensory panel evaluation scores for Nata de coco products for various combination treatments

Parameter[a]	Treatments					
	T_1	T_2	T_3	T_4	T_5	T_6
Flavor	4.88^{b}	4.91^{c}	4.66^{d}	4.86^{bc}	4.23^{bd}	4.52^{bcd}
Texture	4.52^{b}	4.75^{c}	4.33^{d}	4.82^{bc}	4.28^{bd}	4.33^{d}
Color	3.92^{b}	4.97^{c}	3.33^{d}	4.12^{bc}	3.75^{bd}	3.14^{bcd}
Overall acceptability	4.46^{b}	4.87^{c}	4.21^{d}	4.66^{bc}	4.32^{bd}	4.15^{bcd}

[a]Samples presented using a hedonic scale of 1=dislike extremely, 4=neutral, 7=like extremely
[b-d]Means in the same row bearing different superscripts are significantly different ($P<0.05$)

CONCLUSION

Different formulation of substrates significantly influenced the thickness of nata, while the moisture content was no significantly affected by various substrates. The uniqueness bacterial cellulose produced by *A.xylinum* using sugar cane molasses are it could be achieved adequate thickness, good quality of nata with a smooth surface, soft chewy texture and the unique color, look like brown sugar. In, addition this characteristics is the most desirable by panelist. This study suggest, treatment with 7.5% w/v molasses and 0.2% v/v ammonium phosphate concentrations is more appropriate than others concentrations.

The results of this study showed that sugar cane molasses was effective in improving the production rate, nata thickness and yield in a lower concentration. Through utilizing molasses as carbon sources for nata production, will enable efficient utilization of coconut water, a hitherto wasted byproduct of coconut industry and will also provide a new product dimension to the aggrieved coconut farmers who are not getting the right price for their product.

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PROTEASE ACTIVITY OF PROTEIN FRACTION CONTAINING RECOMBINANT ACTINIDIN EXPRESSED IN *Saccharomyces cerevisiae*

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ABSTRACT

Recombinant actinidin is a proteolytic enzyme found kiwi fruit (*Actinidia chinensis*). Recombinant actinidin was expressed in the yeast *Saccharomyces cerevisiae* and the production of recombinant actinidin was acquired at exponential growth phase, precipitated by ammonium sulphate to 30 % saturation. However protease activity of protein fraction containing recombinant actinidin was not reported yet, therefore, it was needed to be analysed. The objective of this study was to know the protein activity of fraction containing recombinant actinidin expressed in *Saccromyces cerevisiae*. Transformant was obtain from previous study. The expresion of recombinant actinidin protein was done by cultivating transformant (*Saccharomyces cerevisiae* BF 307 -10 carrying recombinant plasmid pYSV9~R1) in 5 x 500 ml of YEPD medium. Supernatant protein was harvested at the exponential growth phase (8 hours incubation), then fractionated by precipitation level at saturation of 30 % of ammonium sulphate. The result of fractionation was dialysed and protease activity then analysed. The result of this study showed that protein fraction containing recombinant actinidin had a protease activity. Concentration of soluble amino acid was 9.425 g/l.

Keywords: protease activity, fraction protein, recombinant actinidin, expression, secretion, *Saccromyces cerevisiae*

INTRODUCTION

Recombinant actinidin is a proteolytic enzyme found kiwi fruit (*Actinidia chinensis*). Recombinant actinidin was expressed in the yeast *Saccharomyces cerevisiae* and the production of recombinant actinidin was acquired at exponential growth phase (8 hours incubation), precipitated by ammonium sulphate to 30 % saturation (Anggreni, 2009). However protease activity of protein fraction containing recombinant actinidin was not reported yet, therefore, it was needed to be analysed.

AIM

The objective of this study was to know the protease activity of protein fraction containing recombinant actinidin expressed in *Saccharomyces cerevisiae*.

MATERIALS AND METHODS

Transformant was obtained from previous study. The expression of recombinant actinidin protein was done by cultivating transformant *Saccharomyces cerevisiae* BF 307 -10 carrying recombinant plasmid pYSV9~R1(R) in 5 x 500 ml of YEPD medium. The same treatment was done for *Saccharomyces cerevisiae* BF 307 -10 carrying plasmid pYSV9 (P) and *Saccharomyces cerevisiae* BF 307 -10 without plasmid (BF) as controls. Supernatant protein was harvested at the exponential growth phase (8 hours incubation), then fractionated by precipitation level at saturation of 30 % of ammonium sulphate. The result of fractionation was dialysed, and protease activity (Praekelt method, 1987 *in* Yuwono, 1991) then analysed.

RESULTS AND DISCUSSION

Table 1. Protease activity of protein fraction containing recombinant actinidin was compared with controls

Code	Soluble amino acid concentration (µg/µl)*
R	9.425
P	2.925
BF	2.788

Note : R : transformant *S. cerevisiae* BF 307 -10 carrying recombinant plasmid pYSV9~R1, P : *S. cerevisiae* BF 307 -10 carrying plasmid pYSV9, BF : *S. cerevisiae* BF 307 -10 without plasmid),* showed the enzyme activity

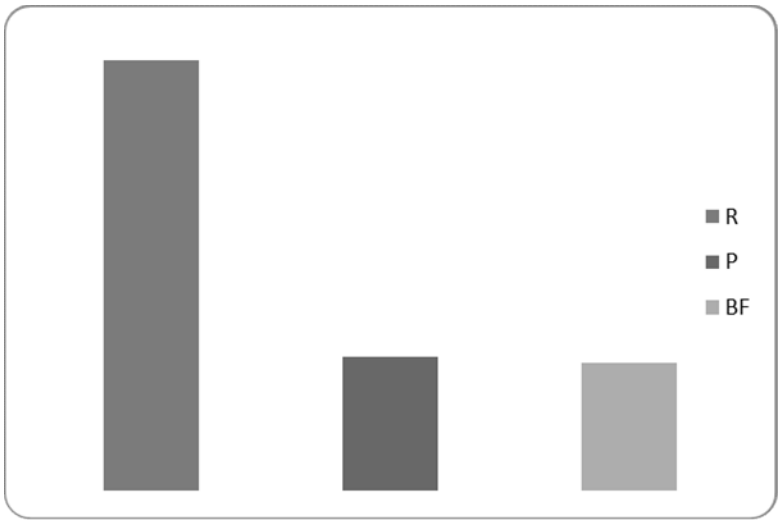

Figure 1. Protease activity of protein fraction containing recombinant actinidin was compared with controls. R (transformant *Saccharomyces cerevisiae* BF 307 -10 carrying recombinant plasmid pYSV9~R1), P (*Saccharomyces cerevisiae* BF 307 -10 carrying plasmid pYSV9), BF (*Saccharomyces cerevisiae* BF 307 -10 without plasmid),* showed the enzyme activity.

Measurement of protease activity was done to know the protein base character in sample. It was proteolytic enzyme or not. Protease activity was measured based on soluble peptide in trichloroacetic acid (TCA) that was formed as a result of casein hydrolysis by enzyme activity. The result showed that protease activity of protein fraction containing recombinant actinidin was higher then both of controls because actinidin is a protease enzyme (Table 1 and Fig.1).

CONCLUSION

Protein fraction containing recombinant actinidin had a protease activity. Concentration of soluble amino acid was 9.425 g/l.

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THE USE OF POLARIMETRIC ASSAY FOR HONEY QUALITY DETERMINATION IN CORELATION WITH ITS TOTAL REDUCTION SUGAR CONTENT

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ABSTRACT

Polarimetric assay which is simple, fast and accurate method have been developed to determine honey quality based on its optical activity properties. In this research, the value of optical rotation of refference honey have been measured which is -32.4o, and its total reduction sugar content was 62.46% (have met National Standard Industry). The addition of external component (such as water, liquid palm sugar, and liquid sugar cane) into the refference honey can significantly reduce the value of optical rotation of the honey. From this research, it is also found that there was a linier corelation between the optical rotation value and the total reduction sugar content of honey. The higher the value of the total reduction sugar content, the higher the optical rotation value. In conclusion, the value of optical rotation can be used as one criteria to determine the quality of honey.

Keywords : Honey Quality, Polarimetric Assay, Reduction Sugar.

DELIGNIFICATION OF SUGARCANE BAGASSE WITH SODIUM HYDROXIDE SOLUTION BEFORE SACCHARIFICATION ENZIMATICALLY USING CRUDE CELLULASE FROM *Aspergillus niger* FNU 6018

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ABSTRACT

Cellulose, the most abundant renewable resource, has received much attention as potential energy and carbon source for the production of useful products. The possibility of converting cellulose in bagasse enzymatically into glucose, after being loosened its complex structure chemically into primary one by using sodium hydroxide was studied. Bagasse was soaked in 6% sodium hydroxide for 12 hours at room temperature. This treatment resulted in loosening some cellulose bundle structure shown by release of lignin and hemicelluloses up to 32.11 and 42.87 %, respectively and high water retention value of 15.90 (w/w). In this condition the delignified bagasse could be saccharified by crude cellulase enzym from Aspergillus niger. Saccharification enzimatically of 2 g delignified bagasse during 120 hours produced reducing sugar of 54.47mg/100 ml.

Keywords: Bagasse, delignification, sodium hydroxide, crude cellulase enzym, *Aspergillus niger*, saccharification

DESTRUCTION MACHINE DESIGN OF MUNICIPAL SOLID ORGANIC WASTE

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ABSTRACT

Machine for destruction organic municipal solid waste was designed by modified system input and system size reduction. By this process size and shape of organic municipal waste were homogen, so that the composting process was effective. Performance of the machine was test at 8 hours. Capacity of destruction and quality of destruction were observe. Before test of machine performance, the functional of every element were test. From functional test get modification large of electricity and the frame of machine. The machine capacity was 22 – 27 kg/hours, number of organic municipal waste through sieving 1.5 x 1.5 cm^2 was 70 – 85 %, and bulk density was 240 – 270 kg/m^3.

SURVIVAL OF FREEZE-DRIED LACTOBACILLUS RHAMNOSUS R21 IN THE PRESENCE SKIM MILK AS PROTECTANT DURING STORAGE

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ABSTRACT

Lactic acid bacteria are the most important bacteria having potential as probiotic. The objectives of the present study were to evaluate influence of skim milk as protectant on survival of freeze-dried Lactobacillus rhamnosus R21 which is isolated from breast milk during storage and to calculate the shelf-life of freeze dried Lactobacillus culture. To predict the shelf life of freeze dried culture, further experiment was conducted by storage the freeze dried of Lactobacillus rhamnosus R21 at RH 75 and RH 90 and shelf life was predicted by sorption isotherm method. Evaluation was done on under act water content, viability, water activity, acidification activity. The result showed that water content and water activity increased during storage from 2.17% db - 21.59% db and 0.099 – 0.801 respectively. Viability and acidification activity of freeze-dried culture decreased from 11.49 log cfu/g – 0 cfu/g. The predicted shelf life of the freeze dried Lactobacillus rhamnosus R21 culture if initial water content is 2.17% db, packaged in aluminium foillaminated by PE (polyethylene) and temperature 30oC are 5.86 years at RH 75 and 5.10 years at RH 80.

Keywords: *Lactobacillus rhamnosus* R21, breast milk, freeze drying, cryogenic, shelf life

MICROBIOLOGICAL, BIOCHEMICAL AND SENSORIAL CHARACTERISTICS OF FERMENTED MILK PRODUCED BY PROBIOTIC *LACTOBACILLUS* SP. SKG34

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ABSTRACT

Application of probiotic is gained interest due to its health promoting significant. Consumption of probiotic strain often hampers by technological production and thus administration of probiotic strain incorporates in food products is preferred. Recently, we have screened *Lactobacillus sp* SKG34 as good probiotic candidate. In order to develop this strain as probiotics, the suitable foods carrier needs to be considered. The objectives of this research were to determine the population of Lactobacillus sp. SKG34 in the fermented milk, biochemical changes, and organoleptic of the product following fermentation by this bacterium. RAPD (Random Amplified Polymorphic DNA) analysis of this bacterium showed polymorphic bands of about 200, 300, and 600 bp. The same analysis on 10 colonies of LAB also gave the same bands as shown by the Lactobacillus sp SKG34, indicating that Lactobacillus sp SKG34 dominated the fermented milk during storage. The total LAB increased from 2.5 x 108 to 7.6 x 109 cfu/ml after 4 days storage, but decreased to 3.1 x 108 cfu/ml when storage was prolonged to 8 days. The growth of LAB in the milk resulted in pH reduction by 0.29 pH unit (from 4.32 to 4.03) within 8 days storage. However, the concentration of the dissolved protein increased from 0.046% to 0.084% after the same period of storage. During storage some free amino acids, such as aspartic acid, glutamic acid, serine, histidine, glysyne, threonine, alanine, tyrosine, methionine, isoleucine, leucine, and lysine were found to increase, but arginine, valine and phenylalanine decreased. In the organoleptic tests, this fermented milk was still acceptable by panelists, although it had been stored for 8 days at 10^{o}C.

Keywords: Probiotic, Lactobacillus, Fermented Milk, RAPD.

GENETIC IDENTIFICATION AND CARBOHYDRATES METABOLISMS OF *LACTOBACILLUS SP.* SKG34, A BILE-SALT HYDROLYZING LACTOBACILLUS ISOLATED FROM SUMBAWA MARE MILK

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ABSTRACT

Introduction and Objectives. *Lactobacillus sp* SKG34, a bile-salt hydrolyzing Lactobacillus was isolated from sumbawa mare milk. The aimed of this research were to identify this Lactobacillus by molecular biology method and to characterize the strain based upon the profile of carbohydrates metabolisms. Material and Methods. *Lactobacillus sp* SKG34 was used in this study. Carbohydrate metabolisms profile of *Lactobacillus sp* SKG34 was carried out using API CHL50 for lactobacilli while genetic identification was performed by sequencing of the 16S rDNA. The genomic DNA was extracted using ISOPLANT II DNA isolation Kit following amplification of the 16S rDNA. Almost entire length of 16S rDNA was amplified and was sequenced using BigDye terminator sequence kit (Applied Biosystems, Japan). The assembled sequence was subjected to homology analysis (http://www.ncbi.nlm.nih.gov/). Results. The results showed that *Lactobacillus sp* SKG34 shared 98% (1471bp identicalout of total 1489bp sequence) similarity with Lactobacillus rhamnosus ATCC53103 (EMBL acc. AP011548.1). Specific carbohydrates metabolisms showed that *Lactobacillus sp* SKG34 was able to metabolize amydon (starch) but not metabolize turanose and lyxose, where the L rhamnosus commonly does not metabolize starch amydon but metabolize turanose and lyxose. These demonstrated the specificity of this strain. Conclusion: *Lactobacillus sp* SKG34 shared 98% homology with *L. rhamnosus* ATCC 53103. Specific characteristics on sugar metabolism, which different from commonly *L. rhamnosus* along with quite low 16S rDNA similarity suggested that the strains highly possible to be a specific strain or belonging to a sub-species of *L. rhamnosus.*

Keywords: Lactobacillus, genetic identification, bile-salt, mare milk

THE CHARACTERISTIC OF BABY BISCUIT WHICH MADE FROM THE KIND OF BANANA'S FLOUR

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ABSTRACT

The main objectives of this research was to know the characteristic of baby biscuit which made from the kind of banana's flour and determined the best biscuit which of consumer's preference. This research applied with randomized completely design with two factors. First factor was the kind of banana's flour (*kepok, raja* and *tanduk*) and the second factor was concentration of adding banana's flour with concentration 25, 20, and 15 percent. All treatments were done with 2 replications that obtained 18 unit treatments. The result of this research indicated that the characteristic of baby biscuit which made from the kind of banana's flour are moisture content range from 16.960 – 27.825% , protein content range from 13.987 – 16.502 %, fiber content range from 6.330 – 15.915% and ash content range from 1.364 – 1.785%. All of baby biscuit are fulfilling of specification quality except moisture content. The best baby biscuit which of consumer's preference is made from 'kepok' banana's four with adding 15%. The characteristic of biscuit are the specially tempeh aroma's (4.05), white – dust colour (3.40), rough texture (2.95) and the consumer's preference is like (3.45).

Keywords : baby biscuit and banana's flour

POSTER PRESENTATIONS:

BIODIVERSITY

AND

ENVIRONMENT

PRELIMINARY STUDY OF CELLULOLYTIC BACTERIA IN RICE STRAW DECOMPOSITION

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ABSTRACT

Cellulolytic bacteria is microorganism that has capability to degrade cellulose to glucose so that this bacteria may can be used as candidate for a good decomposer. The decomposition is expected to produce benefit matter compost. In this research, 79 isolates of bacteria were successfully screened. Among of them, 21 of isolates were categorized as cellulolytic bacteria when assayed semi-quantitatively at medium containing CMC on agar plate. MKA-70 and MKA-79 are best decomposer comparing with others. MKA-70, identified as a Gram-positive bacillus, capable to produce reducing sugars 94.67 ± 9.63 mg/l, 37.80 ± 2.05 mg/l and 36.03 ± 3.27 mg/l when assayed at CMC-pepton, rice straw-pepton and rice straw. But MKA-79 identified as a Gram-negative coccobacillus, produced 97.80 ± 2.05 mg/l, 36.28 ± 0.91 and 32.70 ± 6.27 mg/l respectively.

Key words: cellulolytic bacteria, rice straw, cellulose.

INTRODUCTION

Cellulolytic bacteria are microorganisms that have ability to utilize carbon source from cellulose through decomposition enzymatically. During decomposition the specific enzymes are released [1]. In nature, cellulose degradation is a way of carbon and energy cycle [2]. Some genus of bacteria are known to have cellulolityc activity in board spectrum towards substrates, eg; *Pseudomonas* [3], *Clostridium* [4], *Bacillus* [5] and *Vibrio* [6] where some of them are also found in rice straw waste. Rice straw containing 35% of cellulose [7] is organic source and mainly produced in tropical countries during harvesting of rice paddy. They are released in huge quantities with no economic value. Some of them are incinerated and may increase the greenhouse effect [8]. To manage this waste, a research was done by introducing of cellulolytic bacteria as bioactivator and proved that they can decreased of C/N ratio from 40 to be 15 in two weeks [9]. Other research reported that utilization of bioactivator containing cellulolytic bacteria can be accelerated the decomposting time from 3-4 months to be 2-4 weeks [10]. Based on this references above suggested that some cellulolytic bacteria are known to be potential indigenous microorganism which easily adapt to any environment, and may possible can be obtained from rice straw wastes. Here, our research is try to screen and characterized the cellulolytic bacteria isolated from rice straw wastes.

MATERIALS AND METODHS

Sampling of Isolates; Twenty five grams of composted rice straw sample from different site of Jember District (Patrang, Antirogo, Sumber Pinang, Wirolegi and Ajung) were suspended to 0.9% of NaCl solution 225ml in Erlenmeyer flask. From this suspension, the sample diluted from 10^{-2} to 10^{-8} from initial concentration. For screening, one hundred micro liters of each diluted samples were plated to NA medium and incubated for 48 hours at 30°C. These steps were repeated until getting the single colony of some different type of microorganisms. From this step, 79 isolates of bacteria were successfully screened. All isolates were further pre-culture in the same medium for 24 hours at 30°C.

Screening of Cellulolytic Bacteria by Semiquantively; The Congo-Red method [11] was employed on this analysis. Pre-culture isolates were assayed on CMC media for

screening of cellulolytic activities semiquantively. The clear zones indicate that cellulase hydrolyzed the CMC medium. Activity index was calculated by the comparison of clear zone with the diameter of colony. Five isolates with the highest activity index were then selected for further analysis.

Growth analysis; The pre-culture of five isolates were grown in Nutrient Broth (NB) in 90 rpm at 25°C. In every 6 hours, growth analysis was done by using Total Plate Count (TPC) method. One ml of sample from each isolate was diluted at 10^{-1} to 10^{-16}, and 10 µl of each sample was drop-platted to NA medium and incubated at 30°C for 48 hour. The colony was then counted using colony counter and the total cell per ml of sample was calculated under the formula as follow.

$$\text{Total cell per ml} = \text{mean of total colony per sample } x \frac{1000ul}{10ul} \text{ x } \frac{1}{\text{dilution factor}}$$

Hydrolysis of cellulose from rice straw using selected cellulolytic bacteria; Further analysis of capability of cellulolytic bacteria to decompose cellulose was done under liquid culture using rice straw as substrate. One hundred µl of pre-culture of each isolate was grown in 10 ml suspension containing 1% powdered/pulverized rice straw medium with and without 0.5% peptone. For the comparison, 1% CMC medium containing 0.5% peptone was also used. All assayed were done in duplicate and incubated at 30°C 90 rpm. The culture of each isolate was sampled in 1 ml for every 24 hours and centrifuged at 4000 rpm for 5 minutes to obtain the hydrolyzates. And then the hydrolyzates were analyzed by measuring reducing sugar at 500 nm using the method of Somogyi-Nelson [12, 13, 14]. For the calibration, D-glucose was used as standard.

Microcopies analysis; This analysis is to observe the cell form and was used for an empirical analysis using the method of differentiating bacterial Gram staining (or Gram's Method).

RESULTS AND DISCUSSION

Seventy nine of isolates were successfully screened but among of them only twenty one isolates of bacteria positively had cellulolytic activity which are indicated with the clear zone when they were grown on CMC agar plate medium (Table 1). The clear zone proved that the isolates broke the cellulose binding to be reducing sugar as mono or oligomer of glucose [15] by secreting an extracellular cellulase when they grown using CMC. While the rest of 58 isolates had no ability to grow on the same medium and it's seem they could not utilize CMC as carbon source.

Table 1: The Activity index of cellulolytic bacteria

Isolate	Activity index	Isolate	Activity index	Isolate	Activity index
MKA-04	2	MKA-38	1	MKA-69	1
MKA-13	1.2	MKA-49	3	**MKA-70**	**3.2**
MKA-14	1.2	MKA-55	1.2	MKA-71	1.2
MKA-15	1.2	MKA-61	1	MKA-72	1.2
MKA-16	**5.2**	**MKA-64**	**3.2**	MKA-73	2.4
MKA-23	2	**MKA-65**	**3.7**	MKA-78	1
MKA-27	1	MKA-68	1	**MKA-79**	**5.5**

Five isolates MKA-16 (5.2), MKA-64 (3.2), MKA-65 (3.7), MKA-70 (3.2) and MKA-79 (5.5) gave the highest of cellulase activity index (Table 1). However, this semiquantitative method could not be referred to quantify amount of sugar released during they were grown on CMC plate medium [11].

Growths analyses resulting in various result of each isolate which indicate that every isolate had differences in their metabolism. MKA-64, MKA-65 growth rapidly with the total cell are 1.58×10^{13} and 4.04×10^{13} cells/ml at 12 hours incubation, and MKA-70 reached 2.38×10^{12} cells/ml at 18 hours incubation. However, MKA-79 and MKA-16 growth maximum at 3.62×10^{12} and 2.10×10^{13} cells/ml after 30 hours incubation which grew slower than others.

MKA-70 and MKA-79 released the reducing sugar 94.67 ± 9,63 mg/l and 97.80 ± 2,05 mg/l when they were grown in medium containing 1% CMC and 0.5% peptone for 6 days. But MKA-16, MKA-64 and MKA-65 could release maximum reducing sugar 23,81 ± 1,55 mg/l, 18.15 ± 0,84 mg/l and 13,86 ± 1,48 mg/l although incubation time were extended to 7 days. No significant results were found when they were grown on medium containing 1% rice straw and 0.5% peptone.

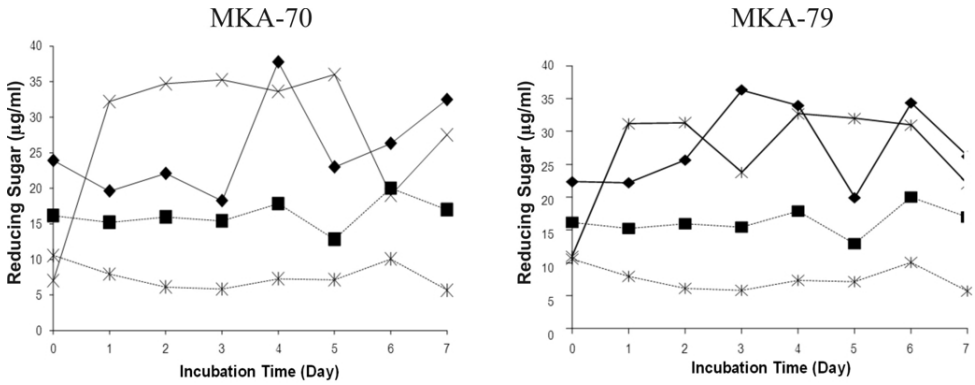

Figure 1. Profiles of reducing sugar production during cultivation of isolates MKA-70 and MKA-79 on various substrates (×) 1% powdered rice straw only and (♦) 1% powdered rice straw only and 0.5% peptone. For controls, the same medium were used (✳ : 1% powdered rice straw only and (■)1% powdered rice straw only and 0.5% peptone without any inoculation.

MKA-70 and MKA-79 still gave highest activity comparing with three others isolates even the reducing sugar produced almost only half (37.80 ± 2.05 mg/l and 36.28 ± 0.91 mg/l) if compare with previous cultures using CMC medium and peptone. This evidence is not indicate the decreasing of cellulase activity from each isolates but these occurrences seemed to be more reflected on the differences of substrates component where rice straw contain only 35% of cellulose. Furthermore, MKA-70 and MKA-79 produced total reducing sugar (36.03 ± 3.27 mg/l and 32.70 ± 6.27 mg/l) almost similar even they grown on rice straw without nitrogen source from peptone (Figure 1). Suggested, these two isolates not only utilized carbon but also nitrogen source from rice straw where the previous research also reported that rice straw containing 4.5% crude protein [16]. These two isolates are they are different type of bacteria. MKA-70 is a bacillus gram positive while MK-79 is coccobacillus gram negative bacteria.

CONCLUSION

Two isolates MKA-70 and MKA-79 identified as a gram positive and negative bacteria are to be potential decomposer. They proved readily degrade the rice straw even no nitrogen sources was added. During hydrolization, MKA-70 and MKA-79 can released reducing sugar 36.03 ± 3.27 and 32.70 ± 6.27 mg/l respectively.

ACKNOWLEDGMENT

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IN-VITRO ANALYSIS OF ISOLATE MICROBES OF STRAW ON PATHOGENS

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ABSTRACT

Compost is produced through decomposition of organic material which makes use of microorganism. Besides, microorganisms are capable to decompose organic material, some microbes may also have capability to inhibit pathogen bacteria. These potential microorganisms have been widely used for biological control agent. In this research, two bacteria named MKA-16 and MKA-79, and two fungi isolates named W-4 and SP-5 derived from straw were assayed for their potential as biological control agent against pathogen microbes *Xanthomonas axonopodis* pv, Ralstonia solanacearum, Fusarium graminearum. Analysis showed that MKA-16 and MKA-79 only have capability to inhibit *X. axonopodis* while W-4 inhibits *X. axonopodis* and *F. oxysporum*. However, SP-5 is much better than the others because of its capability to inhibit the growth of three pathogens species (*X. axonopodis, F. oxysporum* and *F. graminearum*). All isolates only inhibit the growth process but not kill the pathogens so that the types of inhibition of each isolateare bacteriostatic and fungistatic. Among 4 isolates, SP-5 has the highest activity index (2.3) against *X. axonopodis* even lower index activities is detected when it is testsed against *F. oxysporum* (1.1) and *F. graminearum* (1.46).

Keywords: inhibition, pathogen, bacteriostatic, fungistatic

INTRODUCTION

Compost is produced through decomposition of organic material which makes use of microorganism. Besides, microorganism are capable to decompose organic material, some microbes may also have capability to inhibit pathogen bacteria. These potential microorganisms have been widely used for biological control agent (Weller, 1988). Interest in biological control has increased over the past years, driven by the need for alternatives to chemicals-which have often lost their activity due to the development of resistant pathogen populations-and to public pressure to develop production system favorable to the environment (Berg and Hallman, 2006).

Previous research results showed that microbe decomposers from straw which is cellulolytic are bacteria MKA-16 and MKA-79 (Aisyah, 2009); fungi W-4 and SP- (Azizah, 2009). Those microbes will have more value if they have not only a high cellulolytic ability but also an inhibiting ability on the growth of microbial pathogens of plants. Microbe decomposers from straw which have the ability to inhibit the growth of plant pathogens can be developed as a potential starter of compost.

MATERIALS AND METHODS

Time and Place

Research was conducted at the Laboratory of Microbiology, Faculty of Mathematics and Natural Sciences University of Jember. The research was conducted on May until December 2009.

Materials

Microbe decomposers used are bacteria MKA-16 and MKA-79; fungi W-4 and SP-5. The microbial pathogens are bacteria R.solanacearum and X.axonopodis pv. Glycines; fungi F.oxysporum and F.graminearum (Plant Clinic FAPERTA UNEJ collection). Media used consist of media Potato Dextrose Agar (PDA), Nutrient Agar(NA), Yeast Malt Extract Agar (YMEA), and Potato Dextrose Broth (PDB). Reagents used consist of chloroform, 0.5% peptone liquid, and 0.05% tween 80.

The Tests on the Activity of Isolate Decomposers Inhibition On the Growth of Pathogen and among Isolate Decomposers Qualitatively and Quantitatively

Tests for the inhibition of isolate decomposers against microbial pathogens andintra-microbe decomposers was qualitatively done using scratch method (Waksman,1959). Quantitative tests was calculated by using Dual Plating method. Inoculum'sdensity of isolate decomposers and pathogens in supple agar media required for tests was107cells/ml. The inhibiting ability of each isolate decomposers were determined by the inability of pathogen colonies to grow well in every media which previously had been inoculated by isolated decomposers on scratch method and on the size of the inhibition zone index formed at the Dual Plating method.

The test on The Inhibiting Nature of Microbe Decomposers on The Growth of Microbial Pathogen

Agar in the inhibition zone was taken using an ose needle and was inserted into 5 ml of 0.5% peptone liquid for bacteria and fungi by using PDB on Erlenmeyerflask, and then they were incubated at 120 rpm shaker incubator at 300C for 1 to 3 days. Ifpeptone liquid and PDB are turbid after incubation is done, it means that the microbedecomposers are bacteriostatic on bacteria and fungistatic on fungi, in contrast if peptoneliquid remains clear, it means that the microbe decomposers are bacteriocide on bacteriaand fungicide on fungi.

RESULTS AND DISCUSSION

The Inhibition Tests of Isolate Decomposers on the Growth of Pathogens Qualitatively

The results showed that the four isolates decomposers namely; bacteria MKA-16 and MKA-79, fungi W-4 and SP-5 have the ability to inhibit the growth of microbial pathogens. But only three types of the four microbial pathogens testsed which colony growth is inhabited; bacteria X. axonopodis pv. Glycines, fungi F. oxysporum, and fungi F. graminearum. The inhabiting ability of each microbe decomposers on the growth of each microbial pathogen can be seen in Table 1.

Table 1. Inhibiting Ability Tests Results of Bacterial and Fungal Isolates Decomposerss on the Growth of Pathogens Qualitatively

Isolate	Microbial Pathoge		
Decompaserss	*X axonopodis*	*R. solanacearum*	*F. axy*
Bacteria MKA-16	(+)	(-)	(-)
Bacteria MKA-79	(+)	(-)	(-)
Fungi W-4	(+)	(-)	(+)

Bacteria MKA-16 (Figure 1a, 1b, and 1d) and bacteria MKA-79 (Figure 1c.) isolates are able to inhibit the growth of bacteria X. axonopodis pv. Glycines but they areunable to inhibit the growth of bacteria R. solanacearum, fungi F. oxysporum, and F.graminearum. Meanwhile, fungi isolates SP-5 (Figure 1g and 1h) inhibit the growth of bacteria X. axonopodis pv. Glycines, fungi F. oxysporum, and fungi F. graminearum but they do not inhibit the growth of bacteria R. Solanacearum. Fungi isolates W-4 (Figure 1e and 1f) can inhibit bacteria X. axonopodis pv. Glycines and fungi F. oxysporum but they do not have the ability to inhibit bacteria R. solanacearum and fungi F.graminearum.

At this stage of research, there are three possible mechanisms of inhibition by isolate decomposers that are space and nutrients competition, competition for living space and nutrients, such as carbohydrates, nitrogen, growth regulators and vitamins; antibiosis, inhibition or destruction of an organism by metabolites produced by other organisms.Antibiosis mechanism of antagonistic isolates produces antibiotic compounds or other compounds, decreasing environment pH causes microbial pathogens activity to be disturbed and then microbial pathogens cannot survive (Berg and Hallman, 2006).

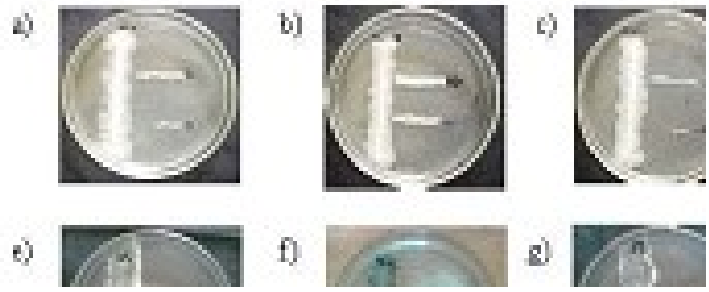

Figure 1. Tests Results of Inhabiting Bacteria Isolate and Fungi Decomposers in the Growth of Pathogens

The inhibiting ability of decomposers fungal isolates W-4 and SP-5 was not only able to inhibit the pathogen, but also capable of inhibiting the growth of isolate decomposers MKA-16 and MKA-79. When decomposers fungal isolates W-4 and SP-5 are used as agent of biology control along with isolate decomposers MKA-16 and MKA-79, they will reduce or even eliminate the inhibiting ability possessed by each isolate. The inhibiting ability possessed by fungi isolates W-4 and SP-5 and bacterial isolates MKA-16 and MKA-79 will work out effectively when they are used separately or not at thesame time.

A Quantitative Tests on the Activity of Isolate Decomposers Inhibition On the Growth of Pathogen and among Isolate Decomposers

The tests is only done to microbial pathogen which growth is positively inhibited by isolate decomposers. The following table 2 shows the result of observation on the testson the activity of isolate decomposers inhibition on the growth of pathogen based on the index of growth inhibition zone.

Table 2. The Activity of Isolate Decomposers Inhibition on The Growth of Pathogen Based on The Index of Growth Inhibition Zone

Isolate Decomposers	Microbial Patho	
	X. axonopodis	*F. Oxysporun*
Bacteria MKA-16	1,67	X
Bacteria MKA-79	1,67	X
Fungi W-4	2,00	1,68
Fungi SP-5	2,30	1,10

Note: X = no tests is done due to isolate decomposers c pathogen

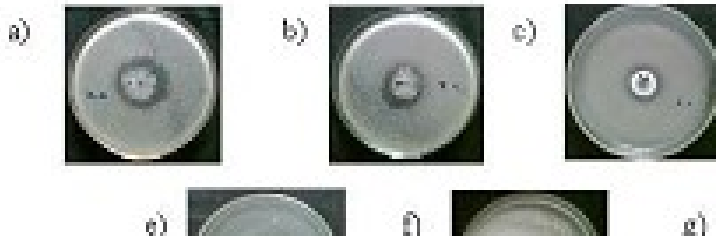

Table 2 and Figure 2 shows that the biggest inhibition is possessed by isolate Fungi SP-5 in inhibiting bacteria X. axonopodis with the value of inhibition as much as 2,30. While the smallest ability is possessed by isolate SP-5 in inhibiting F. oxysporum with index value of 1,10. Index of growth inhibition zone gained is the comparison between the diameter of limpid zone and the diameter of isolate decomposers colony. Isolate SP-5 can inhibit all three pathogen. The more pathogen inhibited the higher the potential of the isolate decomposers to be APH.

The Tests on the Nature of Isolate Decomposers Inhibition on the Growth of Microbial Pathogen

The result of observation on the nature of inhibition in each isolate decomposer that is able to inhibit the growth of pathogen is shown in table 3.

Table 3. The Nature of Each Isolate Decomposers Inhibition on the Growth of Microbial Pathogen

Isolate Decomposers	Microbial Patho	
	X. axonopodis	*F. oxysporum*
Bacteria MKA-16	Turbid	X
Bacteria MKA-79	Turbid	X
Fungi W-4	Turbid	Turbid

The nature of inhibition of microbial decomposers based on the observation in table 3 shows that the inhibiting process of each isolate decomposers on the growth of pathogen is bacteria static and fungi static. Isolate decomposers can only inhibit the growth of the pathogen without being able to kill it. This is seen after being incubated by peptone water or PDB so that it changes from limpid zone to be turbid.

CONCLUSION

Isolate of bacteria MKA-16 and MKA-79 are able to inhibit the growth of pathogen bacteria of X. axonopodis pv. Glycines plant, but are not able to inhibit the growth of bacteria R. solanacearum, fungi F. oxysporum, and fungi F. graminearum. Meanwhile, isolate fungi SP-5 inhibits the growth of bacteria X. axonopodis pv. Glycines, fungi F. oxysporum, and fungi F. graminearum but it does not inhibit bacteria R. solanacearum. isolate of fungi W-4 can also inhibit the growth of X. axonopodis pv. Glycines, and fungi F. oxysporum, but it does not have the ability to inhibit bacteria R. Solanacearum and F. graminearum. The nature of inhibition of each isolate decomposerson the growth of microbial pathogen is bacteria static and fungi static. The tests result on the inhibition between isolate decomposers suggests that fungi W-4 and SP-5 are able to inhibit the growth of isolate bacteria MKA-16 and MKA-79.

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THE DIVERSITY OF BACTERIAL ISOLATES FROM BANDEALIT COASTAL AREA - JEMBER BASED ON BOX-PCR AND BIOLOG GN2 MICROPLATE

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ABSTRACT

The objectives of this research are to study the bacterial diversity based on molecular marker and to investige their metabolic fingerprint in respiring organic substrates. Research methods include water sampling and bacterial isolation, storage of bacterial isolates, colony and cell morphology observation, determination bacterial diversity using BOX-PCR and investigation of bacterial activity (substrates uptake) based on their metabolic fingerprint in respiring carbon sources (BIOLOG GN2 Microplate). A total of 120 isolates have been isolated and 60 of them have been purified. 10 selected isolates have been characterized by cell and colony morphology as well as their genetic profiles based on BOX-PCR. The 10 isolates have the same cell morphology, although then have diference in colony morphology. They also showed different genetic profile after BOX-PCR. this indicated the presence of bacterial diversity based on genetic profiles. Five of them have been tested for their activity in respiring organic substrate on BIOLOG GN2 Microplate. They are BA 011109, BA 041109, BA 061109, BA 091109, and BA 041109*. The bacterial isolates that have the same cell morphology could be different in their genetic profiles. Differences in the genetic profiles related with their diversity in utilization of organic substrates.

Keywords: bacteria, diversity, BOX-PCR, BIOLOG GN2 Microplate

INTRODUCTION

The improvement on the biotechnology sciences has made it possible to observe the microorganism biodiversity to the molecular level like nucleus acid, protein, lipid, and gen (Moore et al., 2004). This molecular-based diversity is available to be used to identify bacterial type, bacterial activity, phylogeny, as well as to examine the potential of the bacteria in the water environment.

Several commonly known techniques used for observing the biodiversity on the DNA-molecular level are: Random Amplified Polymorphic DNA (RAPD), Restricted Fragment Length Polymorphism (RFLP), Degradative Gradient Gel Electrophoresis (DGGE), sequence analysis, and Macrorestricted Fragment Length Polymorphism (MFLP) (Suryanto, 2003). The PCR-based method provides the information regarding the diversity of the genetics profiles of the repetitive sequence found in the genome of the bacteria. The widely used techniques to observe the molecular diversity are the sequence of The Enterobacterial Repetitive Intergenic Consensus (ERIC), and the 154-bp Box Element (BOX-PCR) (Tacao et al., 2005). BOX-PCR is used for the examination on the molecular diversity based on the genetic profiles with the repetitive intergenic sequence target of the genome of the bacteria. The result of the genetic profiles of the BOX-PCR rules the degree of genotype diversity of each bacterium under research. The variation among the genetic profiles resulted from the BOX-PCR examination shows the genotype polymorphism of the bacterial isolates (Oda et al., 2002).

The verification of such bacterial diversity may also be done through the observation to their metabolism activity. The diversity which bases on the metabolism activity is also popularly known as the metabolic fingerprint. BIOLOG is one of the methods to generate the metabolic fingerprint bacteria. The BIOLOG method is designated for identification of bacteria, mold, and yeast based on the variation of respiratory pattern due to distinction of the carbon sources in the 95 micro-plate holes. Each bacterium has its own specific respiratory pattern. The changes of on the

composition of the bacterioplankton community at the York river estuary in the Virginia is examined based on the metabolism activity using the BIOLOG GN (Schultz and Ducklow, 2000). BIOLOG also proves to be able to distinguish the N-substrate usage by the bacterioplankton at the NW marine water of the Mediterranean and Antarctica Sea (Sala et al., 2000). The observation of the bacterial diversity at the Bandealit Coastal water in this research is done by utilizing the molecular approach, which bases on the genetic profiles of each bacterial isolates using the repetitive sequence of the BOX-PCR results. The metabolic fingerprint at the chosen bacterial isolates is further observed using the BIOLOG GN2 Micro-plate

MATERIALS AND METHODS

Sampling collection and Isolation of the Bacteria

The water sample was taken from the Bandealit Coastal area with the region of ±20 from the coastline. Isolation of the bacteria is conducted using the spread plate method at the agar media M1. As many as 10 bacterial isolates were chosen and their colony and cell' morphology are examined further.

Determination of the bacterial isolates diversity based on their genetic profiles

The bacterial diversity based on the genetic profile was determined through the use of BOX-PCR. The Isolation of DNA is done using the modified Freeze and Thaw method of Tsai and Olson's (1991). The Primary BOX-AIR (5'>CTA CGG CAA GGC GAC GCT GAC G<3') (Oda et al., 2002) was used for amplifying the genome of the bacteria. PCR is done in the 25 µl reaction containing 8 µl de-ionized water, 12.5 µl PCR Mastermix (Qiagen Kit, 2.5 primary BOX-AIR [Working Primary]), and 2 µl of the DNA extract (DNA template). The gradient of the temperature used was: initial denaturation 95°C for six minutes, 35 cycles at 94°C for 1 minute, 53°C for one minute, 65°C for eight minutes, and the final extension at 65°C for sixteen minutes.

As much as 10 µl of the DNA fragment resulted from the PCR products was separated through electrophoresis at the 1.5% agarose gel. The DNA tape profile from various bacterial isolates are compared to each other, then 5 bacterial isolates chosen to further examination and test for its usage in the organic substrate use.

Determination of the Bacterial Diversity based on the metabolic fingerprint

The determination of the bacterial diversity based on the metabolic fingerprint was done using the BIOLOG GN2 Micro-plate. The Pre-culture was done by growing the single isolate at the 10 ml liquid media M1 at 21°C for 12 hours. Permanent culture was done through centrifugation at 6000 rpm, 20°C for five minutes. The pellet is washed three times using the physiologic salt solution 0.9% and repeated centrifugation. The final washing buffer was removed and the pellet is diluted into the Ringer 150µl. The bacterial suspension were added into the BIOLOG GN2 Micro-plate wells and incubated at room temperature (30°C for no longer than 48 hours). The positive results of the metabolism test based on the BIOLOG GN2 Micro-plate were characterized by the changing on the culture color in the Micro-plate into purplish, indicating the use of the carbon source.

RESULTS AND DISCUSSION

Isolation and general characteristics of the bacterial Isolates

After isolating 120 bacterial isolates, as many as 60 isolates were purified into a single colony. Next, 10 isolates with different colony morphology were observed for their cell morphology. The results of the observation are shown on Table 1. The bacterial isolates which have been isolated are commonly formed into small round colony with the diameter of ± 0.5-3 cm with various colony colors (yellow, white, and red) but uniform

cell morphology, that is, coccus. The variation on the colony morphology does not account for the variation on its cell morphology. All isolates have uniform cell morphology, which is, coccus.

The existence of the gram positive bacteria in the nature is assumed at 10% of the total population of the bacteria (Purushotaman and Jayalakshmi, no year mentioned). The gram test to the isolates under research shows the result relevancy with the existence of the major amount of bacteria in the nature, commonly known as the gram negative bacteria. The uniform cell morphology does not direct the clue to the exactly the same species (Dwijoseputro, 1998). One of the characteristics of false diversity of the bacterial isolates is indicated by the variation on the colony morphology. Such false finding is further analyzed molecularly to generate the variation of the genetic profile of the bacterial isolates.

Diversity of the bacterial isolates based on the genetic profile

The profile of the genetic diversity of various organisms is also termed genetic fingerprint which is specific to each species (Atlas, 1998). The variation of the genetic profile becomes the ground for the examination on the diversity of the isolates at the species level (Moore et al., 2004). The bacterial isolates' genetic diversity in this research is pointed out by the variation of the migration pattern of the DNA tape at each bacterial isolates resulted from the BOX-PCR.

The bacterial isolates BA 041109 and BA 041109* show the similar migration pattern tape, with the minor variation only on DNA fragment above 1.000 bp. It indicates that both isolates have the close phylogeny. The bacterial isolates BA 011109, BA 061109, BA 091109, BA 031109, BA 351109, BA 201109, BA501109, and BA 051109 show totally different migration pattern tape, indicating that those eight isolates have far phylogeny. The variation on the migration pattern tape of DNA of the 10 bacterial isolates selected from the BOX-PCR shows different genetic profile. It indicates that those 10 bacterial isolates belong to the different species. The variation of the genetic profile of each bacterial isolates shows the existence of the bacterial diversity at the Bandealit Coastal area despite the similar characteristics of the bacterial isolates, particularly on the cell morphology and microscopis morphology.

Diversity of the bacterial Isolates based on metabolic fingerprint

The character of each reaction that occurs in the BIOLOG GN2 Micro-plate wells is specific in nature. The bacterial isolate has the ability to hydrolyze the D-cellobiose, indicating that those 3 bacterial isolates are capable of generating cellulase. This ability plays a vital role in the agricultural waste decomposition which is dominated by the organic wastes resulted from plants and is rich of cellulose.

The bacteria that prove to be able to take the benefits from Tween 40 and Tween 80 are the isolates BA 041109 and BA 041109*. It indicates that those isolates are capable of generating esterase/lipase (Frank and Gerber, 1981) which has the ability to decompose the tween polymer into fatty acid and glycerol in order to be directly used by the microorganism. The organic wastes and food wastes are mostly composed by Tween. The bacterial isolates that are able to take advantage from Maltose (dimmer of the amilum carbohydrate, a α-glycoside bonded by the α-1,4 glycoside) are BA 011109 and BA 061109. Maltase is the enzyme with the ability to hydrolyze Maltose into Glucose. Maltose are easily found on sprouts, milk, and cereals (for example: rice) (Merthin, 2009). The usage of maltose indicates the ability of the bacteria to produce amylase enzyme, which provide the ability to disengage the glycoside binding on the amylum. Thus, the relevancy between the genetic profile and the metabolic fingerprint of the bacterial isolate is that bacterial isolates with uniform cell morphology prove to have different genetic profile, as well as several similarities and differences in taking the advantages from the organic substrates.

CONCLUSION

1. Bacteria with similar or uniform cell morphology are, in fact have different genetic diversity.
2. The variation on the genetic profile diversity shows the variation of the metabolic fingerprint.
3. The bacterial isolates BA 041109*, BA 011109, and BA 061109 have the potential to be used in the bio-remedial action to the water areas which are heavily polluted by the organic wastes for their ability to produce cellulase.

ACKNOWLEDGMENT

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THE DETERMINATION OF ABSORPTION CAPACITY OF ECENG GONDOK (*Eichornia crassipes (Mart)* Solms) TO Pb, Cu AND Cd IN WATER BY THE APPLICATION OF SOLVENT EXTRACTION WITH METHYL ISOBUTHYL KETONE

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ABSTRACT

The absorption capacity of eceng gondok (*Eichornia crassipes* (Mart.) Solms. to Pb, Cu and Cd in water was studied. The metals absorbed by the plant were extracted by the use of ammonium pyrrolidinedithiocarbamate (APDC) as the complexing agent and methyl isobuthyl ketone (MIBK) as the organic solvent. The metals measurements were performed with the technique of atomic absortion spektophotometry (AAS). It was found that during observation (15, 30 and 45 days), the highest absorbtion rate of the three metals occurred within 0 – 15 days of planting. The absorption capacity of the plant to the three metals were varied, the higher the metals concentration in the water the higher the metals absorbed by the plant were.

Keywords: absorbsion capacity, eceng gondok (*Eichornia crassipes* (Mart.) Solms, Pb, Cu and Cd Solvent extraction

INTRODUCTION

Various materials such as water plants, clays, zeolites and cattle bones have been reported to be useful for removing Pb, Cu and Cd from waste water (Suprihatin, et. al., 1997, and Suprihatin and E. Sahara, 1998). Cu is an essential trace metal but toxic to plants at higher level. The effects of acute Pb and Cd poisoning in humans are very serious (Manahan, 1994 and Pallar, 1994). One of the water plants that has been widely used for reducing heavy metals in waste water is Eceng gondok (*Eichornia crassipes (Mart.)* Solms) (Fig. 1)**.** This water plant has been well-known as weeds in agriculture. In water, the rapid growth of this plant can cause eutrofication. This study was aimed to establish the capacity absorption of the plant to Pb, Cu and Cd with the application of solvent extraction. The three metals in water are usually available in very low concentrations. Therefore, solvent extraction was applied to extract the metals, and hence, a higher concentration of the metals could be measured.

Fig. 1 Eceng Gondok (*Eichornia crassipes (Mart)* Solms)

MATERIALS AND METHODS

Chemicals used in this study were: $Pb(NO_3)_2$, $CuSO_4.5H_2O$, $CdCl_2$, HNO_3 conc., H_2SO_4 conc., ammonium pyrrolidinedithiocarbamate (APDC), methyl isobuthyl ketone (MIBK), aquadest.

Apparatus used were: plastic boxes, volumetric pipettes, volumetric flasks, cylindrical glasses, destruction flask, heater, and atomic absorption spectrophotometer.

Eceng gondok were planted into 4 plastic boxes that contained 20 L solutions of Pb, Cu and Cd of 0.5 ppm, 1.0 ppm, 1.5 ppm and 3.0 ppm. As a control, eceng gondok was planted in just aquadest. Absorption was observed in 15, 30 and 45 days after planting.

Approximately 1 gram of dried sample was destructed with 10 mL H_2SO_4 , 10 mL HNO_3 and 100 mL aquadest. Destruction was ended when the solution remain about 25 mL. The solution was then filtered and its pH was adjusted to 3. The solution was transferred quantitatively into a separating funnel and 1 mL APDC and 10 mL MIBK were added. The extraction were carried out three times and after separation, the organic phase was collected. Metals measurement were carried out with the technique of AAS and quantification was made by calibration curve.

Destruction, extraction and metals determination of eceng gondok control were also carrid out in the same way.

RESULTS AND DISCUSSION

As shown by the graphs (Fig. 2-4), generally, it was observed that the highest absorption of Pb, Cu and Cd occurred in the first 15 days after planting. Beyond these days the metals absorption increased gradually.

It was also evident that the higher the metals concentrations in the water, the higher the absorption capacities were. As can be seen from the figures, the metals absorbed per gram eceng gondok increased with concentration. After 45 days, the absorption capacities of Eceng gondok to Pb, Cu and Cd that were planted in various metals concentrations (0.5 - 1.0 ppm) were varied i. e. 0.0330 - 0.1458 mg/g for Pb, 0.0232 - 0.1489 mg/g for Cu and 0.0124 - 0.0421mg/g for Cd. It is obvious that Pb and Cu were more effective to remove than Cd.

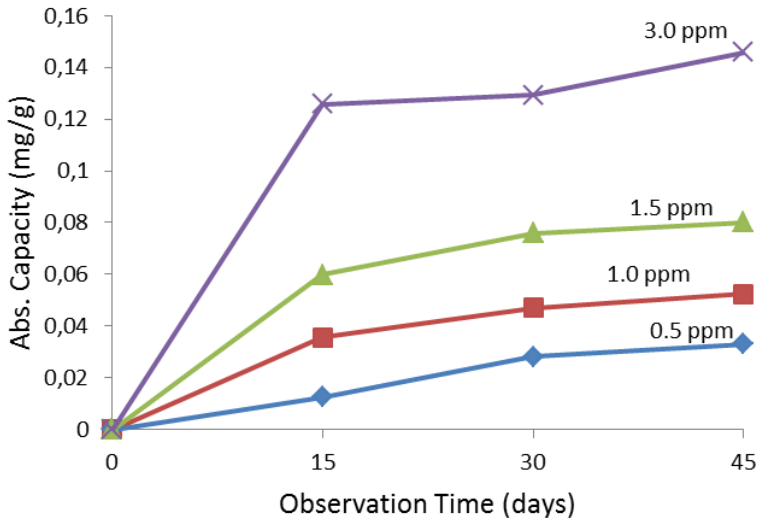

Fig. 2 Absorption Capacity of Eceng Gondok to Pb

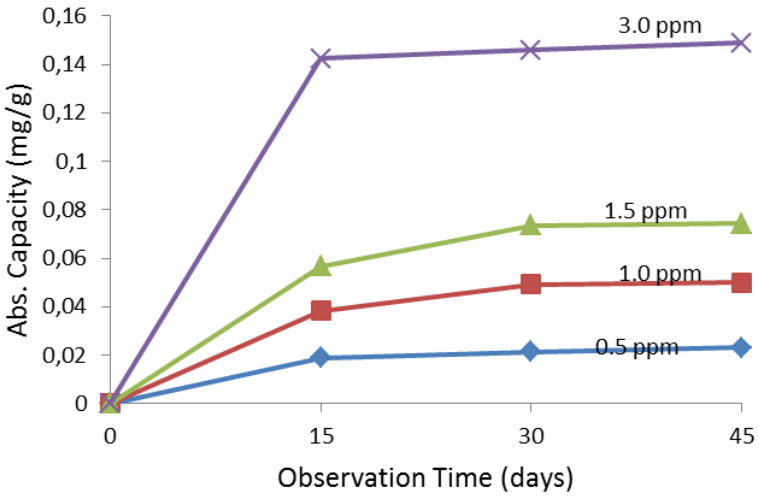

Fig. 3 Absorption Capacity of Eceng Gondok to Cu

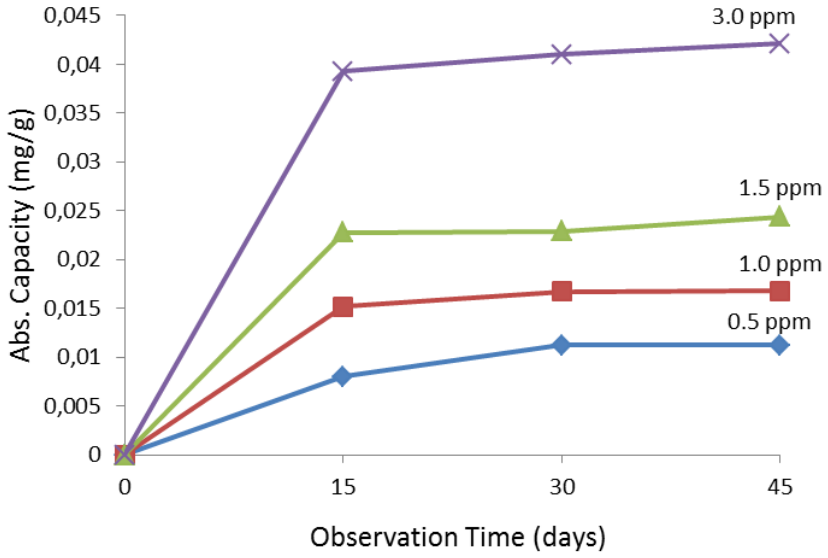

Fig. 4 Absorption Capacity of Eceng Gondok to Cd

Metals absorption by eceng gondok undergoes both passively and actively. The passive one occurs via diffusion in which diffusion takes place from the higher concentration to the lower one. Whereas the active absorption occurs by the formation of chelat that accumulates in the vacuole

In this study, the maximum absorbtion time of eceng gondok to Pb, Cu and Cd has not been reached yet. As indicated by the graphs, the absorbtion capacities of the three metals tend to increase with time.

Statistically, the metals absorbed by the plant that planted in various metals concentrations were significantly different.

CONCLUSION

Eceng gondok (*Eichornia crassipes (Mart.)* Solms) could be used to absorb Pb, Cu and Cd in water. Solvent extraction was also worked well during the determination of the metals. The absorption capacities of the plant were varied depending on the metals spiked to the water and the contact time. The higher the metals concentration in the water the higher the metals absorbed by the plant were and the longer the absorption took place the higher the metals absorbed did.

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DISTRIBUTION OF Pb AND Cu IN SEDIMENT AND SEAWATER ALONG SANUR BEACH

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ABSTRACT

This paper describes the distribution of Pb and Cu in sediment and seawater along Sanur beach. The sediment samples (collected from 10 sampling sites) were digested with the mixture of HNO_3 and HCl (3 : 1) in an ultrasonic bath at 60°C for 45 minutes and then continued by the use of hotplate heating at 140°C for 45 minutes. The seawater samples were aicidified with 10 % HNO_3. The contents of the heavy metals in the sediment and water samples were determined by the technique of atomic absorption spectrophotometry (AAS). It was observed that the distribution of Pb and Cu in the samples were varied in which Pb and Cu more distributed in the sediments than in seawater. The distributions of Pb in the sediments and seawater were 14.85 - 114.06 mg/kg and 0.05 - 0.34 mg/kg, respectively, while the distributions of Cu in the sediments and seawater were found to be 14.96 – 48.08 mg/kg and 0.01 - 0.11 mg/kg, respectively. The highest Pb distribution was obtained in sediment collected from site 10 and in seawater collected from site 9, whereas the highest distributions of Cu were found at site 4 for sediment and at site 2 for seawater.

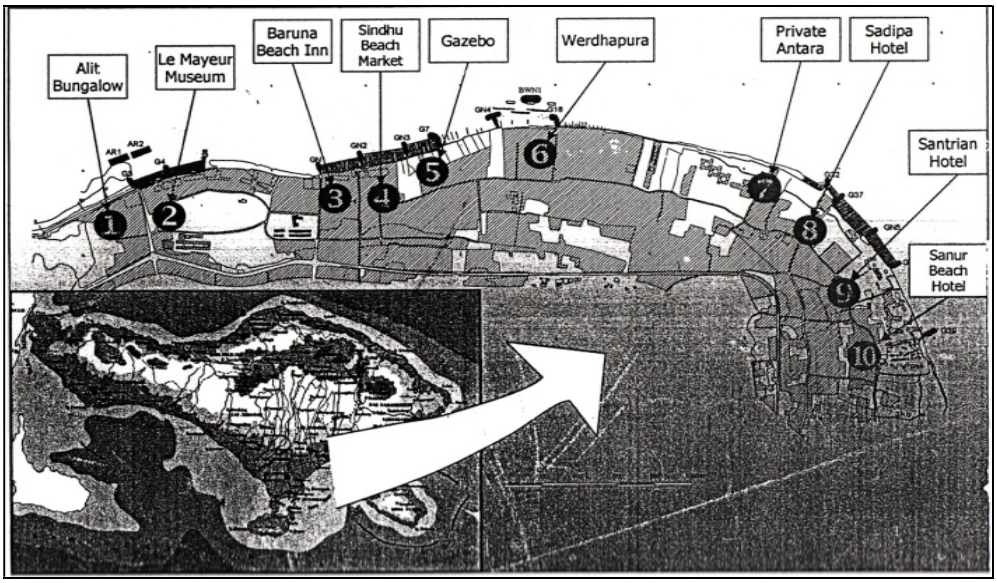
INTRODUCTION

Sanur Beach is one of the beaches that are objects of tourism in Bali (Figure. 1). At this beach, both local and overseas tourists do many activities such as bathing, swimming, sports, water skiing, sightseeing, dipping, and so forth. Besides that, around the beach, there are also hotels and restaurants, agricultural and domestic activities. These activities result in the entrance of the waste into the beach. Due to this, the beach has been facing a pollution threat such as heavy metals, nitrate and nitrite, phosphate, and other harmful chemicals, as well as, pathogenic bacteria such as E. collie. Based on these reasons, heavy metals such as Pb and Cu were determined for recognizing the distribution of those metals both in sediment and seawater. These metals are chosen to be analyzed, due to they are very toxic if they get into food chain in high concentrations. When the metals accumulate in human bodies they can cause of nerves, kidney, and respiration diseases (Darmono,1995; Florence and Batley,1980; Manahan,1990; Palar,1994; Sadiq,1992).

Figure 1. A Small Part of Sanur Beach

METHODS

Sediment and water samples were collected from 10 selected sites along Sanur Beach (Figure 2). The sediments were wet sieved by a 63 µm nylon mesh screen. The fractionated samples (<63 µm) were oven dried at up to 60°C. Approximately 1 g of the fine sediment sample was digested by reverse aqua regia (HNO_3 and HCl, 3:1) at 60°C in an ultrasonic bath for 45 minutes followed by hotplate heating at 140°C for 45 minutes. Seawater samples were only acidified by 10% HNO_3. The determinations of Pb and Cu were carried out by flame-AAS (Siaka,1998; Welz,1985).



Source : Sanur Beach Conservation Project, PT. Penta Waskita

Figure 2. The Selected Sampling Sites at Along Sanur Beach

RESULTS AND DISCUSSION

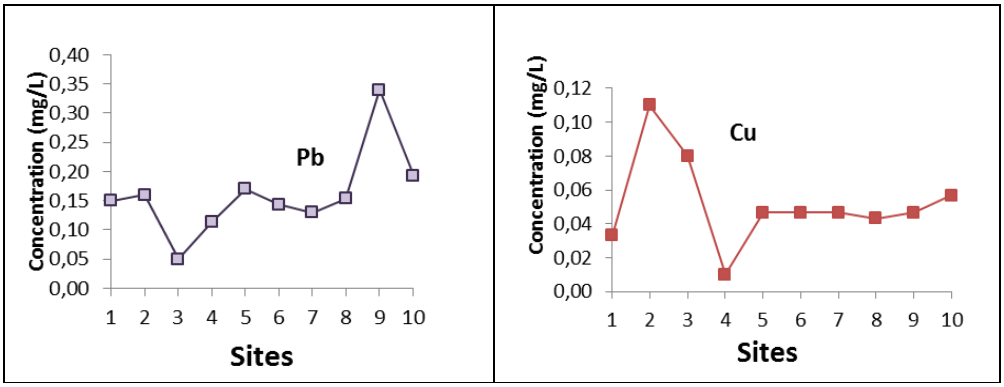


Figure 3. Distribution of Pb and Cu in Seawater

The didtribution of lead (Pb) in seawater collected from 10 selected sites fluctuated as shown in Figure 3. The concentrations were ranging from 0.05 to 0.34 mg/L. The lowest Pb content was found at site 3, while the highest one was distributed at site 9. The concentration of copper (Cu) obtained from the seawater samples (Figure 3)

ranged from 0.01 to 0.11 mg/L. The distribution of Cu at most sites (sites 1, 5-10) was arround 0.04 mg/L. The highest concentration of Cu was found at site 2, while the lowest one at site 4.

Generally, the concentrations of both metals in the water were much higher than the maximum concentration of Pb and Cu allowed according to the decision of Governour of Bali No. 515, year of 2000 about The Quality Standard of Environmental for Tourism and Recreation which is 0.0002 mg/L for Pb and 0.02 mg/L for Cu.

Interestingly, distribution of Pb was higher than that of Cu in the seawater along the Sanur Beach. High level of Pb was mainly produced from human activities arround the costal and on seawater including motor vehicles, boats, skiing jets, and other water vehicles consuming petrol.

The distributions of Pb and Cu in sediments were much higher than that of in seawater. This suggested that at seawater pH of 7 or more, metals tend to form insoluble compound such as: oxides, hidroxides, carbonate, sulfate and accumulate in sediment. Thus, they were available more in sediment than in seawater (Batley,1987). The concentrations of Pb were 14.85 - 114.06 mg/kg. High levels (> 50 mg/kg) of Pb were found at sites 4, 8, 9, and 10, while low concentrations (< 50 mg/kg) were found at sites 1-3 and 5-7) as illustrated in Figure 4.

The concentration of Cu in sediment ranged from 14.96 to 48.08 mg/kg. The highest concentration was found at site 4, while in the seawater of site 4, Cu level was the lowest. This means that most Cu was in the formed of insoluble compounds, so it was less in the water (Batley,1987). Most sites (1, 5 – 10) contained Cu < 20 mg/kg as shown in Figure 4.

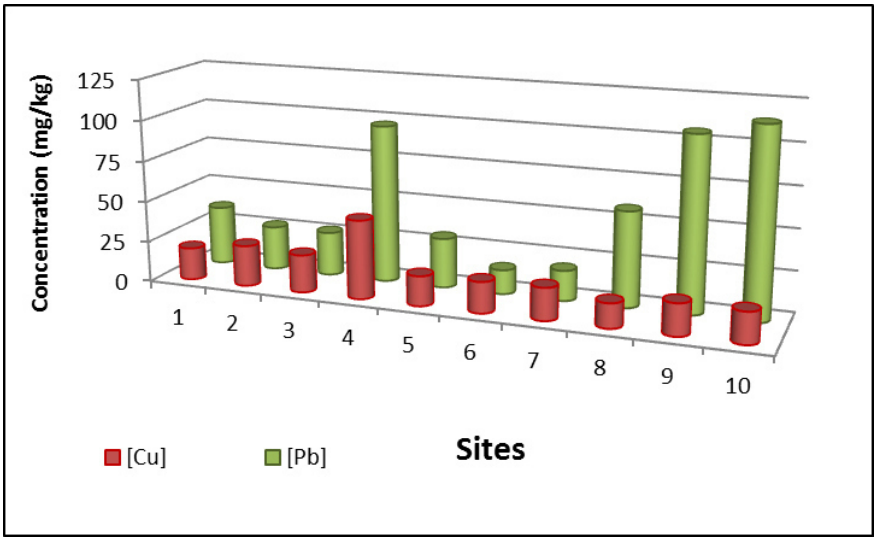


Figure 4. Distribution of Pb and Cu in Sediments

The distributions of Pb in the sediments and seawater along Sanur Beach were 14.85 - 114.06 mg/kg and 0.05 - 0.34 mg/kg, respectively. The highest Pb distribution was obtained in sediment collected from site 10 and in seawater collected from the site 9. The distributions of Cu in the sediments and seawater were found to be 14.96 – 48.08 mg/kg and 0.01 - 0.11 mg/kg, respectively. The highest distributions of Cu were found at site 4 for sediment and site 2 for seawater.

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THE SHELL OF MOLLUSC SOLD AS SOUVENIR ON THE BEACH SOUTHERN PART OF BALI

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ABSTRACT

A study on the shell of mollusc that sold as souvenir has been undertaken on the beach of southern part of Bali island in July, 2008. The study was carried out by undertaking observation on mollusc shells sold on the beach, conducting interview to the souvenir sellers and identifying the species on site. For those that could not been identified on site, their photos were taken and the specimen were bought, and they were brought into the Animal Taxonomy Laboratory at the Faculty of Mathematics and Natural Sciences, Udayana University, Bali for identification. Identification was based on Jutting (1952), Dharma (1988), Dharma (1992), and Vermeulen & Whitten (1998). Origin of the shells and how to get the shells on the beach were revealed from interviewing the sellers. As many as 32 species of mollusc shells were sold as souvenirs on the beach, either as a whole or fragmented, four species of which were identified as protected species. They were *Turbo marmoratus* Linne, 1758, *Cassis cornuta* Linne, 1758, *Charonia tritonis* Linne, 1758 and *Nautilus pompilius* Linnaeus, 1758. In general the sellers bought the shells from other sellers (supliers) who come from outside the island.

Keywords: shell, species, mollusc, souvenir, protected species

INTRODUCTION

Indonesia is a mega-biodiversity country (Noerdjito dkk, 2005), despite in fact many of the species have not been identified. One group of the animals which are found in Indonesia are classified into Mollusc.

Members of Mollusc, known in Indonesia as "siput" or "kerang-kerangan", have been quite popular among community members in this country for their importance. Recently many sea shells have been used for materials of handycrafts and assessories (Leimena, 2002). In Bali the shells have been used as souvenir material by small industries sectors such as for ear rings, gems of neckless, bracelet, bross, rings, and other forms. In addition, sea shells are frequently also sold in a whole, and have been polished so they looks shiny and more attractives.

Bali has been very popular as a tourist destination. On the beach of this island some stands were set where people sells souvenirs, especially on the beach souhtern part of the island where tourism activities predominantly concentrated. From the souvenirs sold there, some were made form shells of Mollusc.

METHODS

Research of shells of Mollusc which were sold as souvenirs on the beach of southern part of Bali island was undertaken in July, 2008. Research was undertaken by visiting the sellers who sold souvenirs made of sells of Mollusc for identifying all specimen of Mollusc sold as souvenirs and handicrafts. Identification was undertaken on site. For those which could not be identified directly on site, their photos were taken, and the sample of specimen were bought for further identification. They were brought to the Laboratory of Animal Taxonomy at Biology Department, FMIPA, Udayana University where further identification carried out. The identification were undertaken based on Jutting (1952), Dharma (1988), Dharma (1992), and Vermeulen & Whitten (1998).

In addition, the sellers were also interviewed. This was carried out to collect information where the shells were got from and how to get the shells.

RESULTS AND DISCUSSION

Table 1. The shell of Mollusc sold as souvenir on the beach southern part of Bali

No	Species	Other name	Family	Class
1.	*Cypraecassis rufa* Linne, 1758	Tempurik (local name), Siput helmet (Indonesian name)	Cassidae	Gastropoda
2.	*Cassis cornuta* Linne, 1758	Kepala kambing (Indonesian name),*Giant Helmet Shell* (popular name)	Cassidae	Gastropoda
3.	*Syrinx aruanus* Linne, 1758	Unam (local name)	Melongenidae	Gastropoda
4.	*Pleuroploca filamentosa* Roding, 1798	Unam (local name)	Fasciolariidae	Gastropoda
5.	*Charonia tritonis* Linne, 1758	Triton terompet (Indonesian name),	Cymatiidae	Gastropoda
6.	*Lambis chiragra* Linne, 1758	Kepala menjang	Strombidae	Gastropoda
7.	*Vasum turbinellus* Linnaeus, 1758	-	Turbinellidae	Gastropoda
8.	*Turbo petholatus* Linne, 1758	Batu lage (local name)	Turbinidae	Gastropoda
9.	*Turbo marmoratus* Linne, 1758	Batu lage, (local name), Batu laga/Siput hijau/Siput mata bulan (Indonesian name), *Green Snail* (popular name)	Turbinidae	Gastropoda
10.	*Bursa rubeta* Linne, 1758	Rukrik (local name)	Bursidae	Gastropoda
11.	*Bursa bubo* Linne, 1758	Rukrik (local name)	Bursidae	Gastropoda
12.	*Cypraea tigris* Linne, 1758	Bulih bali (local name)	Cypraeidae	Gastropoda
13.	*Cypraea arabica* Linne, 1758	Bulih bali (local name)	Cypraeidae	Gastropoda
14.	*Cypraea annulus* Linne, 1758	Bulih bali (local name)	Cypraeidae	Gastropoda
15.	*Melo (melocorona) amphora* Lightfoor, 1786	Palung-palung (local name)	Volutidae	Gastropoda
16.	*Melo (melocorona) aethiopica* Linnaeus, 1758	Palung-palung (local name)	Volutidae	Gastropoda
17.	*Cymbiola (Aulica) nobilis* Lightfoot, 1786	-	Volutidae	Gastropoda
18.	*Tectus pyramis* Born, 1778	Tumpeng-tumpeng (local name)	Trochidae	Gastropoda
19.	*Conus marmoreus* Linnaeus, 1758	-	Conidae	Gastropoda
20.	*Conus thomae* Gmelin, 1791	-	Conidae	Gastropoda
21.	*Conus virgo* Linnaeus, 1758	-	Conidae	Gastropoda
22.	*Conus textile* Linnaeus, 1758	-	Conidae	Gastropoda
23.	*Conus magus* Linnaeus, 1758	-	Conidae	Gastropoda
24.	*Terebra maculata* Linnaeus, 1758	-	Terebridae	Gastropoda
25.	*Chicoreus ramosus* Linne, 1758	-	Muricidae	Gastropoda
26.	*Babylonia spirata* Linne, 1758	-	Buccinidae	Gastropoda
27.	*Haliotis asinia* Linne, 1758	-	Haliotidae	Gastropoda
28.	*Nautilus pompilius* Linnaeus, 1758	Nautilus berongga (Indonesian name), *Pearly/Chambered Nautilus* (popular name)	Nautilidae	Cephalopoda
29.	*Nautilus scrobiculatus* Lightfoot, 1786	-	Nautilidae	Cephalopoda
30.	*Codakia tigerina* Linnaeus, 1758	-	Lucinidae	Bivalvia
31.	*Batissa violacea* Lamarck, 1818	-	Corbiculidae	Bivalvia
32.	*Anadara granosa* Linnaeus, 1758	Kerang darah (Indonesian name)	Arcidae	Bivalvia

Thirty two species of mullusc shells have been identified to be used as souvenir materials on the beach of southern part of Bali island (Table 1). They were sold in a whole or cut in pieces, such as for material of neckless, hair band, belt and spoon.

From the data collected, four species of shells of protected mollusc species were included in souvenir materials. They were classified as protected species referring to Indonesia regulations, "Peraturan Pemerintah Indonesia Nomor 7 Tahun 1999" (Anon. a), namely *Turbo marmoratus* Linne, 1758; *Cassis cornuta* Linne, 1758; *Charonia tritonis* Linne, 1758 and *Nautilus pompilius* Linnaeus, 1758. These are protected because their population has been decreasing.

The use of shells of mullusc as souvenir or handycraft, has providing economic benefits for the sellers, as source of income, including when they were exported. The shells are also likely to be illegally brought overseas. On the other hand, the collection of mollusc from their habitat may cause problem ecologically, especially when the shells from living species were collected continously. In the long term this may cause the population to decrease in their habitat and finally cause the extinction.

CONCLUSION

Thirty two species of shells of molluscs have been used as materials of souvenir on the beach of southern part of Bali. Four of them were taken from the shells of protected species: *Turbo marmoratus* Linne, 1758, *Cassis cornuta* Linne, 1758, *Charonia tritonis* Linne, 1758 and *Nautilus pompilius* Linnaeus, 1758. Generally souvenir sellers bought the souvenir made of shells of mollusc from vendors from outsite the island.

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ISOLATION OF THERMOACIDOPHILIC BACTERIA FROM KAWAH BEUREUM, KAMOJANG, GARUT

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ABSTRACT

A thermoacidophilic bacteria was isolated from hot spring area of Kawah Beureum, Nature Tourism Park Kamojang, Garut. The bacterial strain can grown at temperature between 45 and 65 degree celcius and at pH 3. The amplification and sequencing of 16S rRNA gene of this strain showed that this bacteria belongs to Alicyclobacillus sp. Further characterization and analyzes of potential of the bacterial strain is still in progress.

ANALYSIS OF PHYSIO-ACUSTIC TO DETEMINE OPTIMUM ACUSTIC PARAMETER OF GAMELAN JAWA

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ABSTRACT

Javanese gamelan is one of Indonesia's traditional music, but the study of the characteristics of his music has not done a lot. Musical characteristics that need to be obtained can be used as the basis for the design of concert halls and Javanese gamelan music. This research was conducted to find the time delay and the optimum reverberation time by using the autocorrelation method and fisio-acoustic. From the theoretical autocorrelation method, the value τe to Javanese gamelan music is 25-60 milliseconds. Time Value of Early hum Much after reflections (Tsub) based on theoretical calculation is about 575-1380 milliseconds. Pause Time value after the Early Reflection (Δt1) from theoretical calculations is approximately 25-60 milliseconds. Fisio-acoustic analysis carried out using Electroenchepalogram (EEG). In this study, which will simulate the sound field has been varied in the value of reverberation time and time tundanya. The variation of reverberation time is given on the value of 0000-2000 seconds with step 250 milliseconds. Variation of delay time is given on the value of 25-60 ms with 10 ms step. Results showed the greatest changes to increase alpha waves produced by the variation of time delay 50 milliseconds and 750 milliseconds for the reverberation time point T3, P3, and P4. While at point T4, the variation of time delay 30 ms and 250 ms reverberation time provides the most impact on increasing the alpha wave. This value is close to the theoretical, which fisio-acoustic analysis carried out is still in the theoretical range.

Keywords: Javanese Gamelan, Fisioakustik, EEG, Time Delay, Time hum

POSTER PRESENTATIONS:

HEALTH

CELLULAR SIGNALING OF LEPTIN RESISTANCE IN OBESITY

I G.A. Dewi Ratnayanti, I G.N. Mayun, I.A. Ika Wahyuniari and N.M. Linawati
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ABSTRACT

Obesity, which is defined as a pathologic condition due to the over accumulation of fat, is one of major health problem in the world today. It leads to social, psychological and serious health problems, including cardiovascular disease, the leading cause of death in the world. The mechanism of the imbalance between energy production and expenditure result in obesity is still unclear. Leptin resistance is thought to be one of the possible explanations. Leptin is 16 kDa hormone produced primarily by white adipose tissue. It is sometimes called an adipocytokine due to the shared structure and function with proinflammatory cytokine, such as interleukin-6. The improper response following an induction by leptin is considered as resistancy. This leptin resistance may yield from abnormalities in several levels from the translation through the leptin signaling. Leptin signaling is controlled by intracellular positive and negative feedback regulation. The positive feedback regulation is mediated by an Src homology 2 domain-containing adaptor protein (SH2B1), whereas the negative feedback of leptin signal transduction is facilitated by a suppressor of cytokine signaling-3 (SOCS3) and a protein tyrosine phosphatase 1 B (PTP1B). Dysfunction of those regulators may be the point in which leptin resistance originate and therefore could be the target for future therapy.

Keywords: leptin resistance, obesity, SH2B1, SOCS3, PTP1B.

INTRODUCTION

Obesity is an increasing health problem in the world today, including in the developing country with low or middle income. Around the world approximately 1,6 billion adult (age 15+) were overweight and at least 400 million adults were obese, whereas 20 million children (< 5 years) are overweight in 2005 (WHO, 2006). In the United State alone over 97 million adults are obese (NIH, 2002). In Asia, the prevalence of obesity is relatively lower, even though, it may result from inappropriate cut off point especially for Asian population, considering the high prevalence of obesity related disease in non obese (Chi *et al*., 2008). According the survey in 1997, in Indonesia, 8,1% adult male and 10,5% adult female are overweight; while the prevalence of obesity is 6,8% and 13,5% for adult male and female respectively (Ramadhinara *et al*., 2008)

This condition is a future threat to global health because obesity is related to numbers of disease range from cardiovascular disease, hypertension, dyslipidemia, type 2 diabetes, osteoarthritis, sleep apnea, gallbladder disease and colon, breast, prostate and endometrial cancer. Some of the diseases are major leading cause of death (NIH, 2002). While it may need simple effort to decrease body weight, in the reality, patient and health practitioner are often frustrated to cure this condition.

OBESITY

Obesity is defined as pathologic condition due to the over accumulation fat in the body. Obesity is measured by body mass index (BMI), which is body weight in kilogram divided by the square of height in centimeter. Internationally, BMI ≥ 25 is considered to be overweight and BMI ≥ 30 is considered to be obese. This cut off point is modified for Asian population due to different body composition and size. Although it is still on debate the standard for Asian is BMI ≥ 23 for overweight and BMI ≥ 25 for obese (WHO, 2006; Low *et al.,* 2009).

The etiology of obesity is multi factorial. As much as 40 – 70% is associated with genetic factor. It indicates that obesity is not just a problem of overeating habit and inactive lifestyle, but it may have underlying mechanism. Basic mechanism in obesity is

an imbalance between energy intake and energy expenditure (Scarpace & Zhang, 2009). One of the growing possible pathogenesis is the condition of leptin resistance. There are three phases in obesity related to the leptin resistance. In the first phase, leptin level and function are normal and the function is reduced in the second phase. By the accumulation of fat, leptin level is rise but the effect to control appetite and regulate energy balance is diminished. This condition of third phase is stated as leptin resistance (Martin *et al*., 2008).

LEPTIN SIGNALING

Leptin and Leptin Receptor

Leptin is 16 kDa protein hormone or cytokine produced primarily by white adipose tissue. It's expression also found in other various tissue, such as stomach, placenta, mammary gland, immune cells, vascular tissue, pancreas, skeletal muscle and myocardium. This product of obese gene (ob gene) has similar structure and function with interleukin (IL) such as IL-6, thus it is sometimes called an adipocytokine. The known main function of leptin is to regulate energy balance by modulating appetite and influencing the energy metabolism. It is also involved in glucose metabolism, immune and vascular function (Martin *et al*., 2008; Mantzoros, 1999). The normal level of leptin is 5 – 15 ng/mL and it is increased by overfeeding, insulin, glucocorticoids, endotoxin, and cytokines and is decreased by fasting, testosterone, thyroid hormone, and exposure to cold temperature (Yang & Barouch, 2007).

There are six isoforms of leptin receptor (ObR a-f) and they are closely related to the class I cytokine receptor family. The common receptors are ObRa and ObRb, which can be found in the hearth, whereas the other receptors are expressed scarcely in the body and not well understood (Yang & Barouch, 2007).

Leptin Cellular Signaling

Cellular leptin signaling can be divided into two types according it's target; the central and peripheral leptin signaling. In the central nervous system leptin primarily act on hypothalamus but recent finding shows leptin also work on brain stem to control appetite. In the peripheral, leptin works on skeletal and heart muscle, immune cell, pancreas and liver (Ryoichi *et al.,* 2010; Bjorbaek & Kahn, 2004).

Centrally, leptin will bind to the ObRb and cause phosphorilation of Janus activating kinase 2 (JAK2) which subsequently autophosphorilate the ObRb on Y 985, Y 1037 and Y 1138. Signal transducer and activator of transcription 3 (STAT3) will bind to Y 1138 and be activated by JAK2. After activation, STAT3 will dissociate and form homodimer, then translocate to the nucleus to regulate the transcription of neuropeptides (Bjorbaek & Kahn, 2004; Ramadhinara *et al*., 2008). STAT3 will stimulate anorexigenic peptides, which suppress eating and increase energy expenditure, such as pro-opiomelonocortin (POMC) and cocaine and amphetamine regulated transcript (CART). POMC will undertake further transformation to α-melanocyte stimulating hormone (α-MSH). STAT3 will also inhibit the orexigenic peptides that stimulate eating such as neuropeptide Y (NPY) and agouti-related peptide (AgRP). These processes will decrease apetite and increase of energy expenditure (Ramadhinara *et al*., 2008). Activation of Y 985 ObRb binds SHP-2 and leads to activation of the extracellular signal-regulated kinase (ERK)1/2 pathway and induction of c-Fos expression. Activation of JAK2 also induce insulin receptor substrate 1 and 2 (IRS1/2) – phosphatydilinositol 3-kinase (PI3K). The mechanism of IRS-PI3K pathway is still unclear, but it can suppress the NPY activity. (Bjorbaek & Kahn, 2004).

Negative Feedback Regulation of Leptin Signaling

The activity of leptin is regulated intracellularly by negative and positive feedback mechanism. In the negative regulation, leptin binding to ObRb will activate

JAK2 (Figure 1). The phosphorilated JAK2 will induce self phosphorilation. Phosphorilation on Y1138 will be recognized by STAT3 and cause it's activation by JAK2. The activated STAT3 also stimulate gene transcription of suppressor of cytokine signaling 3 (SOCS3) and protein tyrosine phosphatase 1B (PTP1B). In prolonged stimulation SOCS3 can block the activation of JAK2 and STAT3 through it's binding with the Y985 which in turn inhibit leptin activity. PTP1B can also inhibit the signal transduction of leptin through direct dephosphorilation of JAK2 and STAT3. (Ryoichi *et al.,* 2010; Ramadhinara *et al*., 2008; Bjorbaek & Kahn, 2004; Ronghua & Brouch, 2007).

Positive Feedback Regulation of Leptin Signaling

In basal condition, without leptin binding to ObRb, Src Homology 2 domain-containing adaptor protein 1 (SH2B1) inhibit the activity of JAK2 (Figure 2). Leptin binding autophosphorylates JAK2 in Y813. SH2B1 binds via its SH2 domain to phosphorylated Y813, thus enhancing leptin stimulation of JAK2. SH2B1 (either as monomers or dimers) also directly binds to IRS1 or IRS2, thereby recruiting IRS proteins to JAK2 and/or stabilizing JAK2/SH2B1/IRS protein complexes, which facilitates tyrosine phosphorylation of IRS proteins by JAK2. Although it's exact mechanism is still unkown, this pathway is thought to be involve in energy balance and stimulate reproduction and growth. Another mechanism of SH2B1 potentiates the leptin signaling transduction is by inhibiting the dephosphorilation of IRS1 by protein tyrosine phosphatase (PTP). SH2B1 also potentiate leptin activity through JAK2-STAT3 pathway (Ziqhin *et al*, 2007; Decheng *et al*., 2007).

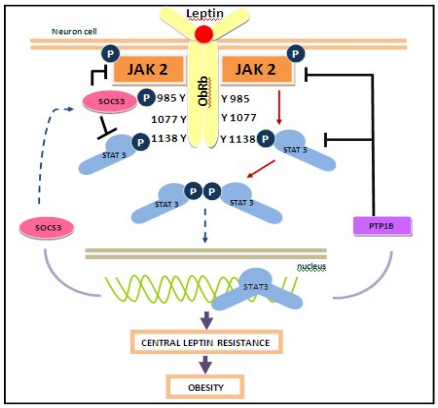

Fig 1

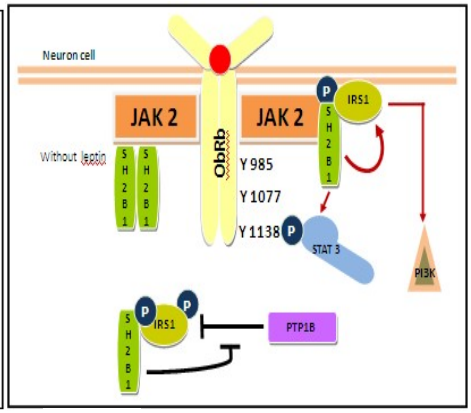

Fig 2

Fig 1. Negative Feedback Regulation in Leptin Signaling. Leptin binding to leptin receptor (ObRb) will cause homodimerization that consequently activate the Janus Kinase 2 (JAK2). The activated signal transducer and activator of transcription 3 (STAT3) will form homodimer and stimulate gene transcription of suppressor of cytokine signaling 3 (SOCS3) and protein tyrosine phosphatase 1B (PTP1B). In prolonged stimulation SOCS3 can block the activation of JAK2 and STAT3 through it's binding with the Y985. PTP1B can also inhibit the signal transduction of leptin through direct dephosphorilation of JAK2 and STAT3. This will cause blunt effect of leptin and result in central leptin resistance that leads to obesity.

Fig. 2. Positive Feedback Regulation of Leptin Signaling. In the present of leptin, JAK2 will be autophosphorylated in Y813. This will change the conformation of SH2B1-JAK2 complex and in turn enhance the activation of JAK2 by leptin. The binding of SH2B1 in Y813 will attract insulin receptor substrate 1 (IRS1) and stabilize the SH2B1-JAK2-IRS1 complex. Another mechanism of SH2B1 potentiates the leptin signaling transduction is by inhibiting the dephosphorilation of IRS1 by protein tyrosine phosphatase (PTP).

SH2B1 also potentiate leptin activity through JAK2-STAT3 pathway. Decrease expression of SH2B1 was shown to result in obesity.

LEPTIN RESISTANCE AND OBESITY

Leptin resistance is the condition of which there is high level of leptin in the circulation but the effect to decrease appetite and regulate energy balance is diminished. This will lead to obesity. Leptin resistance only exists in central nervous system e.g hypothalamus and brain stem. The accumulation of fat in obesity increases the production of leptin. In normal condition the leptin will suppress appetite and increase energy expenditure to keep the energy balance and avoid the storing of energy into fat. This mechanism seems to have it's threshold. To a certain degree of obesity, leptin loose it's effect and the obesity become uncontrolled (Scarpace & Zhang, 2009; Martin *et al*., 2008).

Leptin resistance may yield from abnormalities in several levels from the translation through the leptin signaling. Regulations of the leptin signaling, potentiation by SH2B1 and inhibition by SOCS3 and PTP1B, are changed in leptin resistance. Those disruptions in the regulation will influence the activity of leptin through JAK2/STAT3 pathway or JAK2/IRS/PI3K pathway. Decrease activity of SH2B1 and over activity of SOCS3 and PTP1B are known to associate with uncontrolled eating behavior, imbalance glucose and energy metabolism that result in obesity (Ryoichi *et al*., 2010; Ramadhinara *et al*., 2008; Dencheng *et al*., 2007).

CONCLUSION

Obesity is a major health problem that leads to death-cause diseases. Leptin resistance is involved in the pathogenesis of obesity. Increase body fat rise the leptin level in the body. This increase is not followed by enhancement of leptin effect in balancing the energy metabolism and decreasing appetite. The leptin resistance could be resulted from disruption in the cellular signaling. The intracellular negative and positive feedback regulation of leptin signaling may play important role. Prolonged induction of leptin in the hypothalamus and brain stem stimulate self inhibition of leptin by SOCS3 and PTP1B. Leptin activity also depends on potentiation by SH2B1. Inadequate respond of SH2B1 impairs downstream effect of leptin that leads to leptin resistance.

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DESIGN RECOMBINANT PRODUCTION OF LUMBROKINASE AND PREDICTION OF HOST WITH *INSILICO* MAPPING APPROACH

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ABSTRACT

Lumbrokinase (LK) is a potent plasminogen activator with widespread clinical use as a thrombolytic agent. In this study, we design plasmid which produced high level expression of recombinant lumbrokinase in *E. coli* by expression vector pTZ19R. Genomic DNA of lumbrokinase gene (LK) was goten from NCBI, then amplified by polymerase chain reaction (PCR) method on primer3 and sub-cloned to prokaryotic expression vector pTZ19R with ristriction enzyme digested by Acc651. That is cloned by pDRAW32. The expressed recombinant was tested by agarosa gel. The result of tested are 2862 MW, 788 MW, they are marker of pTZ19R and DNA LK insert respectively. Than *Escherichia coli* were transformed with PTZ19R-lk and gene expression. Our insilico data showed that recombinant lumbrokinase improved by pTZ19R 100% in *Escherichia coli*.

Keyword: Lumbrokinase, insilico, design recombinant, thrombolytic agent

INTRODUCTION

Earthworms have been used as anticoagulant and fibrinolytic medicines in East Asia for several thousands of years. Therefore; in this study, earthworms were investigated to determine the active components of the traditional prescription as starting materials for the development of new antithrombotic and fibrinolytic agents (Mihara et al., 1991). This fibrinolytic agent is Lumbrokinase (LK).

One is plasminogen activator which activate the zymogen plasminogen to generate plasmin for fibrinolysis system (Collen, 1999). Another is plasmin-like protein, which perform fibrinolytic actions directly. Plasminlike proteins were discovered from snake venom(Giron et al, 2008), earthworm(Ge et al, 2005), microorganisms (Peng et al, 2005). They were proven with fibrinolytic, anti-thrombotic activities and attracted much attention in investigators. Recently, production of plasmin-like proteins by fermentation technology and genetic engineering were investigated extensively (Peng et al, 2005). These production processes have given rise to economical manipulation of thrombolytic agents.

In this study, we described the mapping and characterization of Lumbrokinase (LK) from Rubellan by insilico. In addition, the prospective uses of the medical were predicated for design plasmid which produced high level expression of recombinant lumbrokinase in *E. coli* by expression.

MATERIALS AND METHODS

Searching of DNA insert. It is used *database searching* to search for gene data of DNA producing lumbrokinase from earthworm. For this reason, computation on line must be conneted to http://www.ncbi.nlm.nih.gov/html. Then, the similarity searching of the same sequence DNA from the microbe is done by BLAST programme. The BLAST programme is downloaded from the website http://www.ebi.ac.uk/Tools/blast2/index.html. The format of DNA sequence producing lumbrokinase is FASTA.

Design primer gene lumbrokinase. The primer design uses http://www.ncbi.nilm.nih.gov/primertool.cgi.html located in NCBI. The primer candidates is used to determine the presence of restriction enzyme and to prolong or gen amplification

(PCR). The net primer from http://www.premierbiosoft.com/netprimer/index.html is used to determination the primer from PCR

Searching of Plasmid and site ristriction. The plasmid searching to gene recombination is based on restriction enzyme in the Sequence DNA Insert. The data of plasmid is from http://www.fermentas.com/catalog/cloning/index.html#Vectors. Searching of site restriction, After restriction enzyme from primer is known,the restriction of DNA Insert is conducted. pDraw32 software is used to map restriction enzyme of gene sequence producing lumbrokinase.

Ligation of DNA Insert. The ligation of DNA Insert into plasmid is carried out by ligase enzyme with restriction enzyme pattern. pdraw32 software is used to ligate DNA Insert into plasmid

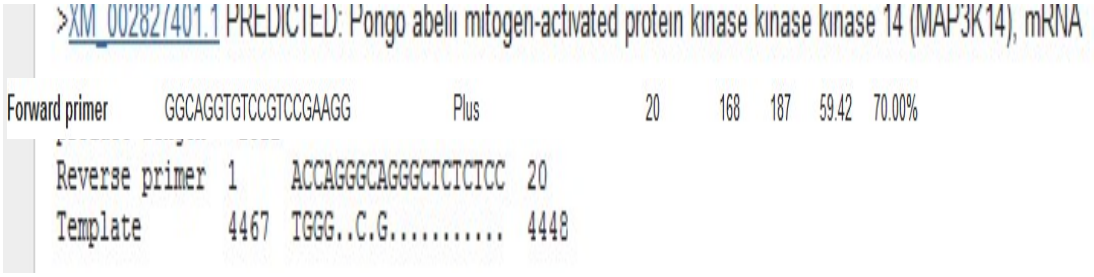
Identification of DNA recombinant. It needs gel agarose electrophoresis DNA for finding evidence wether the DNA recombinant has ligated with plasmid or not. And it is used pDraw32 software to conduct the mapping / sequencing.

Recombinant expression in Host. A software from http://bioportal.weizmann.ac.il/expsysb/suggestES is used to express recombinant into host. This software can determine appropriate host with recombinant.

RESULTS AND DISCUSSION

DNA Insert Gene Lumbrokinase. Computation on line connected with http://www.ncbi.nlm.nih.gov/html is used to search for DNA of earthworm producing lumbrokinase.From searching.we get 17 kinds of earthworm DNA lumbrokinase.Then, they are alignment with BLAST. Using Lumbricus rubellan as a standard,we get the 100% identity comes from Lumbricus bimastus arranged with protein 283 aa and its gene code bank protein dengan kode gene bank is AAL28118.1 GI:16755859

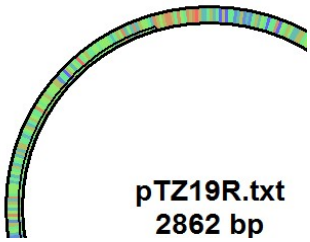
Net primer in http://www.premierbiosoft.com/netprimer/index.html. is used to determine primer of PCR.From the net primer experiment, the forward primer 5'GGCAGGTGTCCGTCCGAAGG3' at sequence 168, is used to make an elongation with reverse primer 5'CCTTCGGACGGACACCTGCC3' at sequence 187.



PCR will amplify of primer. After amplification, it is used as a DNA insert into recombinant DNA. This primer exists between ristriction enzyme. Ristriction enzyme used in the cutting is Acc65I, this enzyme has a sticky side (G'GTAC_C). DNA insert was identified on 794bp and cutting with ACC65I as the Figure 1.

Figure 1. DNA insert gene LK at the restriction ACC65I

Restriction enzyme and Plasmid. The plasmid to be inserted must have restriction enzyme.In this case, plasmid at the resytriction side ACC65I. It is used http://www.fermentas.com/catalog/cloning/index.html#Vectors to searhing plasmid for gene recombination. The result is plasmid Phagemid cloning vector pTZ19R GenBank: Y14835.1 with side restriction ACC65I .Its output from pDRAW32 is as follows:



***Acc*65I (G'GTAC_C) [KpnI,Asp718I], [dcm methylated], Cuts at position 627.**
Figure 2. Plasmid pTZ19R with the side restriction ACC65I at sequence 627

Recombinant and its characterization. Then, ligation is carried out to make recombinan between DNA insert (gene LK) with plasmid pTZ19R. pDRAW program is used for ligation.The result shows that DNA recombinan is combination DNA insert with plasmid 3650 bp.

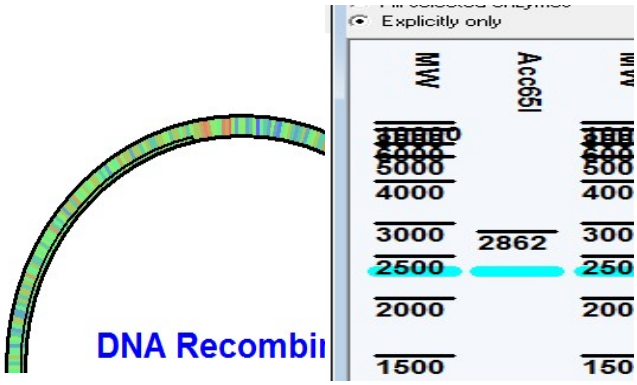


Figure 3. A) Recombinant DNA B) Agarosa Gel Recombinant DNA
Gel agarose is used to check the result of recombinan by using DNA marker Promega 1kb DNA ladder.Gel agarose shows that ACC65I ata 2862bp and 788bp can be identified, therefore DNA recombinant combining successfully, can be seen on Figure 3.

Expression DNA Recombinant
The next step is prediction DNA recombinant into host. For this reason, it is used http://bioportal.weizmann.ac.il/expsysb/suggestES. The result is that recombinant expression will achieve 93,98% in Eschericia colli.

Insilico recombinant DNA is helpful and make laboratory experiment easier. Besides, the lumbrokinase, as a substite streptokinase, can make production of fibrinolytic enzyme cheaper. Lumbrokinase are getting more significant in our daily life nowadays. It is applicable in both experiment and production, such as medical usage and nutritional

production. In the near future, more products based on the lumbrokinase will reach the market.

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ANALYSIS INTERACTION OF HEMAGLUTININ INHIBITOR OF INFLUENZA A FROM SPONGES COMPOUNDS BY MOLECULAR DOCKING APPROACH

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ABSTRACT

Influenza A is a disease caused by infection of influenza A virus. It has become a major health problem in tropical and subtropical countries. In this research, we have conducted the insilico study (docking) of Sponges compounds which has a role as hemaglutinin inhibitor of influenza A virus. From the docking, it is identified that compounds HBIP from sponges *Giodia baretti* have great affinity and ability to inhibit of hemaglutinin. After docking ,there is residu contact between HBIP compound to hemaglutinin. A hydrogen binding also formed at catalytic site of hemaglutinin toward HBIP compounds. The docking result showed that HBIP compounds have better binding energy and affinity than other bioactive compounds and the standards used.

Keywords: inhibitor, molecular docking, influenza A, sponges compounds, hemaglutinin

INTRODUCTION

Influenza virus caused seasonal epidemics and occasional pandemics in humans. Three subtypes of influenza viruses, namely H1N1, H2N2 and H3N2, are able to transmit in humans (Parrish and Kawaoka, 2005; Taubenberger et al., 2007; Webster et al., 1992). Among these three subtypes, H1N1 virus has the longest recorded history of human infection. It caused the 1918 great pandemic which led to 20–50 million dead worldwide (Taubenberger et al., 2007) and continued to circulate after the pandemic until 1957 when it was replaced by the H2N2 virus (Kawaoka et al., 1989). The virus is always mutated, forming new strain that are resistant to antiviral drug, which also has the potential to infect humans in the sense of animal to human transmission or human to human transmission (Moss et al.,2010).

Influenza viruses have a single-stranded nucleic acid, which consists of eight gene segments that encode 11 proteins. Influenza viruses are composed of protein and complex carbohydrates on the envelope which have two glycoproteins, they are hemagglutinin and neuraminidase (Colman, 1994). Neuraminidase is a surface glycoprotein which has an important activity for the replication of influenza A and B virus. This enzyme is responsible for the catalytic cleavage of Sia(α2-6)Gal or Sia(α2-3)Gal protein ketosidic liaison that exists between the terminal sialic acid and amino acid residues. Bond cleavage has some very important effects. First, it allows the release of virus from infected cells. Second, it prevents the formation of viral aggregates after release from host cells (Colman, 1995; Lin et al., 1995).

Various studies of natural compounds, mentioned that the potential diversity of medicinal natural compounds, and as such it presents a great opportunity to serve as good-quality compounds to be developed into anti-influenza drugs. Some natural compounds such as from sponge family have the potential to inhibit hemaglutinin (Ryu et al, 2008). Marine natural products that fail in clinical trials may still be introduced into the market as tools for biomedical research (Folmer et al. 2007). Marine sponges have been considered as a gold mine during the past 50 years, with respect to the diversity of their secondary metabolites. Bioactive compounds from sponge are expected to be further investigated for use as an alternative drug to inhibit the development of influenza virus A, such as natural compounds that contain of active compounds of polyfenol that can inhibit hemaglutinin and neuraminidase (Ryu et al, 2009).

In this research, we have studied the interactions between bioactive compounds from sponge with hemaglutinin, using computer software applications (*in silico*)

(Luscombe et al., 2001). *In silico* is used in the screening process of bioactive compounds as a drug (Kitchen et al., 2004). The interaction with bioactive compounds was done by performed the docking approach (Funkhouser, 2007). We generated the docking as an initial screening process between the molecules of bioactive compounds that can bind to the active site of protein. Analysis was based on Gibbs energy values, inhibition constant (Gohkle et al., 2000), conformation of the structure, affinity, and hydrogen bonding of protein and ligands (Datta, 2002).

MATERIALS AND METHODS

Protein used in this study is hemaglutinin. Hemaglutinin sequences were obtained from NCBI (http://www.ncbi.nlm.nih.gov/genomes/flu/). And then we done multiple sequence alignment method was used ClustalW-program (www.ebi.ac.uk/Tools/clustalw2/index.html website). Homology modeling was performed using the Swiss Model which can be accessed through http://swissmodel.expasy.org/SWISS-MODEL.html. A three-dimensional structure was the highest similarity with hemaglutinin sequences from 3IzgB (PDB Code).

Optimization and minimization of three-dimensional structure of the enzyme were employed using the software of MOE 2008.10. with addition of hydrogen atoms. Protonation was employed with protonate 3D programs. Furthermore, partial charges and force field was employed with MMFF94x. Solvation of enzymes was performed in the form of a gas phase with a fixed charge with a RMS gradient of 0.05 kcal / A^0mol, and other parameters using the standard in MOE 2008.10 software.

Preparation of sponge bioactive compounds as a ligand. Bioactive compounds of Sponge [23-31] were modeled into three-dimensional structure using ACD Labs software. Three-dimensional shape was obtained by storing in the 3D viewer in ACDLabs. Furthermore the ligand was in the wash with compute program, adjustments were made with the ligand partial charge and partial charge optimization using MMFF94 force field. The conformation structure energy of ligands was minimized using the RMS gradient energy with 0.001 Kcal / A mol. Other parameters were in accordance with existing default in the software.

Docking Simulation. The docking process was using a docking program from MOE 2009.10 software. Docking simulations were performed by the Compute-Simulation dock program. Placement method was conducted using a triangle matcher with 2.500.000 repetition energy readings each position and other parameters were in accordance with existing default in the MOE software. Furthermore, scoring functions used affinity DG, refinement of the configuration repetition force field with 1.000 populations. The first repetition of 100 times and the second setting was shown only one of the best results.

RESULTS AND DISCUSSION

Result of hemaglutinin sequence selection is with code bank number ADH01958.1: Hemaglutinin [Influenza A virus (A/Aalborg/INS133/2009(H1N1))]. The result will be used as template for the determination of three-dimensional structure of hemaglutinin. Determination of dimensional structure enzyme hemaglutinin from A/Aalborg/INS133/2009 virus (H1N1) using the Swiss Prot and the templete of protein is 3IzgB (PDB code) with sequence identity 99%.

The ligands from sponge bioactive compounds was resulted from *Giodia baretti, Xestospongia sp , Cryptotethia crypta , Petrosia contignata , Hemiastrella minor* species (Detmer et al., 2005; Faulkner 2000; 2001; 2002; Hedner et al., 2007; Haefner et al.,

2003). These compounds contained of barettin, indole, dipodazine, sterol and alkaloid group.

The docking results showed that from 30 ligands of bioactive compounds from sponge with hemaglutinin, only three bioactive compounds that have a lower Gibbs energy than other bioactive compounds. Furthermore, the docking of the ten compounds is re-done with MTPP (5-acetyl-3-methyl-2-(methylthio)-6-{[(2*S*,3*S*,4*R*,5*R*,6*S*)-3,4,5-trihydroxy-6-(hydroxymethyl)tetrahydro-2*H*-pyran-2-yl]amino}pyrimidin-4(3*H*)-one) ligand from wet lab research as standard (Chien et al, 2009). Analysis of docking results can be seen in Table 1.

Table 1. Docking results and minimized energy of the complexes hemaglutinin ligands

Ligan	HBIP*	BIMP**	MNMB***	Std (MTPP) *
Energi (kcal/mol)	-6.3490	-5.9761	-4.6942	-5.4093
MR	293.326	295.342	314.469	375.402
Log P	2.046	1.354	5.439	-2.688
pKi (μM)	7.261	6.852	4.814	4.805
H Don (catalytic site)	1 (Thr437)	0	1 (Thr437)	1 (Glu476)
H Acc (Catalytic site)	1 (Thr437)	1 (Gln371)	1 (Thr437)	0

* 3-(2,3-dihydro-1*H*-benzo[*e*]indol-1-ylmethyl)piperazine-2,5-dione (HBIP)
** 1-(1*H*-benzo[*g*]indol-2-ylmethyl)piperazine-2,5-dione (BIMP)
*** 2-{[(1*R*,2*S*,4a*R*,8a*R*)-1,2,4a-trimethyl-1,2,3,4,4a,7,8,8a-octahydronaphthalen-1-yl]methyl}benzene-1,4-diol (MNMB)
Std 5-acetyl-3-methyl-2-(methylthio)-6-{[(2*S*,3*S*,4*R*,5*R*,6*S*)-3,4,5-trihydroxy-6-(hydroxymethyl)tetrahydro-2*H*-pyran-2-yl]amino}pyrimidin-4(3*H*)-one (MTPP)

Hydrogen bonding of hemaglutinin complexes with ligands from docking results was identified with MOE 2008.10 software on LigX interaction program. Three ligands interacted with amino acid of hemaglutinin. The HBIP ligand also interacted with site of Thr 437. HBIP ligand showed the active site binding score for hemaglutinin and that complex are 21,3% for Thr437 as donor, score 21,3% for Thr437 as acceptor. The interaction of ligands with hemaglutinin site, HBIP has many interaction with hemaglutinin site, that HBIP ligand has a better interaction than STD ligand (Fig. 1A and 1B), and has a similar interaction with the MNMB ligand (Fig. 1A and 1C). ligand has residue contact to catalytic site can be seen in figure 1.

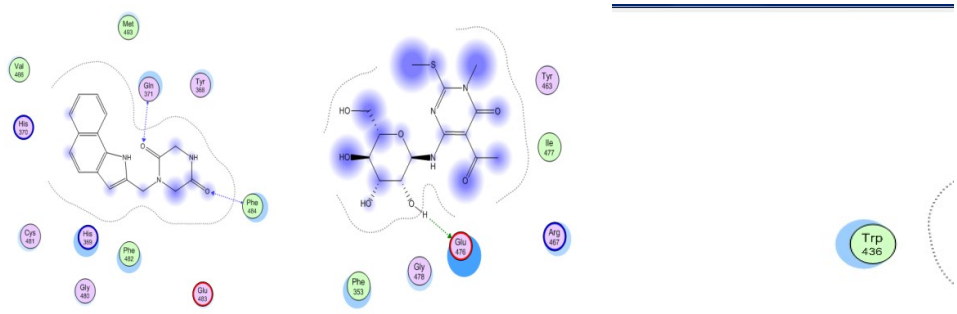
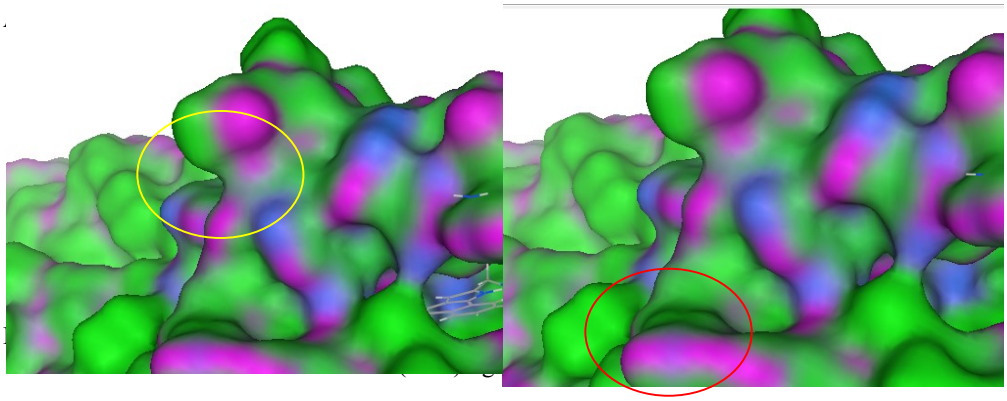


Figure 1. A. Hemaglutinin contact residues with HBIP
B. Hemaglutinin contact residues with STD
C. Hemaglutinin contact residues with MNMB

The conformation of HBIP ligand were able to enter and fill the cavity of hemaglutinin binding site (yellow circle/Figure 2A), HBIP ligand fills the cavity of ligand binding site more than STD ligand (red circle/Figure 2B).



The enzyme has a binding site that can form the cavity with the presence of active compounds. If the active compound is able to enter the enzyme binding site, these compounds can inhibit the catalytic action of hemaglutinin. Based on the spectrum of electrostatic potential, the ligand conformation in the cavity was obtained using the MOE 2008.10 software as shown in Figure 2.

CONCLUSION

Many compounds are known that can act to inhibit hemaglutinin. we indeed find a compound of potent antiviral target candidates. Among them, HBIP ligand which produced 2 hydrogen bonds at catalytic site in hemaglutinin. The binding energy and pKi of HBIP are -6.3490 kcal/mol and 7,261 μM, respectively. Those value are greater than STD as standard. Thus, HBIP has the ability to be lead compound of hemaglutinin antiviral.

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DIFFERENTIATION OF PLASMA IL-10/TNF-α RATIO BETWEEN OF MALARIA FALCIPARUM PATIENTS WITH ANEMIA AND WITHOUT ANEMIA

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ABSTRACT

Background. Anemia is an important complication of malaria, and its pathogenesis is not well understood. High level of the Th2 cytokine (such as IL-10), which counteract the Th1 (such as TNF-α) cytokine, might prevent the development of severe malarial anemia. **Objective.** The purpose of this study was to compare the ratios of plasma IL-10/TNF-α in malaria *falciparum* patients with anemia and without anemia. **Methods.** Concentrations of plasma IL-10 and TNF-α in 16 malaria *falciparum* patients with anemia and 16 malaria *falciparum* patients without anemia from patients at primary health centers, West Lombok and Center Lombok during March until July 2008 were measured using ELISA and were calculated for ratios IL-10/TNF-α respectively.. Concentration of haemoglobin (Hb) was measured using hematology analyzer. Anemia is concentration of Hb < 11 g/dl. The results were analyzed using 2 sample t test with SPSS ver.13.**Results.** The average concentration of plasma IL-10 in malaria *falciparum* patients with anemia was 8.81 ± 3.04 pg/ml while the average concentration of plasma IL-10 in malaria *falciparum* patients without anemia was 47,99 ± 25,26 pg/ml. The average concentration of plasma TNF-α in malaria *falciparum* patients with anemia was 151.7 ± 26.8 pg/ml while the average concentration of plasma TNF-α in malaria *falciparum* patients without anemia was 44.06 ± 15.14 pg/ml. The average ratio of plasma IL-10/TNF-α in malaria *falciparum* patients with anemia was 0.06 ± 0.026 while the average ratio of plasma IL-10/TNF-α in malaria *falciparum* patients without anemia was 1.15 ± 0.46. **Conclusion.** The mean ratio of plasma IL-10/TNF-α in malaria *falciparum* patients with anemia was significantly lower than the mean ratio of plasma IL-10/TNF-α in malaria *falciparum* patients without anemia (p=0.000).

Keywords: malaria *falciparum*, anemia, IL-10, TNF-α, ELISA

INTRODUCTION

The cause of anemia in malaria is multifactorial, such as the red blood cell destruction and decreased production of red blood cells. The mechanism of anemia in malaria vary by age, pregnancy, immunity and genetic, and local endemicity of malaria. Mechanism of decreased production of red blood cells include erythroid hypoplasia, suppression eritropoitin synthesis, may be caused by inflammatory mediators such as tumor necrosis factor (TNF), diserythropoiesis and inbalance cytokine. Concentrations of serum TNF, interleukin 10 (IL-10) and interferon γ (IFN-γ) is generally correlated with severity of disease. High levels of cytokine T helper cells type 1 (Th1) (such as TNF-α, IFN-γ) and *nitric oxide* (NO) has a role in the pathogenesis of cerebral malaria, is also in the mechanism of bone marrow depression, erythrofagocytosis and diserythropoiesis seen in malaria with anemia.[1] High levels of cytokine T helper cells type 2 (Th2) (such as IL-10) which can neutralize the Th1 cytokine, could prevent the development of severe anemia in malaria.[2]

IL-10 can inhibit the synthesis of several cytokines produced by macrophages, NK cells, T-lymphocytes, suppress delayed-type hypersensitivity responses, stimulates B-cell proliferation and differentiation into antibody-producing cells, and inhibit the synthesis of several cytokines that stimulated macrophages after binding to receptors 110 kd cell such as TNF-a, IL-1, IL-6, IL-8, IL-12, granulocyte colony-stimulating factor, MIP-1a, and MIP-2a.[3,4]

TNF-a plays a role in some complications that often occur in people with *Plasmodium falciparum* malaria, especially by such as cerebral malaria and anemia. TNF-a inhibit eritropoitin production as evidenced by studies in vitro and suppresed *stem cell* on bone marrow.[5,6,7]

MATERIALS AND METHODS

The study was observational analytic study with *case control study design.* The population is all Malaria *falciparum* patients with anemia and without anemia in Central Public Health Tanjung West Lombok and Central Lombok. Inclusion criteria for samples study: aged ≥ 17 years old, shows clinical symptoms of malaria: fever, chills, headache, accompanied by the presence or absence of anemia, blood smear examinations was positive *Plasmodium falciparum,* imunochromatography tes for malaria infected with *Plasmodium falciparum* was showed positive results.

Plasma levels of IL-10 measured by ELISA method, in units of pg / ml, using reagent *RayBio ® Human IL-10 ELISA Kit* (Cat # *ELH-IL10-001).*

Plasma levels of TNF-α measured by ELISA method, in units of pg / ml using a reagent *RayBio ® Human TNF-alpha ELISA Kit* (Cat # ELH-TNF a -001).

Anemia is malaria patients with the results of hemoglobin (Hb) showed less than 11 g/dl for all genders. Hb level is determined by using the *hematology analyzer.*

Statistical Analysis

To examine differences in the ratio of a plasma IL-10/TNF- between Malaria *falciparum* patients with anemia and without anemia, first performed normality test with the *Kolmogorov-Smirnov* (KS) was followed by statistical analysis of two independent sample t test. Statistical analysis of this study using SPSS *software* ver.13.

RESULTS AND DISCUSSION

The ratio of a plasma IL-10/TNF- Malaria *falciparum* patients with anemia ranged from 0.03 to 0.13 with mean 0.06 and SD 0.026. The ratio of a plasma IL-10/TNF- Malaria *falciparum* patients without anemia ranged from 0.45 to 2.01 with mean 1.15 and SD 0.46.

Statistical analysis of two independent samples t test showed that the ratio between IL-10 and TNF-a plasma of Malaria *falciparum* patients with anemia and Malaria *falciparum* patients without anemia had a significant difference (p = 0.000). The ratio of IL-10 / TNF-a plasma Malaria *falciparum* patients with anemia (0.06) were significantly lower compared with Malaria *falciparum* patients without anemia (1.15).

Other studies say the average ratio of a plasma IL-10/TNF-α Malaria *falciparum* patients with anemia of age <11 years less than.[8] Study by *Helleberg et al* (2005) on childrens in Africa suffering from persistent Malaria *falciparum* was mention a mean ratio IL-10/TNF-α Malaria *falciparum* patients with anemia who came to the emergency room was 1.9.[9] The results study by Kurtzhals *et al* (1998) showed the ratio of TNF-α/IL-10 was higher in malaria patients with severe anemia compared with other groups, but no mention of the absolute value of the ratio of TNF-α/IL-10.[10] Differences IL-10/TNF-α plasma ratio from previous studies because of differences in age of study subjects who using children as research subjects, while this study used adults (age> 17 year old) as research subjects.

Malaria *Falciparum* patients with anemia who also showed symptoms of fever had significantly higher degrees of parasitemia, elevated levels of TNF-α plasma and increased plasma levels of IL-10 compared with patients who showed no symptoms of fever.[11]

Table 1. Levels of IL-10 and TNF-a plasma Malaria *falciparum* patients with anemia and without anemia

Malaria *falciparum* patients with anemia				Malaria *falciparum* patients without anemia			
No	Concentrations of IL-10 (pg/ml)	Concentrations of TNF-α (pg/ml)	Rasio of IL-10/ TNF-α	No	Concentrations of IL-10 (pg/ml)	Concentrations of TNF-α (pg/ml)	Rasio of IL-10/ TNF-α
1	5.40	131.5	0.04	1	25.20	54.6	0.46
2	5.50	124.1	0.04	2	29.50	65.1	0.45
3	6.00	189.2	0.03	3	30.50	20.9	1.46
4	6.40	155.2	0.04	4	31.05	20.4	1.52
5	6.90	168.4	0.04	5	31.70	41.3	0.77
6	7.45	159.6	0.05	6	36.05	54.8	0.66
7	7.90	144.8	0.05	7	39.10	30.2	1.29
8	8.10	160.2	0.05	8	39.20	42.6	0.92
9	8.30	132.9	0.06	9	40.10	40.1	1.00
10	8.70	126.7	0.07	10	41.50	50.7	0.82
11	9.20	199.4	0.05	11	41.60	36.4	1.14
12	9.80	197.2	0.05	12	43.80	21.8	2.01
13	10.80	120.9	0.09	13	46.00	44.5	1.03
14	11.10	164.1	0.07	14	94.85	55.6	1.70
15	11.95	122.4	0.10	15	95.70	60.9	1.57
16	17.50	130.6	0.13	16	101.95	65.1	1.57
	Mean: 8.81 SD: 3.04	Mean: 151.7 SD: 26.8	Mean: 0.06 SD: 0.026		Mean: 47.99 SD : 25,26	Mean: 44,06 SD: 15,14	Mean: 1,15 SD: 0,46

Increased activity of Th2 cytokines such as IL-10 in the early phases tends to increase the sensitivity and exacerbation of parasite infection. This situation occurs because IL-10 can inhibit Th1 cytokine production and inhibits the function of macrophages that important role in the host body's defense mechanism against malaria parasite infection in the acute phase. Th2 cytokines as a protection in chronic phase of infection by *Plasmodium* or healing period with a powerful way of inducing the formation of antibody response against parasite antigens.[12]

Result studies by Othoro *et al* (1999) in children suffering from malaria in malaria holoendemic region (Western Kenya) was found the average ratio of IL-10/TNF-α malaria patients without anemia at 4.64. The existence of this high ratio was found in patients with high parasite densities but did not experience complications of severe malaria such as anemia or cerebral malaria. The ratio IL-10/TNF-α in malaria patients with moderate anemia (Hb <8 g/dl to> 5 g/dl) were not statistically significant different from the malaria patients with severe anemia (Hb <5 g/dl). The study also mentions that children who live in areas holoendemic with mild disease (uncomplicated malaria with low parasite density or high) has a ratio of IL-10/TNF-α significantly higher compared with patients with severe malaria (malaria with anemia) ($P<0.05$). The findings are consistent with results obtained in the present study. The balance between Th1 cytokines (like TNF-α) with Th2 cytokines (like IL-10) plays an important role in the pathogenesis of malaria complications with *P.falciparum* infection.[13]

Different results obtained in studies conducted by Dodoo *et al* (2002) in *vitro* experiments which showed that a high ratio between pro-inflammatory cytokine with anti-inflammatory cytokines (both TGF-β and IL-10) associated with an increased concentration of hemoglobin. This is different with previous theory showed that high levels of inflammatory cytokines and low levels of proinflammatory cytokines as a protective factor against the occurrence of anemia in malaria patients. These circumstances may be said to relate to antiparasitic effects rather than pro-inflammatory cytokine that can prevent the occurrence of anemia due to the parasites in the blood.[14]

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FORMULATION AND TEST OF STERILITY STERILE COMBINATION GEL ALOE VERA EXTRACT (*ALOE BARBADENSIS* MILL.) AND THE BANANA'S STEM EXTRACT (*MUSA PARADISIACA* LINN.)

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ABSTRACT

Damaged skin burns could be treated by using a natural drug from plants. Plants, which are commonly used empirically to treat burns and has been tested its activity are Aloe vera (Aloe barbadensis mill.) and banana tree (Musa paradisiaca Linn.). Preparations for the treatment of burns, especially on the second and third degree of burns, should be free of microbes. In this research, it had been formulated sterile gel for burns containing 6% extracts of aloe vera and 2% extract of banana stem with two methods, by aseptically process and using gamma radiation. This research showed that all of the formula fulfilled the requirements of the sterility test and no physical changes during 56 days of storage although there were differences in pH and viscosity values. Result of qualitative analysis using Thin Layer Chromatography showed that all of sterile gels still had the same component with extract. The physical appearance of gels which were formulated aseptically and using gamma radiation gave a different result especially in color and its consistancy.

Keywords: Aloe vera, Burns, Extract of banana stem, Sterile gel

INTRODUCTION

Skin damage can occur due to various reason, one of them is burns. Degrees of burns is divided into three, first, second and third degree burns (Marzoeki, 1991). Plants hich are commonly used empirically to treat burn are Aloe vera and banana. Dosage form that is used to treat burns is usually a topical preparation, such as gels. Preparations for the treatment of burns, according to the International Pharmaceutical Federation (FIP), must be free of microbial (Wibowo, 2008). Based on that problems, then conducted research on sterile gel formulation for the treatment of burns from aloe vera extract and extract of banana stem.

MATERIALS AND METHODS

1. Collecting Material and Plant Determination
   Banana stem plants were collected from Cicalengka, Bandung. Those plants were then determined in the Laboratory of Herbarium, Biology Department of Faculty of Mathematics and Natural Sciences, Padjadjaran University.
2. Banana Stem Extraction
   Banana trees are cut and dried, then it is soxhleted for minimum 5 hours using 95% ethanol. Then the extract is collected and evaporated with a rotary evaporator in low pressure at 30-40ºC temperature until thick.
3. The Formula Design
   Gel formula that is used in the formulation of sterile gel from extracts of Aloe vera (*Aloe barbadensis* Mill.) and the extract of Banana stem (*Musa paradisiaca* Linn.) can be seen in Table 1.

Tabel 1. Sterile Gel Formula

Material (%)	F0	F1	F2	F3	F4
Aloe vera extract	-	6	6	6	6
Extract of Banana Stem	-	2	2	2	2
Carbopol® 980	1.5	1.5	1.5	1.5	1.5
Triethanolamine	3	3	3	3	3
Propylenglycol	10	10	10	10	10
Glyserin	10	10	10	10	10
Acnibio®	0.25	-	-	0.25	0.25
Honey	5	-	5	-	5
Aquabidest sterile ad	100	100	100	100	100

Sterile gel formulation was done in two methods of sterilization by aseptic process using a bacterial filter and terminal sterilization with gamma radiation.

4. Preparations Gel Formulation

Fifth gel formula that has been designed in Table 1 then formulated in the following manner:

A. Formula Zero (F0)

In the space of Laminar Air Flow, gel was made with cold process. 1.5 grams of Carbopol® 980 was sprinkled in aquabidestilata, then slowly stirred using a stirrer shaft with added triethanolamine (TEA) drop by drop until the mass is formed a gel. Then put propylenglycol and glycerin with stirring slowly. After the gel base is formed and then added acnibio® and honey in small increments while stirring until homogenous.

B. F1, F2, F3, F4

In the space of Laminar Air Flow, gel was made with cold process. 1.5 grams of Carbopol® 980 was sprinkled in aquabidestilata, then slowly stirred using a stirrer shaft with added triethanolamine (TEA) drop by drop until the mass is formed a gel. Then put propylenglycol and glycerin with stirring slowly. After the gel base is formed, then added the extract of Aloe vera and banana stem extract. Stirring the mixture until homogeneous. Finally, added preservatives (acnibio® or honey) in accordance with a formula that will be made.

5. Sterilization dosage

Sterilization processes include sterilization of instruments used during the formulation, sterilization of gel base and sterilization of the final dosage form. Sterilization of the equipment are using oven, autoclave and direct sterilization in fire. The base of gel used for gel formulation is sterilized with an autoclave at a temperature of 121°C for 15 minutes.

Sterilization of dosage is done by two methods, sterilization by aseptic process using a bacterial filter and terminal sterilization with gamma radiation.

In the method of sterilization with aseptic process, aloe vera extract and extract of banana stem that will be used first dissolved in sterile distilled water and sterilized with filtration technique using 0.22 μm bacteria filter. The extracts then mixed with a gel base that has been sterilized using the autoclave. This process is done in the Laminar Air Flow aseptically.

Sterilization using gamma rays are done after all the formulas were formulated. All the formulas that have been made filled into the tube, and then sterilized using gamma radiation with dose of radiation 25kGy.

6. Sterility Testing

Sterile gel preparation was inoculated into reaction tube containing medium Thioglycolate and incubated at a temperature 30-35 ° C for not less than 14 days. Occurrence of turbidity in the tubes was observed daily.

Into a reaction tube containing the medium Soybean Casein Digest, inoculated the sterile gel preparation then incubated at a temperature of 20-25 ° C for not less than 14 days. Occurrence of turbidity in the tubes was observed daily.

7. Evaluation of Physical Properties and Qualitative Analysis

a) Observations Organoleptic

Organoleptic observations observation changes the consistency, color and odor of gel on days 1, 3, 7 and subsequently carried out every week to 56 days of storage.

b) pH Measurement

Measurements carried out using a pH meter 744 Methorm by dipping the electrode in the pH meter into the gel which has been diluted with distilled water. After the electrodes submerged, turn on the pH meter and then left untouched until the display shows the pH meter at a steady rate. Measurements made during the time of storage on days 1, 3, 7 and subsequently carried out every week to 56 days of storage.

c) Viscosity Measurement

Measurements carried out using Viskotester Rion VT-04 by dipping the rod into the spindle no.2 gel and numbers on screen are recorded when the needle on the screen does not move anymore. Measurements conducted during the time of storage on days 1, 3, 7 and subsequently carried out every week to 56 days of storage.

RESULTS AND DISCUSSION

Gel Formula

Table 1.Sterile Gel Formula

Materials (%)	F0	F1	F2	F3	F4
Aloe vera extract	-	6	6	6	6
Extract of banana stem	-	2	2	2	2
Carbopol® 980	1,5	1,5	1,5	1,5	1,5
Trietanolamin	3	3	3	3	3
Propilenglycol	10	10	10	10	10
Glyserin	10	10	10	10	10
Acnibio®	0,25	-	-	0,25	0,25
Honey	5	-	5	-	5
Aquabidest ad.	100	100	100	100	100

The Result of Sterile Gel Formulation

Fig 1. Sterile gel formulated aseptically

Fig 2. Sterile gel sterilized using gamma rays

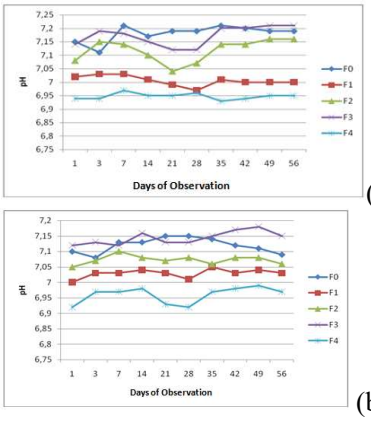
Result of Sterility Test

Table 2. Result of Sterility Test of Sterile Gel

Formula	Aseptically Process		Gamma Radiation	
	FTM Media	TSB Media	FTM Media	TSB Media
F0	(-)	(-)	(-)	(-)
F1	(-)	(-)	(-)	(-)
F2	(-)	(-)	(-)	(-)
F3	(-)	(-)	(-)	(-)
F4	(-)	(-)	(-)	(-)

The result of sterility test showed that all formulas which are formulated aseptically and sterilized using gamma rays meet sterility requirements.

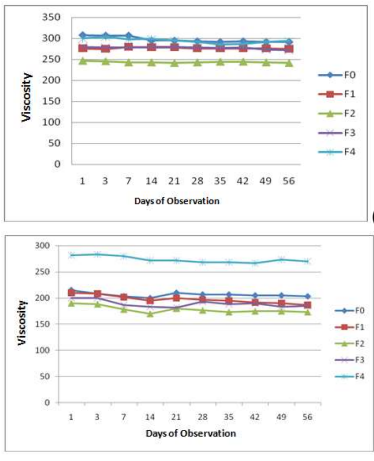
Result of Physical Evaluation



(a)

(b)

Fig 3. pH of sterile gel formulated aseptically (a) and sterilized using gamma rays (b) during 56 days of storage



(a)

(b)

Fig 4. Viscosity of sterile gel formulated aseptically (a) and sterilized using gamma rays (b) during 56 days of storage

Results of evaluation of physical properties showed that no changes to the physical qualities in each gel for 56 days of storage although there are significant differences in pH value and viscosity.

Description: F0, F1, F2, F3 dan F4: Sterile Gel Formula; (-): No microbial growth

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DIFFERENCES IN PLASMA ADIPONECTIN LEVELS IN PATIENTS WITH TYPE 2 DIABETES MELLITUS ON VARIOUS LEVELS OF HBA1C CONCENTRATION AS A CRITERIA OF DIABETES MELLITUS MONITORING

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ABSTRACT

Background. Insulin resistance is a major contributor to the pathogenesis of type 2 diabetes and plays a key role in associated metabolic abnormalities, such as dyslipidemia and hypertension. Adiponectin appears to enhance insulin action and ameliorate a variety of detrimental vascular responses in insulin-resistant patients. Adiponectin plasma concentrations are decreased in patients with type 2 diabetes. Despite the fact that adiponectin is an adipose-specific protein, as an individual becomes more obese, the adiponectin concentration in the blood is decreased and insulin resistance increases. **Objective.** The purpose of this study was to compare the concentration of plasma adiponectin in type 2 diabetes mellitus patients with grade several of HbA1C level. **Methods.** Concentrations of plasma adiponectin in 30 type 2 diabetes mellitus patients from patients at General Hospital Sanglah Denpasar, during February until April 2009 were measured using ELISA. Concentration of HbA1C was measured using chemical analyzer. There were three groups of sample population: group of HbA1C < 6.5%, HbA1C 6.5-8%, and HbA1C > 8%. The results were analyzed using ANOVA with SPSS ver.13. **Results.** The average concentration of plasma adiponectin in type 2 diabetes mellitus patients with HbA1C < 6,5% was 7.33 ± 2.63 μg/ml; with HbA1C 6,5% - 8% was 3.80 ± 0.79 μg/ml; with HbA1C > 8% was 2.35 ± 0.64 μg/ml. **Conclusion.** The mean of plasma adiponectin concentration in type 2 diabetes mellitus patients with HbA1C < 6,5% was significantly higher than the mean of plasma adiponectin concentration in type 2 diabetes mellitus patients with HbA1C 6,5-8% and HbA1C > 8% (p=0.000), while the mean of plasma adiponectin concentration in type 2 diabetes mellitus patients with HbA1C 6,5-8% was not significantly different than the mean of plasma adiponectin concentration in type 2 diabetes mellitus with HbA1C >8%.

Key words: Type 2 diabetes mellitus, HbA1C, plasma adiponectin, ELISA.

INTRODUCTION

Diabetes mellitus (DM) is a disease characterized by an increase of blood glucose levels caused by genetic and environmental factors. Diabetes mellitus type 2 occurs because the two causes of disruption of insulin production by pancreatic beta cells or insulin resistance on the cell network target.[1]

Obesity also causes increase of insulin resistance , leptin, IL-6, resistin, PAI-1, TNF-α and decrease of adiponektin.[2] In addition to PAI-1, excessive adipose tissue also increase the release of serum fibrinogen, von Willebrand factor, factor VII and thrombin that can stimulate aterogenesis and causing vulnerability to experiencing cardiovascular events such as acute coronary syndrome. The existence of atherogenic lipoprotein phenotype, lipotoksisitas and circumstances that stimulate aterogenesis explain that obesity associated with insulin resistance and cardiovascular disease.[3,4]

Adiponectine present in human blood circulation and healthy rats with high levels. Levels reached approximately 0.01% of total plasma proteins, approximately three times greater than leptin. Unlike other adipokin levels increases in proportion to the fat content decreased in obesitas. Adiponectine level decrease due to interaction of genetic and environmental factors that cause obesity. Decrease in activity is also caused by a decrease in adiponectin receptors are linked to obesity. Decrease in adiponectine activity

is a cause of insulin resistance, type 2 diabetes mellitus, metabolic syndrome and atherosclerosis.[5]

Fat tissue (adipose tissues), which was originally known only as a triglycerides depocit, recently shown to have a variety of roles following the discovery of leptin by Friedman and colleagues. Currently adiposit viewed as a dynamic endocrine cells that release free fatty acids and secreting substances called adipokines, including tumor necrosis factor-α (TNF-α), interleukins, plasminogen-activator inhibitor type1 (PAI-1), leptin, resistin, and adiponectine. These adipokins have role in various regulatory processes ranging from appetite and insulin sensitivity to inflammation and atherogenesis. Adipokin have a central effect on the level of the central nervous system and peripheral tissue such as skeletal muscle and liver.[6]

In humans and experimental animals, reduced adiponectine levels in individuals with type 2 diabetes and showed a strong negative correlation with insulin resistance. Adiponectin level decrease also correlated with decreased vascular function and increased coronary artery disease (CAD). This study was conducted to determine the differences of plasma adiponectin levels in patients with type 2 diabetes mellitus of various concentrations of HbA1c as a criteria of diabetic mellitus monitoring.

MATERIALS AND METHODS

This study was done by consecutive sampling technique until the required samples were achieved.Case were recruited from the internal outpatient clinic of Sanglah Hospital Denpasar from February until April 2009. The inclusion criterias are:

a. Diabetic mellitus patients with symptoms polyphagia, polidipsi, polyuria and weight reduce.
b. Patients who had been diagnosed as type 2 diabetic mellitus in internal medicine of Sanglah Hospital.
c. Appropriate laboratory criteria of diabetic mellitus.

Plasma of the patients were collected, and measured for HbA1C and adiponectine. HbA1c were measured by autoanalyzer and adiponectine were measured by ELISA *reader* with ELISA double sandwich methods. Data of adiponectine concentration were analysis by ANOVA.

RESULTS AND DISCUSSION

Based on the research that has been done in Sanglah Hospital, were collected 30 samples of type 2 diabetes mellitus with details as follows: type 2 diabetes mellitus patients with HbA1c <6.5%: 10 (33.33%); type 2 diabetes mellitus patients with HbA1c between 6.5 to 8%: 10 (33.33%) and type 2 diabetes mellitus patients with HbA1c> 8%: 10 (33.33%).

Adiponectin levels in type 2 diabetes mellitus patients with HbA1c <6.5% ranged between 4.63 ug / ml up to 13.22 ug / ml with a mean of 7.33 ug / ml and SD 2.63 g / ml. Adiponectin levels in type 2 diabetes mellitus patients with HbA1c 6.5-8,0% ranged between 2,39 ug / ml up to 4,95 ug / ml with a mean of 3,80 ug / ml and SD 0,79 g / ml. Adiponectin levels in type 2 diabetes mellitus patients with HbA1c >8,0% ranged between 1,61 ug / ml up to 3,45 ug / ml with a mean of 2,35 ug / ml and SD 0,64 g / ml.

Statistical analysis by ANOVA showed that plasma adiponectin levels between patients with diabetes mellitus at various levels of HbA1c concentrations have a significant difference (p = 0.000).Plasma adiponectin levels of type 2 diabetes mellitus patients at concentration of HbA1c < 6,5% (7.33 0,± 2,63 µg / ml) were significantly

higher than the concentration of HbA1c 6.5 to 8.0% (3,80 ± 0,79µg/ml) and the concentration of HbA1c> 8.0% (2,34 ± 0,64µg/ml) Meanwhile, plasma adiponectin levels of type 2 diabetes mellitus patients with concentration of HbA1c 6.5 to 8.0% non-significant difference (p = 0.056) with concentrations of HbA1c> 8.0% (2,34 ± 0,64µg/ml). This situation indicates that there is an inverse relationship between plasma adiponectin levels with type 2 diabetic mellitus monitoring level (HbA1c levels). Increased plasma adiponectin levels correlated positively to insulin sensitivity. Giving adiponectin is a novel therapeutic strategy for insulin resistance and type 2 diabetic mellitus. Adiponectin may have several therapeutic uses including as antidiabetic drugs such as those used in the clinic today. First adiponectin has anti-diabetic and hypolipidemic effects and serves as a potential anti-inflammatory ingredients that will prevent or slow the aterogenesis. Second, adiponectin did not result in weight gain.

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THE ANALYISIS OF HEPATITIS B VIRUS (HBV) SUBTYPES ON S (Surface) REGION GENES FROM PATIENT IN MENGWI DISTRICT, BADUNG REGENCY, BALI

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ABSTRACT

Hepatitis B virus (HBV) infection is a major worldwide problem as well as in Indonesia. Indonesia has a high Endemic level of hepatitis B infection. The subtypes HBV can show some differences in the aspects of geographical distribution and clinical and virological characteristics and provide historical information on the migration pattern of local's ancestor. Mengwi has become a developed region with the rapid growth of social economic, demographic and dynamic inhabitants. This condition can contribute the varieties of HBV subtype. The purpose of this research was to analyze the HBV subtype on patients who possessed hepatitis signs and symptoms and came to Puskesmas Mengwi I, Badung, Bali. The research subject was taken from 75 patients who came to Puskesmas Mengwi I during collecting sample period. All serum samples were examined for ALT/SGPT level and detection for HBsAg by using enzyme-linked immunosorbent assay method. Serum of HBsAg Positive samples were used to identify the HBV subtype. We carried out DNA extraction from serum of HBsAg Positive samples. Some parts of the viral S gene were amplified by polymerase chain reaction (PCR) first-round with primers P7 and P8. If the PCR Amplification gad been negative, a second-round (nested) PCR would have been carried out by using primers HBS1 and HBS2. Then, they were purified, labeled, and sequenced. The obtained nucleotide sequences were converted into amino-acid sequences and we conducted multiple alignment. HBV subtype were determined by using the analysis of amino acid substitutions at positions 122, 127, 134, 159, 160, and 177 of S gene and done with computer program Genetyx for windows version 9.0. The analysis of HBV subtype showed that subtype *adw2* was found on ten of eleven isolates (90,9%), followed by subtype *adrq+* was found only on one sample (9,1%). The previous study by Utama A et al, (2009) found that HBV subtype *adw2* and *adrq+* were found predominantly on HBV genotype B and C infection in Indonesia. Interestingly, in this study we found all of genotypes B were subtype *adw2*, and genotype C sample was subtype *adrq+*.

Keywords: Hepatitis B virus (HBV), subtypes, Mengwi area

INTRODUCTION

Hepatitis B virus (HBV) infection is still become major health problem in the world including in Indonesia. Worldwide, more than 350 million people chronically are infected with HBV, some of whom develop severe liver disease. The prevalence of HBV infection is generally high in Asia and Africa[1].

Indonesia has a moderate to high endemicity of HBV infection. Indonesia consists of thousands of islands inhabited by more than 400 ethnic groups. Accordingly, the prevalence of HBV infections varies widely from one part to another, ranging from 5 to 10% for HBsAg[2,3]. The carrier rates among apparently healthy populations in Indonesia have been reported to range from 4.0 to 20.3%[4].

HBV infection prevalence varies by island. In general, areas outside of Java Island have a higher prevalence of hepatitis B surface antigen (HBsAg) (9.2%) than areas within Java (5.0%) [5]. HBV isolates of different genotypes and subtypes appear to show different geographical distributions[6,7], virological characteristics, and, possibly, clinical outcomes [8]. They can also provide historical information on the migration pattern of the ancestor of a local population [9,10].

Mengwi is the capital city of Badung regency, located 25 km north of Denpasar, the capital city of Bali Province. Since more than ten years, Mengwi district is become a

capital city of Badung Regency. Thus, development of social-economic and people's mobility is increasing and more dynamic in Mengwi. There has been no previous report on the prevalence of HBV and HCV infections in the Mengwi population. This is the first report on the epidemiology of these viruses in Mengwi, Bali. The new and representative Information and analysis of HBV subgenotype and HCV subtype in Mengwi, Badung are needed

The objective of this study was to determine the distribution of HBV subtypes among patient with hepatitis symptoms in Mengwi, Bali. Another interest of our current study lay in the possibility that we might find a new subtypes of HBV suggested by others previous study that a diverse variety of subtypes HBV were exist in Bali and in Indonesia.

MATERIALS AND METHODS

Serum samples

Serum samples collected from a total of 75 subjects with hepatitis symptoms who visited Puskesmas Mengwi I Bali during the four months from July to October 2009. Ethical approval of this investigation was obtained from the ethics committees of the Airlangga University in Surabaya, Indonesia. All subjects signed an informed consent form and participated voluntarily in this study. The level of ALT/SGPT was tested by commercial kit (IFCC mod, Diasys, Germany). The presence of HBsAg was determined by ELISA commercial assay kits (Axiom Diagnostic). The sera that positive for HBsAg were subsequently analyzed for HBV genotypes and subgenotypes as described below.

HBV DNA extraction and PCR amplification.

DNA extraction and amplification were done as described previously [11]. In brief, HBV DNA was extracted from 200 µl of serum samples by use of commercial kit QIAamp DNA Mini kit (Qiagen, Inc). Part of the viral S gene (nucleotides 256 to 796) was amplified by PCR with primers P7 and P8 (Table 1) as reported previously [11]. When the PCR amplification was negative, a second-round (nested) PCR was carried out using primers HBS1 and HBS2 (table 1).

Table 1. Oligonucleotide primers used for first round and second round PCR amplification of The HBV genome

Primer	Gene (position)	Polarity	
P7	S (256 to 278)	Sense	5′-GTGGTGG
P8	S (796 to 776)	Antisense	5′-CGGTAWA

* M= A or C ; W= A or T [8,12]

Both first-round and second-round PCRs were performed for 40 cycles, each consisting of 1 min at 94°C, 1 min at 55°C, and 2 min at 72°C. Amplification products were visualized on a 2% agarose gel stained with ethidium bromide.

Analysis of HBV subtypes.

Nucleotide sequences of the amplified fragments were determined with the BigDye deoxy Terminator v1.1 cycle sequencing kit (Applied Biosystems) and an ABI Prism 310 genetic analyzer (Perkin Elmer) as described previously [8]. The obtained nucleotide sequences were converted into amino-acid sequences and we conducted multiple alignment to compared to those from the international DNA data bank (DDBJ/EMBL/GenBank). HBV subtype were determined by using the analysis of amino acid substitutions at positions 122, 127, 134, 159, 160, and 177 of S gene and done by use

of the computer software Genetyx-Win v9.0 (Genetyx Corporation, Tokyo, Japan).

RESULTS

A total of 75 serum samples collected from subjects with hepatitis symptoms (45 male and 30 female; range 11 to 75 years) were analyzed. Eleven (14.7%) of the 75 sera tested positive for HBsAg and were subsequently analyzed for HBV subtypes. One of the 11 (9,1%) HBsAg positive sera was female and the other were male. From the ethnic origin we found that nine of the 11 (81,8%) HBsAg positive sera ware Balinese ethnic origin. Two (18,2%) of the 11 HBsAg positive sera were found had twice upper normal limit (UNL) of ALT level (Tabel 2)

Table 2. HBsAg and ALT/SGPT level test result

ALT/SGPT	HBsAg positive	HBsAg negative
> 2 x UNL	2	0
≤ 2 x UNL (Normal)	9	64
Total	11/75 (14.7 %)	64/75 (85.3%)

*UNL: Upper Normal Limit (Diasys,Germany)
Male = < 41 U/l
Female = < 31 U/l

HBV DNA of the S gene was detected in eight from 11 (72.7%) samples after the first-round PCR and in three from 11 samples (27.3%) after the second-round PCR. The analysis of amino acid substitutions at positions 122, 127, 134, 159, 160, and 177 were showed at the figure below (Figure 1)

Fig. 1. Multiple alignment of amino acid sequences of HBsAg (positions 116 to 183) of HBV isolates from Mengwi, Bali (code SM; shown in bold) and those from the international DNA data bank (indicated with the accession numbers and countries of origin). Genotypes, subgenotypes,and subtypes are also indicated.
*** : residues that determine subtypes [13, 14, 15, 16].**

Figure 1 depict the multiple alignment of amino acid sequences at positions 116 to 183 (nt 500 to 703) in HBsAg of the HBV isolates analyzed in this study and the 38 reference sequences of the ten HBV genotypes (A to J) obtained from the international DNA data bank.

Table 3. Distribution of HBV subtypes from samples

HBV Subtypes	
adw2	*adrq+*
10/11 (90,9%)	1/11 (9,1%)

Based on the amino acid substitutions at positions 122, 127, 134, 159, 160 and 177, it was found that the *adw2* HBV subtype was predominant (90.9%), followed by *adrq+* (9,1%) in Mengwi, Bali (Table 3).

The 11 isolates of HBV subgenotype B from samples, 10 isolates (90,9%) (SM 169, SM 187, Sm 214, SM 228, SM 230, SM 231, SM 232, SM 234, SM 235 and SM 236) had lysine at position 122 (K122), proline at posisition 127 (P127), phenylalanine at position 134 (F134), alanine at position 159 (A159), lysine at position 160 (K160), and valine at position 177 (V177) in HBsAg (Fig. 3). This combination is considered to be the expression of the *adw2* HBV Subtype. The other isolate (9,1%) which is SM 233 had lysine at position 122 (K122), proline at posisition 127 (P127), phenylalanine at position 134 (F134), alanine at position 159 (A159), arginine at position 160 (R160), and valine at position 177 (V177) in HBsAg (Fig. 3) and is considered to be *adrq+* HBV Subtype. Interestingly, in this study we found all of genotypes B were subtype *adw2*, and genotype C sample was subtype *adrq+*.

DISCUSSION

The analysis of HBV subtype showed that subtype *adw2* was found on ten of eleven isolates (90,9%), followed by subtype *adrq+* was found only on one sample (9,1%). This result has similarity with the previous observations that *adw* subtype was predominant in Denpasar, Bali, Indonesia followed by *adr*, *ayw* and *ayr* subtypes[3].

The previous study by Utama A et al, (2009)[17] found that HBV subtype *adw2* and *adrq+* were found predominantly on HBV genotype B and C infection in Indonesia. On the other hand, it was reported that subtype *adw* (*adw2 and adw4*) was most prevalent in Sumatra, Java, the southern part of Kalimantan, Bali, Lombok, Ternate, and Morotai [8]. Subtype ayr was not found in this study and was rarely found.[8] Thus, this study has assumpted that, mengwi district is within the *adw* zone.

HBV genotypes and subtypes have been used as an instrument to identify migration patterns of the ancestors of local populations in certain geographical areas.[19] There are some theories about the origin of the Indonesian population, one of the most accepted theories is that proposed by Brandes [18]. According to Brandes, the inhabitants occupying Indonesia consist of people who originated from language speakers of Stocks Austronesia. Stocks Austronesia is divided into Substocks West Austronesia and East Austronesia. The Substocks West Austronesia populations occupied the western part of Indonesia from Sumatera to Kalimantan, Bali and West Nusa Tenggara Islands, where B3/*adw* is predominant [3], while Substocks East Austronesia populations occupied the eastern part of Indonesia from Sulawesi to Nusa Tenggara and South Moluccas, where B7/*ayw* and B8/*ayw* prevail[3]. Speculated that the ancestors of the inhabitants of the mengwi district, Bali came most likely from substock west austronesia, where HBV subtype *adw* was predominant[3]. However, one isolate we found had *adrq+* subtype, which is the most typical subtype for Melanesia and Polynesia.[3]

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REALLY NECESSARY FOR THE RECONSTRUCTION OF PENIS ENLARGEMENT?

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ABSTRACT

The penis is composed predominantly by the connective tissue. Starting from the urethra, erectile tissue corpora cavernosum, corpus spongiosum and the tunica albuginea, and smooth muscle fibers and muscle fibers with few stripes. Since reaching the peak of male puberty at the age of 16-17 years old, penis size can no longer change. Physically, the size of the penis does not affect the achievement of female orgasm and sexual satisfaction, as long as the penis under normal circumstances that based on normal developments. Female orgasm and sexual satisfaction is more determined by the quality of erection, ejaculation and the ability to control the emotional involvement of the spouse. Recently, Enhancement Phalloplasty and Girth Enhancement is a surgical procedure performed reconstruction by extending the penis and enlarge its diameter. The trick is cutting the suspensory ligament of the penis, which fix the base of the penis on the pubic bone. Part attached to the bone to be released so that it will be a fall and look longer. Meanwhile, to make the penis thicker involves removal of fat from the thick muscular body, buttocks or stomach and inject the fat into the penis. Cutting the suspensory ligament can cause the penis to be unstable. Injections of fat into the penis there is no proven benefit. In fact there are potential risks of these techniques, such as infections, loss of skin sensation, excessive bleeding to loss of function of the penis. There is no independent study monitored the objective of safety or success of these methods, so the mentioned operation is nothing more than an experimental surgery (trial operation) alone and most patients who undergo this operation are not satisfied with the results.

Keywords: penis enlargement, enhancement phalloplasty, girth enhancement, sexual satisfaction

INTRODUCTION

21 years old, comes with irregular swollen penis after the "orang-aring" oil injected by his friend. This case is almost similar to some victims of previous liquid silicone injections. Then, a man 42-year-old arrived with his wife aged 24 years complained his penis pain, swelling and numbness. Apparently at the base of his penis inserted a black rubber band tied tightly on the base of the penis. His wife did not even notice and just learned that day. Of course some cases this is only a fraction of the effort to enlarge the penis.

Besides functioning as a means of reproduction, the penis was also instrumental in running the recreation function in sexual relationships. Always appeared many endless discussions about the penis, including those regarding the ideal size to be able to bring sexual pleasure for both men and their partners. A popular myth about the penis size and is still perceived by many men is "bigger, better" (Hanifah, 2007). From generation to generation in many ways illogical and unscientific, have been made to extend the size of the penis, which can apparently lead to adverse effects, eventually even compromising the function of the penis itself. Of course this is a myth that needs to be straightened. By histology, penis composed predominantly by connective tissue (starting from the urethra, erectile tissue and corpora cavernosa, corpora cavernosum and tunica albuginea) and the smooth muscle fibers and only a few stripes of muscle fibers. Because more connective tissue and few striped muscle, since puberty is reached peak at 16-17 years of age, penile size was no longer experiencing the change. Whereas before the age of penis size could grow because of the influence of hormones, especially testosterone.

Categorized as normal penis size is how long and how much in diameter, not much is officially documented. Even if there are to do the study, the results still vary widely. One of them, mentioning that the size of the erect penis length is not the average

ranged from 7.6 to 10.2 cm. Meanwhile, when the average erect penis length is 12.7-17.8 cm (Francoeur, 1991). For the Indonesian data has not even officially exist. Likely to be different from the average standard of research mentioned by Francoeur. In a post, an andrology expert doctors had mentioned that the average penis size of Indonesian men, likely range is 7 inches when not erect and doubled when erect (N Setiawan, 2008). Fear of men that the size of his penis looks too small to satisfy her partner during intercourse often becomes an excuse plural. However, it was a number of studies show that most men who think their penis is too small indeed to have a normal-sized penis (JP Mulhall, 2001). Another reason that often arises is to increase your confidence.

Physically, penis size does not affect the achievement of female orgasm and sexual satisfaction, as long as the penis under normal circumstances should be based on developments. Orgasm and female sexual satisfaction is more determined by the quality of erection, ejaculation and the ability to control the emotional involvement of the spouse (Pangkahila W, 2008).

Misleading Practices On Penis Enlargement

There have been many efforts made wrong man to do this for penis enlargement. Unwittingly, this has become the target circles are not responsible for the commercial benefit that is not logical. This has been going down through generations and hundreds of years, even going around the world. Names such as "Mak Erot" is also very famous as an icon in the Indonesian penis enlargement. Though it was clear in many cases, which occur after the procedure is much penis enlarge penis deformity who experience both physical and sexual function (Pangkahila W, 2008).

Assistive devices are commercially targeted for penis enlargement can be harmful to male sexual health that can cause permanent damage to the penis. If you try again what can be observed due to the efforts to penis enlargement are as follows:

- Liquid silicone injection is of course very dangerous because it can damage the penis permanently.Some studies also mention the allegation of liquid silicon with the incidence of cancer.
- Hands massage (manual massage), which traditionally is often combined with the provision of certain foods (eg rice lemang) and the penis is inserted into a particular device (eg bamboo) can still cause adverse effects such as abrasion, swelling to pain.
- Pumps (vacuum), popular as a means of therapy had sexual dysfunction, but when used in a longer time than is recommended instead can damage elastic tissue in the penis. Indeed using the pump only creates the illusion that the penis looks to be bigger, but rarely succeed permanently.
- Pill, ointment, paste drug (patch) and other materials (such as tea marinade). None of these products are proven to work and some may be harmful if the dose is arbitrary, for example, which contains the hormone.
- Stretch with the load (traction) method is very risky because it can cause permanent damage to the penis.There is no scientific evidence that this technique can increase penis size.
- Operation or surgical penis (phalloplasty) can still be medically recommended for non-cosmetic purposes. Operations can be done to reconstruct a penis who experience severe injury, for example due to an accident, or for example a penis cut (as in the popular John Bobbitt case). While for the interests of penis enlargement with penis cut floor muscles is not recommended. It can be seen in the penis become longer but the results are not satisfactory and may even be bad because when the muscles are not strong base of the penis, penile erection was not going to be fine.

Enhancement Phalloplasty and Girth Enhancement

Enhancement Phalloplasty and Girth Enhancement is an operation to enlarge the penis which can be done today. This procedure is divided into two, namely, extend and enlarge the diameter. To increase the penis length is principally made cuts on the suspensory ligament of the penis, which is a base of the penis to fixate tissue at the pubic bone. With these procedures inherent in the bone to be released so it will be hanging and longer.

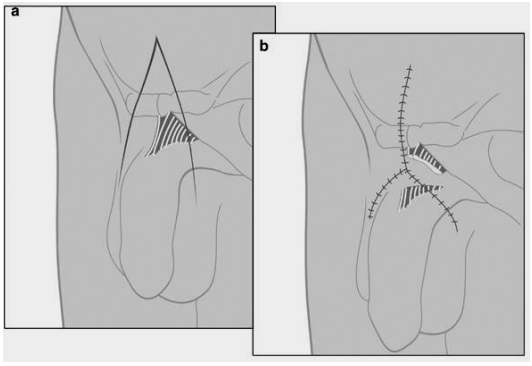

Figure 1: Cutting the suspensory ligament of penis

Meanwhile, to enlarge the diameter of a few ways that can be achieved one of them is by injecting fat taken from the patient's own body, is usually part of the inner thigh. There are using dermal fat graft is also taken from the patient's own body part but in a slightly different technique. However, this method is not always satisfactory because not all fat cells can still survive, approximately 33% to 50% fat cells that are injected are not able to survive in a few months after the procedure. Later also used AlloDerm, which is the matrix of human skin from donors in the form of slabs are said to have a higher survival rate compared with previous methods, with a more expensive price of course.

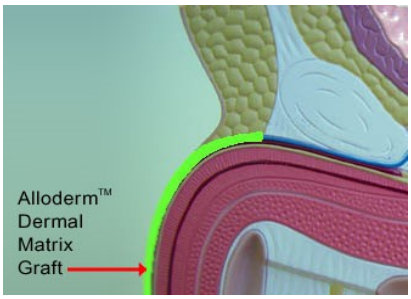

Figure 2: Addition of AlloDerm dermal matrix

DISCUSSION

From the description above we can see that there are still ways you can do to enlarge penis size is considered safer than the traditional way. Although, every action still has a respective risk. Cost of an expensive operating procedures, (ranging from tens to hundreds of millions of rupiah) with results that do not necessarily satisfying, and certainly not comparable proportion. This can be caused by anatomical penile shape will change to something like "fall" or hanging because the networks that sustain the position of the penis cut off. Erect position on a particular case can be changed, can not erect and rather leads to the bottom due to the loss of this support network, even in some cases

erect penis size actually increased short because that is not healing well, forming scar tissue and this procedure also raises the operation scar disturbing for some people. While the addition of the diameter of the penis thing that often happens is that the size of the penile shaft and glans (head) penis is not proportional, because the addition of trunk diameter at all. When using fat is sometimes uneven distribution so that it looked from the outside that the surface of the penis is a bit bumpy. Injections of hyaluronic acid on the glans penis several times tried to get a balanced proportion, but the results are not satisfactory and hyaluronic acid is absorbed rapidly so that only temporary. What is important to note, that there has been no scientific publications are the official objective of this operation method, either on the level of safety, complications, degree of success, client satisfaction, long-term effects, and so forth. The Society for the Study of Impotence states there is no independent study monitored the objective of safety or success of this method, so the mentioned operation is nothing more than a experimental surgery (trial operation) only. Also noted that most patients who undergo this operation are not satisfied with the results.

Many communities that are trapped in the myth about a bigger penis size can be more satisfying couple. Whereas the truth is far more powerful is the quality of erections and ability to sustain an erection. Penis size, it can be said so far is nothing more than physical appearance alone and do not represent the function and quality of sexual relations, for its size is still within the normal range. This is one example of dozens or even hundreds of misleading myths about sex and even widely circulated among the community health practitioners. This proves that the knowledge about sexuality in society remains low. What precisely is very important to do now is educate the whole society in order to have adequate knowledge, including about sexuality. On the basis of good knowledge of this would form the basis of perception of healthy and unhealthy sexual behaviors. Finally, it is expected also to the media in order to consciously sorting out the correct information can be selectively include ad mengeksklusi kontraedukatif and cultivating a myth, given the mass media function as agents for the intellectual community. So do not follow deceiving the public. Because up to now all the ads about penis enlargement is a public fraud.

During intercourse, penis size is more than average can cause a sense of discomfort to pain. In many cases, penis size is a matter of personal preference for men and their partners. A more mainstream is the sexual relationship and sexual communication is good, so not only the physical size alone. Particularly important to realize that the sensitive vaginal stimulation (G-spot) is located in the outer third of the vagina. So, in fact quite irrelevant to extend or enlarge the size of the penis to the satisfaction of the couple. Indeed many other things can be done to enhance confidence and understanding with good sexual partner. The following things can be done:

- Communicate better with a sexual partner. Including trying to open discussions about all that is on ourselves and on the couple.
- Improve personal appearance. Regular exercise can certainly make a big difference. Also healthy habits, balanced eating, stop bad habits (smoking, alcohol), maintaining ideal body weight remained. Better physical fitness not only makes it look more attractive, but also can restore a better sexual performance.
- Trim pubic hair. Pubic hair or pubic hair is not neatly around the base of the penis can make the penis does not look attractive. Bahkann pubic hair shaving can increase the sensitivity around the base of the penis.
- Talk with a doctor who understands sexual health. Many men will eventually feel comfortable with the belief that they are "normal" on the recommendation of an expert.

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THE POTENCY OF L-AMINO ACIDS AND DIPEPTIDES AS POTENTIATOR OF $GABA_B$ RECEPTORS IN RAT NEOCORTICAL SLICES

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ABSTRACT

The activity of seven L-amino acids and two dipeptides as potentiator of $GABA_B$ receptors have been evaluated, using grease-trap recording of baclofen modulation in rat neocortical slices. These selected L-amino acids and dipeptides were reversible, stereospecific, potentiator of $GABA_B$ receptors mediated hyperpolarizing to baclofen (3-100 μM), and these responses were reduced by (+)-(s)-5,5-dimethylmorpholinyl-2-acetic-acid (Sch50911) (30μM). The most potent were acidic amino acid L-glutamine (EC_{50} 0.2 μM), and L-asparagine (EC_{50} 1.0 μM), followed by basic amino acid L-ornithine (EC_{50} 10.0 μM) while the neutral L-amino acids with branch or aromatic side chain such as L-phenylalanine, L-leucine, and L-isoleucine were less potent which all had EC_{50} 50.0 μM. Replacing phenyl with a 1-naphthyl (L-Napa) markedly increase the activity which gave EC_{50} of 10.0 μM. The dipeptides L-phenylalanine-phenylalanine (EC_{50} 10.0 μM), and L-phenylalanine-leucine (EC_{50} 10.0 μM) were more potent than their parent compounds but equipotent to L-Napa.
Keywords: L-amino acids, dipeptides, $GABA_B$ receptor potentiators

INTRODUCTION

Metabotropic γ-aminobutyric $acid_B$ ($GABA_B$) receptors belong to Family 3 of the G-protein-coupled receptors which share the characteristic of large extracellular amino terminal domain that contain a so-called "Venus flytrap" ligand-binding site (Bockaert and Pin, 1999). $GABA_B$ receptors control neuronal excitability and transmission in the nervous system and are of crucial importance in many physiological activities such as autonomic function, memory, and cognition, as well as motor and sensory control including pain regulation and epilepsy (Bowery *et al* 2002). Indeed, $GABA_{(B1)}$ subunit knockout mice lacking these receptors exhibit defects in all processes, resulting in premature death (Prosser *et al*, 2001). Other members of Family that show a degree of similarity to $GABA_B$ receptors include metabotropic glutamate,Ca^{2+} sensing, some pheromone, and taste receptors (Couve *et al*, 2000).

Biological sensing of L-amino acids plays key roles in coupling changes in whole body protein and amino acids metabolism to appropriate physiological responses. Conigrave (Conigrave *et al*, 2000) reported that some L-amino acids, especially aromatic amino acids including L-phenylalanine, and tryptophan, positively modulated calcium sensing receptors. They also found that very high milimolar concentration of the active aromatic amino acids are required for the potentiation to occur, and some (e.g. branched amino acids) were virtually inactive. The mode of action of these acids at the receptor is unusual, in that they bind at the VFT terminal domain where their binding site overlaps that for calcium itself. It is clear that the amino acids act at a the different site from that used by the phenylalkylamines (Kerr and Ong, 2005).

So far, there have been no report of amino acids or dipeptides as potential modulator of $GABA_B$ receptors. Preliminary studied carried out by Kerr and Ong showed that a number of neutral L-amino acids but not D-amino acids were effectively modulator at $GABA_B$ receptors. Of these L-phenylalanine, and L-cystine were the most active, whilst tryptophan, lysine and histidine were inactive (Kerr and Ong, 2003). The aim of this study is to explore in a preliminary way, the $GABA_B$ receptor potentiating action of other L-amino acids including acidic, basic, and neutral amino acids as well as small peptides.

MATERIALS AND METHODS

Materials

Racemic (±)-baclofen and (+)-(S)-5,5-dimethylmorpholinyl-2-acetic acid (Sch50911) purchased from Sigma whilst dipeptides were synthesized in our laboratory. CGP7930 was a gift from Novartis.

Methods

Preparation of rat neocortical wedges

The rat neocortical slices were prepared from halothane anaesthetized outbred male adult Sprague-Dawley rats (250-350g) which were decapitated using a previously established procedure (Ong *et al*, 2001). The brains were rapidly dissected out and immersed for 30 minutes in ice cold oxygenated Krebs solution gassed with 95 % O_2, 5 % CO_2 (pH 7.4) of the following composition (in nM): NaCl 118, KCl 2.1, KH_2PO_4 1.2, $CaCl_2$ 2.5, $NaHCO_3$ 25, glucose 11, $MgSO_4$ 1.3. Cerebral cortical slices (400 µm thick) were prepared by cutting coronal section using a vibraslice microton (Campden Instrumen UK), and radial wedges were cut from each side of the dorsal mid-line to yield slices of cingulate cortex and corpus callosum 2-3 mm wide. The slices were subsequently equilibrated in gassed Krebs solution at room temperature (20-30ºC) for 1 hour prior to experimentation.

Grease-gap experiment

Following the equilibrium period, wedge-shaped slices from neocortex were placed in a two-compartment prespex perfusion chamber, where each wedge was placed across a septum, separating pools containing the cortex and white matter by a grease seal, using superfusion method based on a grease-gap system described previously (Horne *et al*, 1986, Smart, 2001). The Grey matter was then continuously superfused with gassed Krebs medium at 25ºC, delivered by a peristaltic pump at 1 mL/min, while the white matter was maintained in a stationary pool of the same medium. Differential recording (mV) between the cortex and white matter, on either side of the septum, were measured with Ag/AgCl electrodes, and the DC potential was monitored on a chart recorder using a high input impedance DC amplifier. Here Mg^{2+}containing Krebs medium was used throughout the experiment to eliminate the spontaneous discharged since the latter tended to complicate the hyperpolarizing responses.

After 60 min equilibrated the $GABA_B$ receptor agonist were added to the superfusing medium, and applied to the cortical side of the tissue for 3 min to achieve steady-state concentration within the recording chamber. Each preparation was allowed to a minimum of 30 min recovery between drug applications. When examining the modulatory effects of a potentiator, the latter was superfused for 5 min and then added together with the agonist for a further 3 min before tissue wash-out. In some experiment where $GABA_B$ receptor antagonist Sch50911 was used, it was first superfused alone for 3 min, and then added together with the test compounds and agonist. In each experiment, control responses to the agonist were re-established, after each drug application. Results were quantified, and value expressed as percentage of the maximum hyperpolarization obtained with the agonist alone from the chart recording. Concentration-response curves were constructed, in the absence and presence of the test agents. To test the positive modulatory activity of the compounds, they were applied with ascending concentration and a fix concentration of the agonist. Concentration-response profile.

For all potentiators were constructed by measuring the peak amplitude during application of the compound and a fixed concentration of agonist (EC_{50} of the agonist), calculating the percent increase relative to the agonist (alone) response and plotting the data as a function potentiator concentration.

In other experiment, the concentration-response curves of the agonist were constructed, in the absence and presence of differing concentration of potentiator. The EC_{50} values were then calculated from the concentration-response curve, where the EC_{50} is the concentration giving a response equal to 50% of the maximally effect concentration. All numerical data on the concentration-response curve as means ± S.E.M. Each experiment was repeated on slices obtained from 6-10 different animals. Comparison of the data was made using student t-test with $p<0.05$ being significant.

RESULTS AND DISCUSSIONS

As seen in from Figure 1, in a typical examples of $GABA_B$ receptor potentiation by a branched chain amino acid (L-leucine), the agonist baclofen at 10µM (which is the EC_{50}), applied alone for 3 min induced a control hyperpolarization. In this preparation, 30 min after wash-out, subsequent application of the L-leucine (100 µM) alone for 5 min had no significant effect on the membrane potential, but when co-superfused with baclofen (10µM) for 3 min, there was a marked potentiation of the baclofen-induced hyperpolarizing response, giving an initial spike followed by a more prolonged recovery. The control response then required some 45-60 min wash-out before it could be re-established; indeed, we emphasize that potentiation of responses to re-applied baclofen is still seen during the ensuing 30-40 min after wash-out of the potentiator. Also, The $GABA_B$ receptor antagonist Sch50911 (30µM) abolished the potentiated response, indicating that GABAB receptors were involved. As a reference compound, the known modulator CGP7930 (10 µM) was active in potentiating $GABA_B$ receptors with an EC_{50} 10 µM.

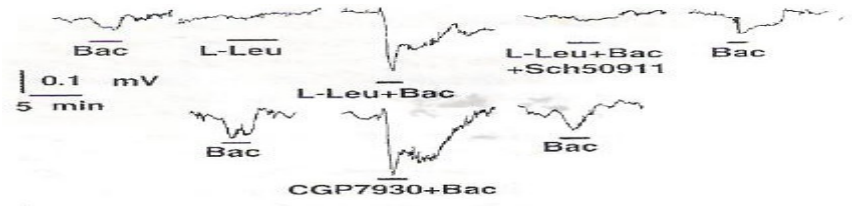

Fig. 1. Discontinuous records of the hyperpolarizing effects of baclofen (Bac; 10 µM) in a rat neocortical slice and the potentiating effect of the branched chain amino acid L-Leu (100 µM) on the baclofen-induced response, which was abolished by Sch50911 (30 µM). L-Leu (100 µM) when applied on its own had no significant effect on the membrane potential. The control response to baclofen was subsequently re-established upon tissue wash-out within 60 min. The interval between drug applications was at least 30 min. For comparison, the more potent modulator CGP7930 (10 µM) was shown to significantly and reversibly potentiate the response to baclofen (10 µM) in a separate slice.

The activity of selected amino acids and dipeptides in potentiating the $GABA_B$ receptor ($EC5_0$) was summarized in Table 1.

Table 1. The EC_{50} values of the selected L-amino acids and dipeptides

No	Structure	EC_{50}
1	L-glutamine	0.2 µM
2	L-asparagine	1.0 µM
3	L-ornithine	10.0 µM
4	L-leucine	50.0 µM
5	L-isoleucine	50.0 µM
6	L-phenylalanine	50.0 µM
7	L-naphthylalanine	10.0 µM
8	L-phenylalanine-phenylalanine	10.0 µM
9	L-phenylalanine-leucine	10.0 µM

As summarized in Table 1, the most active potentiator of $GABA_B$ receptor-mediated action in rat neocortical slices were the acidic amino acid L-glutamine and L-asparagine with EC_{50} of 0.2μM, and 1.0μM respectively, followed by the basic amino acids L-ornithine which had EC_{50} of 10.0 μM. Whilst, the neutral L-amino acids with branched or aromatic side chain, i.e. L-leucine, L-isoleucine, and L-phenylalanine were less potent with all had EC_{50} of 50.0 μM. Replacing phenyl with a 1-naphthyl (L-Napa) markedly increase the activity which gave EC_{50} of 10.0 μM. The dipetides L-phenylalanine-phenylalanine, and L-phenylalanine-leucine both gave EC_{50} of 10.0μM, therefore they were more potent than their parent compounds but equipotent to L-Napa. The potentiating actions of those L-amino acids and dipeptides were selective to $GABA_B$ receptor, having no effect on $GABA_A$. In the presence of the modulators, the hyperpolarization generated by a given baclofen concentration was not only increase in amplitude but the duration also become prolonged. This suggested that the modulator in some way prevented desensitization, and that dissociation of the agonist was delayed by the modulator, or that activation of the G-protein was prolonged.

CONCLUSION

Selected L-amino acids and dipeptides are effective potentiator of $GABA_B$ receptor mediated-action in rat neocortical slices

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RESISTANCE OF *EXTENDED-SPECTRUM BETA LACTAMASES* (ESBLs) PRODUCTION AMONG *Escherichia coli* AND *Klebsiella pneumonia* TO THE THIRD-GENERATION CEPHALOSPORIN IN CLINICAL LABORATORY SANGLAH HOSPITAL DENPASAR

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ABSTRACT

Identifying organisms that produce *extended-spectrum ß-lactamases* (ESBLs) is a major challenge for the clinical microbiology laboratory. Due to the variable affinity of these enzymes for different substrates and inoculum effect, some ESBLs isolates may appear susceptible to a third generation cephalosporin in vitro. Because of this ESBLs enzyme mediated plasmid, the gene that encodes this enzyme easily transferred between different bacteria, this resistance mechanism is much going on *K. pneumonia* and *E.coli*. Several studies have reported that ESBLs enzyme-producing bacteria, has been resistant to third generation *Cephalosporin*. This study was designed to describe the prevalence of ESBLs producing *E. coli* and *K. Pneumonia* isolated from different clinical specimens and the drug resistance pattern of ESBLs, performed the data collection and analysis of antibiotic resistance in the Clinical Laboratory Sanglah Hospital during January-December 2007. The study showed, there were 573 isolates of *E. coli*, and 103 isolates. *Escherichia coli* isolates resistant to *Ceftazidime* (CAZ) was 30.16%, *Cefotaxime* (CTX) was 36.35%, and both were 24.54%; *Cefoperazone/Sulbactam* (CSL) was 8,57%; and all three (*Ceftazidime*, *Cefotaxime*, *Cefoperazone/Sulbactam*) were 3,57%. *Klebsiella pneumonia* isolates resistant to *Ceftazidime* was 48.96%, *Cefotaxime* was 49.48% and 39.58% against both; *Cefoperazone/Sulbactam* (CSL) was 16%; and all three were 8%.

Keywords: ESBL, *Escherichia coli, Klebsiella pneumonia,* Ceftazidime, Cefotaxim, Cefoperazone, Sulbactam

INTRODUCTION

Emergence of resistance to β-lactam antibiotics began even before the first β-lactam, penicillin, was developed. The first β-lactamase was identified in *Escherichia coli* prior to the release of penicillin for use in medical practice.[1] Investigations into the mechanism responsible for this resistance have revealed the presence of extended-spectrum *ß*-lactamase (ESBLs) enzymes derived from the widespread TEM-1/2 and SHV-1 family.[2,3] The TEM-1 enzyme was originally found in a single strain of *E. Coli* isolated from a blood culture from a patient named Temoniera in Greece, hence the designation TEM. Another common plasmid mediated β-lactamase found in *Klebsiella pneumoniae* and *E.coli* is SHV-1 (for sulphydryl variable). The SHV-1 β-lactamase is chromosomally encoded in the majority of isolates of *K. pneumoniae* but is usually plasmid mediated in *E. coli*.[3,4,5] To date, there are over 110 derivatives of TEM *ß*-lactamases and more than 63 derivatives of SHV *ß*-lactamases.[2] Due to alterations at the active site caused by specific point mutations, these extended-spectrum beta-lactamases (ESBLs) are also able to hydrolyze oxyimino-_-cephalosporins (e.g., cefotaxime, cefpodoxime, and ceftazidime) and the monobactam aztreonam.[6]

In epidemiological, ESBL found in many countries around the world with different prevalence depending of antibiotic usage patterns. In 1988 in Asia, ESBLs bacteria was found at 5-8% in Korea, Jepang, Malaysia, Singapura, but in Thailand, Taiwan, Philipina and Indonesia amounted to 12-24%. The SENTRY study at 1998-1999 showed the prevalence of *E.coli* ESBL in Hong Kong 13%, *K. pneumonia* 8%; in Philippines, *E.coli* 13%, *K. Pneumonia* 31%, and *Enterobacter* 1.8%; in Singapore,

K. Pneumonia 18%.[7] During a 6-month period in 2004 at a teaching hospital in southern Thailand, ESBLs production was detected in *K. pneumoniae*, *E. coli*, and *E. cloacae*, at rates of 16/36 (44.4%), 3/59 (5.1%), and 2/13 (15.4%), respectively.[8] The study at 2004 in showed that the prevalence of ESBLs *E. coli* in Surabaya was 10.8%.[7] Study at Dr Kariadi Hospital, Semarang showed that bacterial culture positive rate was 34.76% with predominance bacteria was gram negative bacteria (59.6%), in which ESBLs detected in 50.6%. The most predominance bacteria were *P. aeruginosa*, *E. aerogenes* and *E. coli*. Antibiotic sensitivity patterns mostly sensitive to meropenem, aminoglycocide and quinolone.[8]

Due to the variable affinity of these enzymes for different substrates and inoculum effect, some ESBLs isolates may appear susceptible to a third generation cephalosporin in vitro. Cefpodoxime and ceftazidime have been proposed as indicators of ESBLs production as compared to cefotaxime and ceftriaxone.[9] In Bali, including in Sanglah hospital, there aren't study to describe the prevalence of ESBLs. *E. coli* being commonest organism after *Klebsiella* to exhibit ESBLs, complicates the problem, unless a definitive policy of detecting ESBLs producing isolates and in time reporting to clinicians is communicated, so that appropriate treatment is instituted and the resulting morbidity and mortality are substantially reduced. Therefore, this study are designed to describe the prevalence of ESBLs producing *E. coli* and *K. Pneumonia* isolated from different clinical specimen and the drug resistance pattern of ESBLs.

MATERIALS AND METHODS

Data retrieved retrospectively from *E. Coli* and *K. pneumonia* in-patient isolates that tested in the devision of Clinical Microbiology, Clinical Laboratory Departement, Sanglah hospital during January to December 2007, were screened for potential ESBL activity. These strains were isolated from different clinical specimens. All specimens were processed and identified using Gram strain, biochemical tests and mini API ® test.

Based on routine antibiotic disk sensitivity tests, isolates that exhibited resistance to any one of the third generation cephalosporin were shortlisted to detect ESBLs but these result didn't confirm to confirmatory test of ESBLs producers. The inclusion criteria for ESBLs bacteria are *E. Coli* and *K. pneumonia* resistant to one or more of the paper disc (disk) antibiotic ceftazidime (CAZ) 30µg, cefotaxim (CTX) 30µg, and cefoperazone / Sulbactam (CSL) 75µg/30 µg.

RESULTS AND DISCUSSION

During January to December 2007, there were 573 isolates of *E. coli*, and 103 isolates of *K. Pneumonia* which division based on clinical specimens can be seen in the table below.

Table 1. *E.coli* or *K.pneumonia* isolates based on clinical specimens

Clinical specimens	Urine	Sputum	Pus	Rectal swab	Blood	Feces	Tissue	Throat swab	Others	
E.coli	436	50	30	15	14	12	4	1	11	573
K.pneumonia	5	62	-	-	25	-	1	4	6	103

Escherichia coli isolates resistant to *Ceftazidime* (CAZ) was 30.16%, *Cefotaxim*e (CTX) was 36.35%, and both were 24.54%; *Cefoperazone/Sulbactam* (CSL) was 8,57%; and all three (*Ceftazidime, Cefotaxim, Cefoperazone/ Sulbactam*) were 3,57%.

Table 2. *Escherichia coli* isolates resistance to the third-generation *Cephalosporin* respectively.

E.coli	CAZ	CTX	CSL
R	168 = 30,16%	197 = 36,35%	24 = 8,57%
I	24 = 4,31%	54 = 9,96%	-
S	368 = 66,07%	291 = 53,69%	-
Sum	557 = 100%	542 = 100%	280 = 100%

R= Resistance, I = Intermediet, S = Susceptible

Klebsiella pneumonia isolates resistant to *Ceftazidime* was 48.96%, *Cefotaxime* was 49.48% and 39.58% against both; *Cefoperazone/Sulbactam* (CSL) was 16%; and all three (*Ceftazidime, Cefotaxim, Cefoperazone/Sulbactam*) were 8%.

Table 3. *Klebsiella pneumonia* isolates resistance to the third-generation *Cephalosporin*

K.pneumonia	CAZ	CTX	CSL
R	47 = 48,96%	48 = 49,48%	8 = 16%
I	3 = 3,13%	10 = 10,31%%	-
S	46 = 47,91%	39 = 40,21%	-
Sum	96 = 100%	97 = 100%	50 = 100%

R= Resistance, I = Intermediet, S = Susceptible

Identification of ESBLs bacteria is a big challenge for clinical microbiology laboratories. ESBLs affinity toward substrate and inoculum effects vary widely so that the sensitive isolates in vitro, probably will fail in the clinic. The use of sensitivity tests third-generation cephalosporin alone is not enough to determine ESBLs, only used as a screening and still need to be confirmed. According to NCCLS (National Committee on Clinical Laboratory Standards) for the detection of ESBLs is to compare the addition of clavulanic acid on the antibiotic, suspected of being germ ESBLs, as a phenotype confirmation test of ESBLs production, using 6 discs are put on the plates: ceftazidime (30 µg), ceftazidime/clavulanic acid (30/10 µg), cefotaxime (30 µg), cefotaxime/clavulanic acid (30/10), cefpodoxime (10 µg), and cefpodoxime/clavulanic acid (10/1 µg). An increase in zone size >5 mm in the disc containing clavulanic acid versus its zone size when tested alone was accepted as confirmation of ESBL production.[8,10] The use of more than one of the five antimicrobial agents suggested for screening will improve the sensitivity of detection. Cefpodoxime and ceftazidime show the highest sensitivity for ESBLs detection.[14]

In Clinical Microbiology, Clinical Pathology Laboratory, Sanglah Hospital, ESBLs screening was done by disk diffusion susceptibility tests using cefotaxim, ceftazidime and cefoperazone/ Sulbactam. Furthermore, these results must be confirmed by phenotypic (phenotypic confirmation) using a paper disk, cefotaxime and ceftazidime 30µg/ 30µg with/ witout the addition of clavulanic acid. There are advantages and disadvantages of this phenotypic confirmation tests, i.e. when the test result is not ESBLs and the patient was given medication carbapemen, hence causing it was unnecessary. Conversely, if clinicians wait for the results to provide a phenotypic confirmation carbapemen treatment, then a delay of treatment that may cause harm to patients. Therefore required a rapid confirmation tests, the results are reliable and can be done as a routine test. Kuntaman in Surabaya prove that double-diffusion technique has the accuracy of the VITEK 2 ESBLs by 90%.[8] This automated short-incubation system appears to be a rapid and reliable tool for routine dentification of ESBLs-producing

isolates of *Enterobacteriaceae*. Although further studies are needed to evaluate its overall performance, *Enterobacteriaceae* isolates indicates that the VITEK 2 ESBL test system is a reliable time-saving tool for routine identification of ESBLs-producing strains.[12] The MicroScan clavulanate synergy test proved to be a valuable tool for ESBLs confirmation. This study demonstrated that the MicroScan MIC difference test, using both ceftazidime and cefotaxime, is a sensitive tool in its ability to confirm the ESBLs status of a given Gram-negative isolate.[11]

Antibiotic options in the treatment of ESBLs-producing organisms are extremely limited, this problem requires limitations on extended-spectrum cephalosporin usage. While carbapenems are clearly the treatment of choice for infections caused by these pathogens, empirical use of piperacillin/tazobactam has been associated with reduction in the prevalence of cephalosporin resistance.[15] Carbapenem is an effective antibiotic for bacteria resistant ESBLs because β-lactam hydrolysis, but the use of carbapenem freely have become resistant because of the risk can be hydrolyzed by the enzyme Metallo β-lactamase. Meropenem is the most active and its MIC smaller than imipenem, although the effect is not clinically different. Those which are still in the evaluation is Ertapenem that has long halflife, so can be given once a day, and Faropenem is an oral dosage form. Other antibiotics which reportedly also effective for ESBLs germs are aminoglycoside class.[1,8] This investigation has demonstrated how important it is for clinical microbiology laboratories to have the ability to detect and report on ESBLs production in clinical isolates of gram-negative bacteria. Routine disk diffusion should be carefully monitored for ESBLs production, particulary with *K. pneumoniae* and *E. coli*.[8]

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FORMULATION OF BURN INJURY GEL FROM AMBON BANANA STEM FRACTION (MUSA X PARADISIACA LINN) AND ALOE VERA EXTRACT

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ABSTRACT

Burn injury is a trauma caused by heat, electric current, chemical substances that come into contact with skin, mucosa and inner tissue. Burn injury can be treated with sintetic and natural substance . Plants used traditionally in a burn injury treatment are Aloe vera (Aloe barbadensis Mill.) and Ambon banana's (Musa x paradisiaca Linn.). This research aimed to made a formulation for burn injury dosage from Aloe vera extract and Ambon banana's stem fraction. The experimental method of this research has a following steps extraction and fractionation Ambon banana's stem, effectiveness test of fraction group, formulation of burn injury gel, effectiveness test of gel contained the combination of Ambon banana's stem fraction and Aloe vera extract, gel evaluation and safety test. The research showed quality of gel is meet a standard, effectiveness of dosage F3 is 64,6% differ from negative control which have percentage 44,28% and the gel made is not skin irritant.

Keywords: Burn injury, Ambon banana's, Gel, Aloe vera

INTRODUCTION

Burn injury is the third leading cause of death due to accidents in all age groups (Hidayat, 2009). Burn injury 65.7% in children caused by hot water or steam heat (Pharmacist, 2007).

Nowadays the use of herbal preparations more wide with no exception the use of herbal preparations to cope with burn injury, one of it is aloe vera. The using of topical aloe vera gel extract showed significant results in wound healing in animal experiments. Wounds on experimental animals better within 14 days. This activity is based on the content of mannose in aloe vera that play a role in the increased activity of the macrophages and accelerates wound healing (Subramanian, 2006).

In addition to aloe vera, a plant that has traditionally been used for healing of burn injury is a banana. The sap that is produced by a banana tree can cure the wounds on the skin because the sap of the banana tree can increase blood flow to injured areas and also stimulate the fibroblasts in response to wound healing. Banana tree stems extract contains saponins (compounds that have the ability as a cleansing and antiseptic), flavonoids (anti-inflammatory) and tannin (anti septic and adstringen) (Priosoeryanto, 2006; Sirait, 2007).

Based on the research Agarwal flavonoids contained in bananas are known to reduce lipid peroxidation. Besides flavonoids showed adstringen activity and antimicrobial role in accelerating wound healing and increase the formation of new tissue in the wound area (Agarwal, 2009).

Selection is based on the effect of gel which cools, because many contain water, so is expected to help accelerate the healing process (Wardani, 2009).

MATERIALS AND METHODS

Material

Plant materials: The stem of banana tree that has been chopped and dried obtained from area of Cicalengka West Java, and determined in the Department of Biology, Science Faculty, The University of Padjadjaran, while aloe vera extract that is used aloe vera extract that has been marketed (Exsymol ®, Nardev Chemie). **Animal experiments:** the

white race rabbits of New Zealand with average weight 1.5 kg obtained from the Faculty of Animal Husbandry, The University of Padjadjaran. **Chemical Substance**: n-hexane, ethyl acetate and ethanol 70% (Bratachem) redestilation result, reagent ethanol sulfate, silica gel TLC plates 60 F254 (Merck) Art No. 05 554, silica gel 60 pro column, 70-230 mesh ASTM Art No. 07734 (Merck), methanol (Bratachem), chloroform (Bratachem), Carbophol 980 (MERCK), propylenglycol (Brataco), glycerin (Brataco), triethanolamine (Brataco), Acnibio ® (Nardev Chemie), aquabidest (IKA Pharmindo), ethanol 95% (Brataco), aquadest.

Method

Extracts Preparation: Dried Ambon banana's stem, which has been weighed and then extracted by soxhletasi for ± 8 hours with 95% ethanol, then evaporated using a rotary evaporator to obtain viscous extract.

Fractionation Extract: Extract thick Ambon banana trees obtained, then the fractionation using Vacuum Liquid Chromatography (KCV) and eluted with gradient solvent use 13 comparisons solvent such as n-hexane and ethyl acetate (100:0, 90:0; 80 : 20; 70:30, 60:40, 50:50, 40:60, 30:70, 20:80, 10:90, 0:100) and ethyl acetate and ethanol 95% (50:50, 0:100). Then fractions are monitored by Thin Layer Chromatography (TLC) and by grouping fractions based on TLC and the spot pattern similarity and Rf score.

Fraction Group Effectiveness Test: The test is performed by making a formula for the fraction of each group that have been grouped based on the results of TLC, then tested its effectiveness to animal experiments.

Burn injury Gel Formulation Preparations: The gel formula used containing a combination of the active fraction of Ambon banana trees and aloe vera extract (Exsymol ®). Preparations made by developing Carbophol 980 in aqua bidesteril then add TEA little by little to form a gel, then enter glycerin and acnibio.

Homogeneous gel base that has been added to the fraction of banana trees that previously had been dissolved in propylenglycol and aloe vera extract, stirring until homogeneous.

Evaluation of Preparations Gel Burn Injury: Evaluation conducted on preparation cover organoleptic examination, pH and viscosity during the 56 days of storage.

Testing of Burn Injury Gel Effectiveness: Rabbits are classified into two groups, each group consists of two tails and to the respective groups were treated the following:

Group 1 was induced three burn injury at a different location. The first injury as a negative control, second wound lubricated F0, the third wound lubricated F1. Group 2 was induced three burn injury at a different location. The first wound lubricated F2, the second wound lubricated F3, the wound as a positive control given aloe vera gel that has been marketed.

Burn Injury Gel Qualitative Testing: Testing is done by Thin Layer Chromatography. Developers that used n-hexane: ethyl acetate (7:3) and spray reagent used was 10% sulfuric acid in ethanol, the observed spots are marked and counted its Rf.

Burn injury Gel Safety Testing: Testing safety were made to the preparations formula showed the best quality data during storage with the method of 4 8-H Irritancy Test. Irritation test conducted on 25 volunteers. Preparations lubricated to the particular location, then left for 24 hours if no irritation occurs, the observation was continued until 48-72 hours.

RESULTS AND DISCUSSION

Extraction: The extraction of Ambon banana tree stem (1.2 kg) in Soxhletasi with 95% ethanol obtained Ambon banana tree stem thick extracts 150 g, so the rendement 12.5%..

Extract fractionation: Results of the fractionation of 30 g of viscous extract of Ambon banana's stems obtained 13 fraction. TLC results showed that there were three groups of fractions. Fraction 2-fraction 5 (group 1) with rendement of 3.11%, the fraction of 6-

fraction 9 (group 2) with rendement of 3.31% and fraction 10-fraction 13 with rendement of 4%.

Fraction Group Effectiveness: The effectiveness of the test groups fraction result showed that the group fraction 1 had the highest effectiveness can be seen from wound diameter were observed for 14 days.

Effectiveness Test Preparations Gel Injury: Testing the effectiveness of the gel containing a combination fraction of Ambon banana trees and aloe vera extract showed significant effect on the healing of burn injury.

Statistical analysis result by using Random Sub-Sampling Design showed that the fraction of gel containing Ambon banana's stems and aloe vera extract had effectiveness to the healing of burn injury with $\alpha = 0.05$ F3 showed the percentage of healing wound amounted to 64.6% was different from the control negative that influence 44.28%.

Evaluation Preparations Gel: Evaluation results of gel preparation based on organoleptic, pH and viscosity during the 56 days of storage shows that the relatively stable gel can be seen from tables and figures as follows:

Table 1. Observation results of Organoleptic Gel Preparations Burn injury During 56 days storage

Formula	Consisteny	Colour	Smell
F0	K	Tbw	Tb
F1	K	Km	Bl
F2	Ak	Ku	Bl
F3	Ak	Km	Bl

Notes:

F0 : Gel without a fraction of Ambon banana trees stem and aloe vera extract (Exsymol ®)

F1 : Gel containing 0.5% fraction of Ambon banana trees stem and 4% aloe vera extract (Exsymol ®)

F2 : Gel fraction containing 0.75% Ambon banana trees stem and 4% aloe vera extract (Exsymol ®)

F3 : Gel containing 0.5% fraction of Ambon banana trees stem and 6% aloe vera extract (Exsymol ®)

K : viscous; Ak: less viscous; Tbw: Colourless; Km: young Yellow; Ku: Yellow

Tb : No smell; Bl: Smells weak

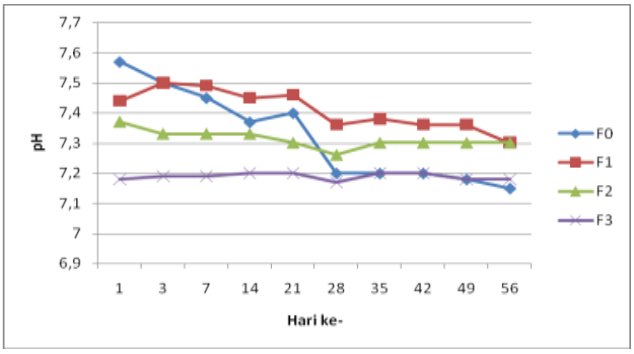

Figure 1. Averaged change of pH gel preparation during 56 days storage

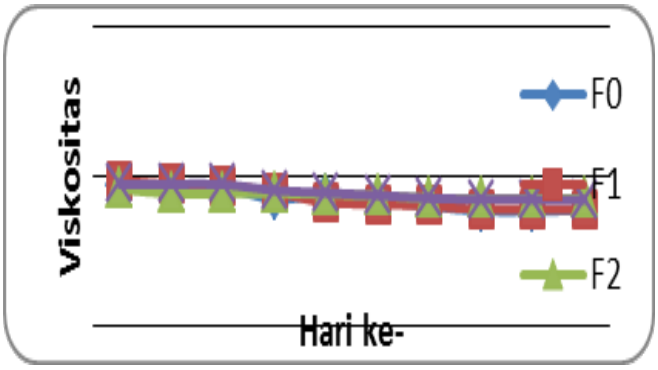

Figure 2. Averaged change of viscosity gel preparation during 56 days storage

From the statistical analysis, F3 showed the smallest standard deviations for changes in pH during 56 days of storage, this means F3 stocks relatively more stable than the other formulas because the standard deviation shows the magnitude of change that occurred during storage. While the viscosity of the preparations for F2 which has the smallest standard deviation, this means F2 preparation relatively more stable compared with other formulas. While organoleptic observations during 56 days of storage showed that the gel has made no change in color, consistency, and odor/smell.

Qualitative Test Preparations Gel Burn injury: Results of a qualitative test based gel burn injury TLC showed that the compound contained in the extract is still on the preparation during 56 days of storage time.

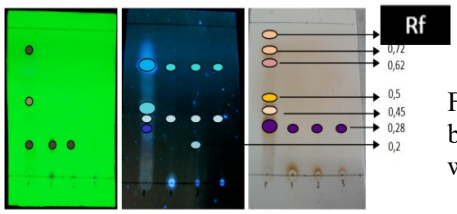

Figure 3. Results of TLC fraction Ambon banana trunks and burn injury gel preparation with n-hexane: ethyl acetate 8:2

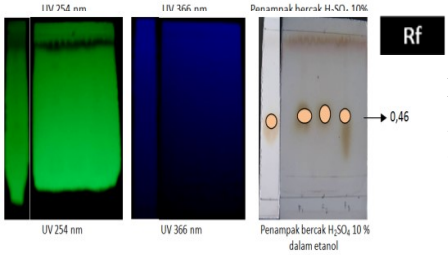

Figure 4. Results of TLC aloe vera extracts and gel burn injury with a developer of ethyl acetate: methanol: water 100:13,5:10

Burn Injury Gel Safety Test: Results of testing the safety of gel to 25 volunteers with method of 48-H Irritancy Test, showed that the gel is made safe because it does not cause irritation to the skin lubricated with gel preparation.

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CHROMOGENIC METHOD IN ENDOTOXIN TESTING FOR INTRAVENA INJECTION PREPARATION

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ABSTRACT

A chromogenic endotoxin test using Limulus Amoebocyte Lysate has been done. The objective was study the application of the chromogenic method of endotoxin determination of ascorbic acid injection dosage form. In the chromogenic method the colour was formed as a result as the reaction between endotoxin and LAL reagent; the colour intensity and the speed of colour formation rate is proportional to the concentration of endotoxin. In this test, Escherichia coli endotoxin standard with concentration of 50, 5, 0.5, 0.05, and 0.005 EU/mL were applied. From the test, it was found that endotoxin content 1.686 and 0.324 EU/mL for Product A and Product B respectively.

Keywords: Chromogenic, Endotoxin, Ascorbic Acid injection, *Limulus Amoebocyte Lysate*

INTRODUCTION

Injection is a sterile dosage in form of solution, emulsion, suspension or powder that must be dissolved or suspended before use, which is injected into the tissue by ripping into the skin or through the skin or mucous membranes (Depkes RI, 1979). As sterile preparation, injection must meet the following requirements: germ-free, free of solvents that are physiologically not neutral, isotonic, isohydri, free from floating materials and for large volume injection should be free of pyrogens (Voigt, 1995).

Pyrogen is a substance capable of causing fever and ever contaminate pharmaceutical preparations (Suwandi, 1988). Gram negative bacteria sre the most potent procedure of endotoxin (Jones, 2001). Usually, human are sensitive to endotoxin that often cause pyrogenic respond. The exist of endotoxin in blood stream can cause fever, inflamation, and often shock (Joiner, et al., 2002).

To guarantee that there is no pyrogen in an injection preparation, endotoxin testing should be done. Endotoxin test is an importan part of quality assurance or quality control of large volume injection dosage. The first endotoxin test conduted was rabbit test according to the USP. In this time, in vitro test had been developed which have higher sensitivity than the rabbit test, the test using Limulus Amoebocyte Lysate (LAL). LAL is a liquid extract of crab's (Mottar, et al., 2006). There are several types of endotoxin test using LAL, such as gel-clot method, chromogenic method and turbidimetry method (The Official Compendia of Standards, 2002).

Method that to be used in this research is the chromogenic method. This method measures the reaction time required for the formation of colour intensity after the release of colours from the peptide complex chromogenic appropriate by endotoxin lysate using a spectrophotometer set at wavelength 405 nm (Her Majesty's Stationery Office, 1980). The stronger the colour, the greater the measured absorbance value. Endotoxin concentration of endotoin in units of ng/mL or EU/ml (Akers, 1994).

Preparation that will be tested the level of endotoxin in this research is the small volumes injection containing Vitamin C.

MATERIALS AND METHODS

Instrument for this research are cuvet, sterile syringe, thermometer, test tube, refrigator, laminar airflow (LAF), Uv-vis Spectrophotometer (Specord, Analytical Zena), Peristaltic pump, watrbath (Memert), and all general labolatory glasses equipment.

Materials: A class vitamin C injection, B class Vit C injection, Pyrocrome LAL test, (chromogenic test), LAL reconstruction Buffer, endotoxin control standard, (e . Coli), LAL reagent water, 70% alcohol spiritus,

Method:

Endotoxin preparation and LAL Kit:

Endotoxin preparation. Preparation endotoxin standard solution in concentration of 50 Eu/ml ; 5 Eu/ml ; 0,5 Eu/ml ; 0,05 Eu/ml, and 0,05 eu/ml to make endotoxin standard curve.

LAL preparation. Pyrochrome LAL 60- test kit reagent used kin this research pyrocrome is soluted in 3,2 ml LAL reconstruction buffer after being soluted, LAL is used to test endotoxin in vitamin C injection

Chromogenic Method Procedure

Wavelength determination.

Prepareation the endotoxin standard solution as test solution, pathogen free aqua pro injection with LAL reagent blanko, blanko is sterilized and store in same temperature, as test solution than the test solution and blanko solution, are put in each cuvet to detarmaining the wavelength that make maximum absorbation, the absorbation is being measued at 405 nm wavelength.

How to make standard curve/standard curve process

Put 50 eu/ml endotoxin standard solution that has been prepared as many as 0,3 ml into cuvet. After that add LAL reagent as many s 0,3 ml in to the cuvet and than put cuvet to Uv-vis spectrophotometer which has been connected to waterbath with 37^0C temperature, measure the initial time when the maximum peak absorbance wave is formed. Do the same treatment for 5, 0.5, 0.05, and 0,005 eu/ml standard solution. thus obtained caliberation curve in each initial time when the peak formed

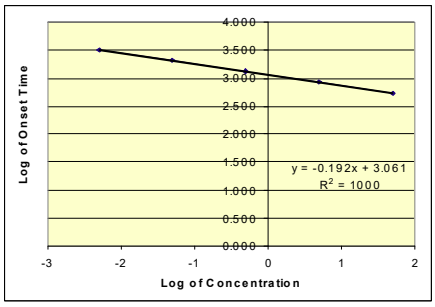
Level evdotoxin in vit C injection. Determiantion a sample of 0,3 ml vit c injection is inserted in to cuvet, then add 0,3 ml LAL reagent put the cuvet into Uv-vis spectrophotometer which has beeb connected to waterbath in 37^0C, measured the initial time when the curve peak is formed. Do the same treatment to every vit C injection Product twice each product

Data Analysis. To find out the endotoxin level in vit c injection, data that was obtained is calculated using linier regression equation in calaiberation curve

RESULTS AND DISCUSSION

Onset Time Endotoxin Standard Solution

Concentration (EU/mL)	Onset Time (second)
0.05	3181
0.05	2065
0.5	1312
5	837
50	546

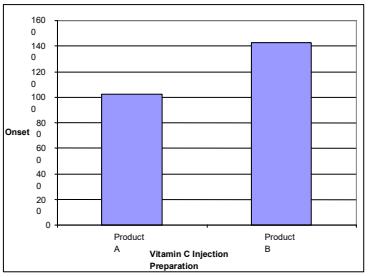


Standard Curve of Endotoxin

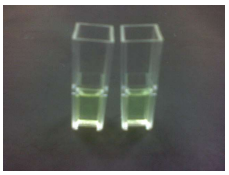
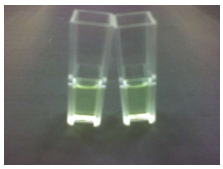
Endotoxin standard curve generated from dilution of the concentration of endotoxin in 50 EU/mL, 5 EU/mL, 0,5 EU/mL, 0,05 EU/mL and 0,005 EU/mL which reacted with pyrochrome Measurement of onset time (time of initial formation of colour) endotoxin standard solution carried out at wavelength of 405 nm. Provided linier regression equation y = -0,192 x + 3,061. Where y = Log T (time of initial formation of colour) and x = Log C (endotoxin cncentration) with r = 1,000.

Onset Time Vitamin C Injection Preparation at 405 nm Wavelength

Vit. C Injection Preparation	***Onset Time* (second)**		
	I	**II**	**Mean**
Product A	1150	932	1041
Product B	1502	1355	1428.5



The result of the endotoxin in the injection dosage of vitamin C with pyrochrome produce yellow colour.

 (product A)  (product B)

From the graph and the picture above can be seen that the onset times dosage injections of vitamin C of product A is faster than product B. And intensity of the colour formed on the injection dosage of vitamin C of product A is more powerful than product B. This shows that product A have more endotoxin than product B. Because the less time the formation of colour and stronger the colour produced showed greater levels of endotoxin contained in a sample.

The level of endotoxin contained in the dosage of vitamin C injection of product A as much as 1.686 EU/mL and product B as much as 0.324 EU/mL.

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ANTIMICROBIAL ACTIVITY OF MOTHER STARTER KEFIR TOWARDS *SALMONELLA, STAPHYLOCOCCUS* and *E.COLI* IN VITRO

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ABSTRACT

Antimicrobial activity of mother starter kefir towards *Salmonella* and *Staphylococcus* in vitro was carried out. The study was designed by using a Completed Randomized Design with five treatmens (0, 3, 6, 9 and 12 days) fermentation and five replications. At all treatments (0-12 days fermented) of mother starter kefir affectively inhibited the growth of tested as shown by the clear zone from 1.00-1.46 mm for *Salmonella*; 1.08-1.20 mm for *Staphylococcus* and 0.76-0.98 mm for *E.Coli*. The highesth antimicrobial activity of mother starter kefir at 9 days fermented for *Salmonella* , *Staphylococcus* and zero days for *E.Coli.*

Keywords*:* mother starter kefir, antimicrobial activity

INTRODUCTION

Kefir is a fermented milk product, a natural products which involve microbial activity (lactic acid bacteria and yeast) that can support human and animal health (Farnworth, 2005). Kefir and yogurt are different because in addition to inhibiting the growth of pathogen bacteria, also inhibits the growth of yeast pathogen (Surono, 2004), so that when the kefir is consumed, can balance the intestinal microbes.

Mother Starter kefir is a fermented milk product as starter maked kefir, because countains grains kefir. Lindawati (2005) states that kefir (kefir contains grains) had inhibition zone / broad spectrum of bacteria inhibit the growth of gram positive and negative (*Salmonella, Staphylococcus*, *Coliform* and *E. coli*) that produced antimicrobials can be use to cure bacterial infections.

The aim of this research is to determine how much highest activity of antimicrobial mother starter kefir beverage daily with different age fermentation bacteria capable of inhibiting the growth of *Salmonella*, *Staphylococcus* and *E.coli.*

MATERIALS AND METHODS

1) The material used in this study, fresh cow's milk pasteurization. The grains is used as a stater kefir in kefir production. Bacteria test used is a bacterial pathogen (*Salmonella*, *Staphylococcus* and *E. coli*).
2) Preparation stater kefir is done by way of fresh milk is sterilized (121^0C for 3 minutes), then cooled until the temperature of 27^0C. Milk is added to the grains kefir, then incubated at a temperature of 22^0 C for 20 hours (Ot'es and Cagindi, 2003).
3) Making mother starter kefir , fresh milk is heated at a temperature of 85^0 C for 30 minutes, then cooled until the temperature of 22 ^{0}C. Milk added kefir starter as much as 3%, then fermented for 20 hours. (Ot'es and Cagindi, 2003) to get kefir fermentation time 0, 3, 6, 9, 12 days.
4) Test the activity of antimicrobial mother starter kefir towards bacterial pathogen (*Salmonella, Staphylococcus* and *E. coli*) by the well diffusion method (Yin and Cheng, 1997).
5) The data were analysis variance, if at significantly different between treatments ($P<0.05$) followed by Duncan test (Steel and Torrie, 1993).

RESULTS AND DISCUSSION

The activity of antimicrobial mother starter kefir with different fermentation times (0, 3, 6, 9 and 12 days) has broad spectrum of gram positive bacterial pathogen and negative (*Salmonella*, *Staphylococcus* and *E. coli*) can be seen in Table 1

Table 1.The activity of antimicrobial mother starter kefir towards *Salmonella*, *Staphylococcus* and *E.coli* in vitro.

Treatments (day)	Inhibition zone (mm)		
	Salmonella	*Staphylococcus*	*E. Coli*
0	1.00 ab	1.12 a	0.98 b
3	1.20 ab	1.10 a	0.80 ab
6	1.18 ab	1.08 a	0.94 ab
9	1.46 b	1.20 a	0.76 a
12	1.14 ab	1.12 a	0.70 b

Description; - Values with different letters in the same column, significantly different (P <0.05)

Tests for antimicrobial activity of mother starter kefir showed the highest at the age of 9 days fermentation for *Salmonella* and *Staphylococcus*. It is mother starter kefir affectively as antimicrobial and ecpected the process of fermentation in kefir optimal at 9 days, because kefir countais avariety of microbes such as lactic acid bacteria homofermentatif, heterofermentatif and yeast (Ulusoy *et al*., 2007). Added Ot'es and Cagindi (2003) that the optimal fermentation of kefir, in case simbiosis between microbes kefir, resulting bioactive components complete such as lactic acid, acetic,butyric and alcohol. This results are consistent with the results of research Lindawati and Rarah (2005) that the antimicrobial kefir affectively of inhibiting the growth of *Staphylococcus* (bacteria that cause acne). This is also supported by the results of research Lindawati et al (2009) until 9 days fermentation the number of lactic acid bacteria of kefir concumption highest. Bised, it also caused the test bacteria are gram positive bacteria, which means the thick cell wall so that the dispersal of its bioactive components diffuses slowly. The highest antimicrobial activity for *E.coli* affectively at the zero days fermentation, because E.coli including gram negative bacteria, which means a thinner cell wall of gram positive bacteria, so that the diffusion of bioactive faster. Besides bioactive fermented early formed acid only, because Fanworth (2005) initial fermentation is active in biodegration only lactic acid bacteria.

CONCLUSION

It was concluded that the highest antimicrobial activity of mother starter kefir of *Salmonella and Staphylococcus* occurred in day-9 fermentation with inhibition zone of 1.46 and 1.20 mm. Zero day fermentation for *E.coli* with inhibition zone 0.98 mm.

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ETHANOL LEVEL IN BLOOD OF WISTAR RATS AFTER ACUTELY PERORAL ALCOHOL CONSUMPTION

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ABSTRACT

Alcohol in beverage consumption was increasing in Indonesia. Drinking of alcohol is constanly can be caused health disolders such as liver diseases. Recently, a lot of people in Bali was death after drinking alcohol because the contents of beverage were ethanol and methanol. The Governor of Bali reported cases as unusual to the Central Government because the promise of alcohol beverage just ethanol content. The experimental study to find ethanol level in blood of Wistar rats after peroral alcohol consumption. Rats randomly distributed according to experimental design were treated daily for a week (acute) with 5% alcohol by gas chromatography has been conducted. Gas chromatography used in the research was GC-agilent Technologies 6890-N, carrier gas helium. DB-Wax capillary column (30m x 0.25 µm x 250 µm) with polyethylene glycol stationary phase, and flame ionization detector (FID). As standard compound was ethanol and internal standard was isopropanol with the retention time were 3.532 minutes and 3.450 minutes minutes respectively. Resolution between isopropanol with ethanol is 1. The injection alcohol solution is only 1 µL into gas chromatography. After repeated acute alcohol treatment, the average of ethanol levels analysed by GC-FID in blood collected at 6 hours were 0.0000 ppm in control rat, 3.2636 ppm in rat with alcohol 5% and at 24 hours, the etanol level 0.0000 ppm in control rat is 0.0081 ppm in rat with, alcohol 5%. alcohol treament.

Keywords: ethanol, isopropanol, Wistar rats, and gas chromatography

INTRODUCTION

Repeated peroral consumption of alcohol can cause health disolders in human. When alcohol is consumed acutely period can determine blood alcohol concentration (BAC). That it is very important to determine whether a person is alcoholic or not.

The examination of alcohol as prevention liver damaged as the result of alcohol consumption. The alcohol abuse can be caused heavy toxic and the decrease awareness (Fernandez, 2007; Sutarni, 2007).

In Switzerland and the United Kingdom, a person is prohibited from driving a car on a highway when the BAC has reached a level of 80 mg/100mL or more and the Urine of Alcohol Concentration (UAC) of 107 mg/100mL (Sutter, 2002; Shepherd, 2003). In Indonesia, the limit level of alcohol comsumption has not been established, although the consumption of alcohol in human is increasing every year. Determining the level of alcohol consumption in human is generally done by examining of ethanol level in the blood. The objectives of this research were: to know ethanol level in blood of Wistar rats after acutely peroral alcohol consumption.

MATERIALS AND METHODS

The materials of chemicals are ethanol, and isopropanol as pro analysis (p.a). The method is used to separate by gas chromatography *GC-agilent Technologies 6890-N Network GC System*, coloum DB-Wax size 30m x 250 µm x 0,25µm, polyethylen glycol as stationary phase, flame ionization detector, helium (He) gas and make-up nitrogen gas.

RESULTS AND DISCUSSION

Gas chromatography used on the research is GC-Agilent Technologies 6890-N, carrier gas Helium, make-up gas Nitrogen, DB-Wax capillary column (30m x 0,25µm x 250µm) with polyethylene glicol stationary phase, and flame ionization detector (FID). Gas chromaography is conditioned on initial temperature column 50^0C which restrained for two minutes in that temperature, then increased until 10^0C per minute until temperature reaches 200^0C, and ratio split 20. Injector temperature is 200°C and detector temperature is 300°C. The linear rate column is 17 cm/second flow rate constant 0.6 mL/minute. The helium flow rate in gas chromatography is 40 mL/minute, the nitrogen flow rate is 50 mL/minute, and air rate is 450 mL/minute.

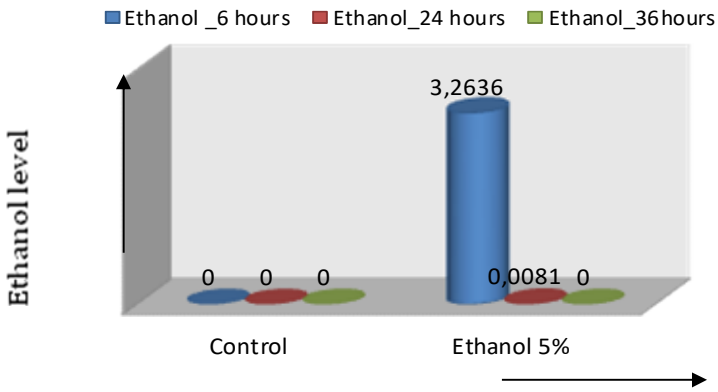

Figure 1. Ethanol level in blood of Wistar rats after 6, 24, and 36 hours (acutely) peroral alcohol 5%

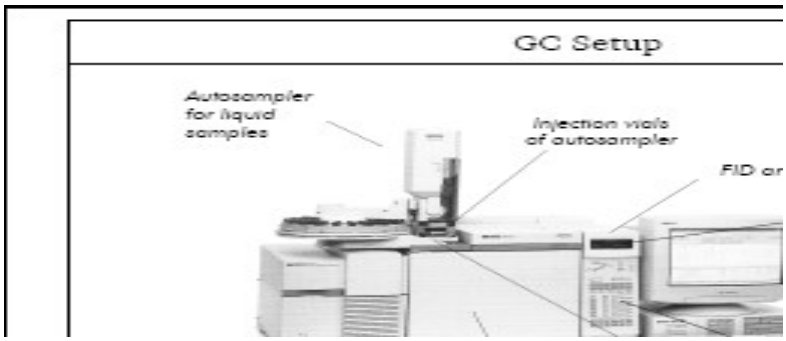

Figure 2. Gas Chromatograph

Table 1. The equation of regression line ethanol of the standard compound

Standard	Equation regression line: y= bx +a	Coefisien correlation (R^2)
Ethanol	Y= 0.062 x	0.9940

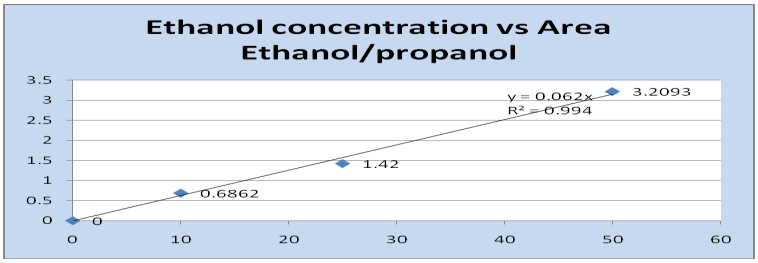

Figure 3. Ethanol Callibration Curve

The chromatogram of ethanol and isopropanol as internal standard is shown in figure 5.

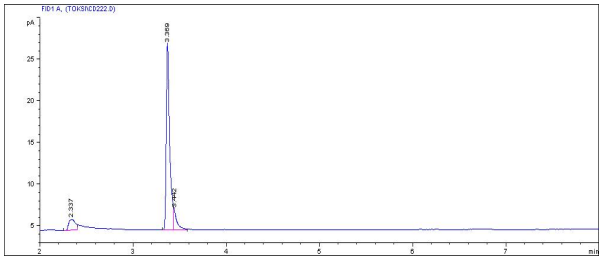

Figure 4. The chromatogram of ethanol and isopropanol

The optimation analysis of ethanol and isopropanol as internal standard by gas chromatography has been conducted. Resolution is one of all optimation separation as a fundamental of chromatography. The resolution Rs of a column provides a quantitative measure of its ability to separate two analytes.

The resolution of ethanol with isopropanol as internal standard was 1 throught base line. The retention time of propanol and ethanol were 3.450 minutes and 3.532 minutes.

Ethanol level decrease in blood of Wistar Rats between 6 hours and 24 hours after acutely peroral Alcohol consumption because ethanol is rapidly disappearing from the blood and is formed acetaldehid. Marchiti et al. (2008) stated that the aldehyde is a reactive molecule that can be oxidized by ALDH into acetate. Higher acetaldehyde level in the body were toxic to various organ/tissues such as Liver disease (Hoek et al., 2004; Lieber.2005; Moon et al.,2007).

CONCLUSION

Ethanol in blood of Wistar rats were increasing ethanol level after 6 hours peroral alcohol 5% (3.2636 ppm) then decreasing ethanol level after 24 and 36 hours peroral alcohol 5% were 0.0081 and 0.0000 ppm respectively.

ACKNOWLEDGEMENT

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THE CORRELATION OF WORK STRESS, NUTRITIONAL STATUS, AND METABOLIC SYNDROME IN ADULT MALE WORKERS

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ABSTRACT

The objectives of the study were to investigate correlation of work stress index, nutritional status, and metabolic syndrome in adult male workers. The study was a cross sectional. Thirty two subjects joined from thirty five subjects which were selected using certain criteria. Data collection covered age, physical activity, smoking, alcohol consumption, body mass index, mid upper arm circumstance, abdomen circumstance, tricep skinfold, suprailiaca skinfold, glucosa, high dense lipoportein (HDL), and trigliseride levels. Statistical analysis used correlation test and the level of significance was 5%. Median of age was 42 (25-46) years old, and average of BMI was obese I (27,49 kg/m^2). Median of stress index was low (score 62,5), and physical activity index was moderate. There was not significantly correlation between stress index and metabolic syndrome. The correlation were significantly negative between weight (p=0,025), height (p=0,003), suprailiaca skinfold (p=0,014), and HDL levels. Abdomen circumstance was significantly correlated with trigliseride levels (p=0,035). There were significantly correlation between weight (p=0,024), body mass index (p=0,018), abdomen circumstance (p=0,009), and trigliseride HDL ratio in which the ratio as plaque atherosclerosis indicators. In conclution, the correlation between nutritional status and metabolic syndrome were statistical significant but there were not clinical implication because there should be three minimal risk factor present.

Keywords: adult male worker, stress index, nutritional status, metabolic syndrome.

INTRODUCTION

High work load and limited time of work were one of the etiology of work stress. Work stress and obesity were correlated, because work stress will change into unhealthy diet, less of activities, and finally will increase body weight. The etiology of obesity is still unclear, but perhaps there were correlation between biologic and enviromental factors. The prevalence of obesity increase over the world. Obesity was one of risk factor of metabolic syndrome. The prevalence of metabolic syndrome was 68% in cronic stress workers and in male were higher than female. Base on National Cholesterol Education Program (NCEP) Adult Treatment Panel III (ATP III), the risk factors of metabolic syndrome include central obesity, dyslipidemia (high trigliseride and low HDL), high blood pressure, insulin resistency (high blood glucosa), prothrombosis and proinflamatory state (high fibrinogen and C-reactive protein). Diagnosis of metabolic syndrome were done if more than three of risk factors were present.

The study by Chandola et al. (2006) about correlation of work stress and metabolic syndrome in 10.308 subjects during 14 years, there were positive correlation between work stress and metabolic syndrome. Cronic work stress would increase the risk of metabolic syndrome two fold (OR 2,25; 95% CI 1,31-3,85). This study showed that work stress was one of importance factor of metabolic syndrome. Work stress would change body metabolism and nutritional status. Kouvonen et al. (2005) found that the correlation of work stress and body mass index (BMI) as nutritional status in 45.810 subjects, there were mild correlation between low work stress and high BMI. This study showed that there were mild correlation between work stress and BMI. Several factor influenced work stress, nutritional status, and metabolic syndrome such as male, adult (30-55 years old), married status, smoking, alcoholism, less of activities and job letter.

METHODS

Subjects

Design of the study was a cross sectional to detemine correlation between work stress index, nutritional status, and metabolic syndrome in adult male workers. The subject was adult male workers ages 25-55 years old base on research criteria. There were 32 subjects from 35 subjects whose written informed concent were attained.

Study Measurements

Data collection was done in last week of July. Based on protocol, selected subjects completed of demographic information include ages, school status, married status, smoking, alcoholism. Work stress, nutritional status, and metabolic syndrome were examined later. Work stress examination were performed by complete questionnaire, in which subject asked to complete stress index questionnaire. Nutritional status examination included weight, height, tricep and suprailiaca skinfold, abdomen and mid upper arm circumstance. Risk factor of metabolic syndrome were examined by blood pressure, fasting blood glucosa, trigliseride and HDL level. Subject were asked to fast 10-12 hours (overnight fast) before blood were drawn to minimize of bias.

Statistical Analysis

All statistical analisys were performed using Statistical Package for Social Science (SPSS 11.5 version) programme. Test of normality were done using Kolmogorov-Smirnov test. Differences in mean values were assessed by Pearson correlation test for the normal distributed data or Spearman for the abnormal one. Values of $p<0,05$ were considered to indicate statistical significance.

RESULTS

Median and minimum-maximum of ages was 42 (25-46) years old. Most of subjects (28 subjects) were undergraduate level (87,5%) and the others were senior high school (12,5%). Based on married status, most of them (30 subjects) had been married (93,75%) and only 6,25% had not been married. There were eight subjects (25%) always smoking 2-4 piece per day, and other 24 subjects were not smoking. Only two subjects always drank alcohol and the others were never. There were 23 subjects (78,88%) had moderate physical activities and just nine subjects (21,12%) had mild physical activities.

Table 1. Base Line Characteristic Data

Variable	Value	P
Age (years old)	42 (25-46)#	0,000
Physical activity	32 (2-48)#	0,000
Weight (kg)	77,75 (57,20-113,00)#	0,036
Height (cm)	167,20±5,89	0,070*
BMI (kg/m^2)	27,49±3,39	0,150*
Trisep skinfold (mm)	13,23±4,72	0,200*
Suprailiaca skinfold (mm)	26,06±8,45	0,200*
Mid upper arm circumstance (cm)	29,26±2,33	0,200*
Abdominal circumstance (cm)	89,43±9,96	0,200*
Stress index	62,5 (50-102)#	0,009
Fasting glucosa level (g/dL)	92 (77-349)#	0,000
HDL level (mg/dL)	41,81±8,81	0,200*
Trigliseride level (mg/dL)	167 (68-375)#	0,043
Trigliseride and HDL ratio	4,56±1,92	0,200*
Sistolic (mm Hg)	116,96±14,22	0,077*
Diastolic (mm Hg)	79,16±10,99	0,082*

* = significant; # = median (minimum-maximum)

There were no significant correlation between work stress index and metabolic syndrome. The correlation were significantly negative between weight (p=0,025), height (p=0,003), suprailiaca skinfold (p=0,014), and HDL levels. Abdomen circumstance was significantly correlated with trigliseride levels (p=0,035). There were significantly correlation between weight (p=0,024), body mass index (p=0,018), abdomen circumstance (p=0,009), and trigliseride and HDL ratio in which the ratio as plaque atherosclerosis indicators.

Table 2. Correlation of Work Stress Index, Nutritional Status and Metabolic Syndrome

Variable		Glucosa	HDL	Trigliseride	Sistolic	Diastolic
Stress	r	- 0,180	- 0,112	- 0,040	- 0,110	- 0,094
	p	0,325	0,543	0,828	0,548	0,610
Weight	r	0,179	- 0,396	0,246	0,032	0,135
	p	0,328	0,025*	0,828	0,862	0,462
Height	r	- 0,167	- 0,505^{P}	- 0,141	- 0,260^{P}	- 0,208^{P}
	p	0,362	0,003*	0,443	0,151	0,253
BMI	r	0,266	- 0,222^{P}	0,272	0,058^{P}	0,191^{P}
	p	0,140	0,223	0,132	0,753	0,295
Upper Arm	r	0,150	- 0,108^{P}	0,187	0,045^{P}	0,096^{P}
Circumtance	p	0,412	0,555	0,307	0,806	0,602
Abdominal	r	0,268	- 0,332^{P}	0,374	0,094^{P}	0,223^{P}
Circumtance	p	0,113	0,063	0,035*	0,608	0,220
Tricep	r	0,121	- 0,283^{P}	0,192	0,102^{P}	0,092^{P}
Skinfold	p	0,511	0,117	0,293	0,580	0,617
Suprailiaca	r	0,274	- 0,431	0,125	- 0,034^{P}	- 0,041^{P}
Skinfold	p	0,192	0,014*	0,497	0,855	0,825

* = significant; p = Pearson Correlation

DISCUSSION

Limitation of work stress index examination using questionnaire was not completed by subject because of time limit so the results would be bias. Before laboratory examination, subjects asked to fast 10-12 hours to minimize food factor influence, but four subjects did not fast so laboratory examination were done later. There were negative weak correlation between stress index and metabolic syndrome which was not statistical significant, it means that there reversed correlation between stress index and metabolic syndrome. As known, cronic stress exposure would increase cortisol hormon secretion so it would increase risk of central obesity, insulin resistancy and dyslipidemia.

In this Study, stress examination were done only once and strength or length of stress exposure were not incluced in this qustionnaire, so how strength or length stress exposure were not known in subjects. Kivimaki et al found that the more strength and length of stress exposure the increase of metabolic syndrome risk. The study results reversed with Kivimaki et al study. As a theory, sample size influenced strength of intervariable correlation, but in this study the subjects partisipated less than sample size (91,43%). Mean of stress index was mild, so its correlation with metabolic syndrome was weak because subjects did not completed stress index examination carefully.

The correlation of weight and HDL level was negative and statistically significant, meanwhile base on height, there were negative correlation between height and HDL level and statistically significant. According to BMI, there were weak correlation between BMI and metabolic syndrome which was not significant. Negative significant correlation between weight and height with HDL level showed that the reversed correlation intervariable, althought the metabolic syndrome risk was not clinically significant.

Nutritional status examination such as weight, height and BMI showed general nutritional status. As known, BMI was more descriptive to metabolic syndrome risk than weight or height alone because BMI used two variable ratio. Mean of BMI subjects was obese I (27,49 kg/m^2), it means obese I was very importance risk factor of metabolic syndrome. There were positive correlation between abdomen circumstance and trigliseride level which was significant. Mean of abdomen circumstance was normal (89,43 cm), it means that there were central obesity. As known in central obesity, dyslipidemia showed high lipid storage so it would increase risk of metabolic syndrome. Trigliseride level showed lipid storage also particulary in central obesity. This results approved Steptoe et al study, the higher abdomen circumstance the higher trigliseride level.

The correlation of suprailiaca skinfold and HDL level were negative significant. Suprailiaca skinfold showed subcutaneous lipid storage in abdomen regio. As a theory, the bigger abdomen circumstance the bigger suprailiaca skinfold. It means, high of suprailiaca skinfold would increase metabolic syndrome risk. This results found that there were significant correlation between nutritional status and metabolic syndrome, but it had not clinically approved because it would be three minimal risk factor present.

Cronic stress exposure would change lifestyle, for example food choises would be high calori and big size because of limitation of time to manage healthy lifestyle. High calori food contained of simple carbohidrate and trans faty acids which known increase metabolic syndome risk. Beside that, this kind of food had relaxation effects because it would inhibit corticotropin realising factor (CRF) hormon secretion. The inhibition would make confort during stress. Meanwhile, lunch pending habbit would increase of food portion on dinner so it would make obesity.

In this study, there were positive and significant correlation between weight, BMI, and abdomen circumstance with trigliseride and HDL ratio. This ratio showed vascular atherosclerosis plaque development degre, in which increase of the ratio would increase atherosclerosis plaque risk. Abdomen circumstance and BMI were more descriptive than weight alone. In conclusion, the correlation between nutritional status and metabolic syndrome were statistical significant but there were not clinical implication because there should be three minimal risk factor present.

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PROTEIN PROFILE OF ANOPHELES SUNDAICUS SALIVARY GLAND AS POTENSIAL TARGET FOR TRANSMISSION BLOCKING VACCINE (TBV) AGAINST MALARIA

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ABSTRACT

Malaria is a mosquito-borne disease of world-wide concern as well as in Indonesia causing 1.5 to 2.7 millian people dying each year. Many attempts to overcome this diasease have been conducted included with vaccine. The ideal malaria vaccine strategy should include several stage of parasit life cycles during infection i.e. pre-erithrocytic, erithrocytic and transmission. Recently, it has been showed that mosquitoes salivary contains components which are immunogenic, thus it would be very potential to serve as targets for the development of *Transmission-Blocking Vaccine* (TBV). The objective of this research was to characterize the protein profile of *Anopheles sundaicus* salivary gland and to collect mRNA as template for RT-PCR to construct cDNA library.*Salivary gland* (SG) of *A. sundaicus* has been isolated from the mosquitoes following landing collection. SDS-PAGE was conducted to elucidate crude protein profile of Salivary Gland Extract (SGE) for preliminary detection of the existing of immunomodulator proteins. Furthermore, SG was then extracted to collect mRNA as template for RT-PCR to construct cDNA library. Protein profile investigation showed that there were protein bands corresponding with the putative immunomodulatory proteins which are previousely published from Anopheles Salivary Glands.Using *Micro-FastTrack mRNA isolation kit* (Invitrogen, San Diego, CA, USA) showed negative result.This research is a preliminary research to moleculary and functionally characterize the salivary components from salivary gland of *A. sundaicus* which are responsible as a immunomodulatory factor as a potential target for the development of TBV.Therefore it was suggested to use another method instead of using mRNA kit for further isolation of RNA from *A. sundaicus.*

Keywords: salivary gland, immunomodulator, TBV,malaria, Anopheles sundaicus

INTRODUCTION

Malaria is a mosquito-borne disease of world-wide concern as well as in Indonesia causing 1.5 to 2.7 millian people dying each year (Greenwood and Mutabingwa, 2002). The disease is caused by Plasmodium parasites and naturally transmitted by competent Anopheles mosquitoes. There are about 80 species of Anopheles in Indonesia, however, only about 24 are vectors of the malaria parasites (Harijanto, 2003). *Anopheles sundaicus* is one of the major malaria mosquito species complex in Indonesia, particularly in Java and Sumatra islands (Dusfour et al, 2004).

Many attempts to overcome this disease have been conducted included with vaccine. Malaria vaccine development showed a relatively slow progress. The complex biology of malaria parasites cuopled with antigenic polymorphism, poor antigen immunogenicity, and parasite-induced immunosuppression distinguishes the quest for a malaria vaccine as extraordinarily daunting (Donovan et al, 2007).The ideal malaria vaccine strategy should include several stage of parasit life cycles during infection i.e. pre-erithrocytic, erithrocytic and transmission. The vaccine that inhibit transmission of pathogens using antigens originated from vector (arthropodes, e.g. mosquitoes) is called Transmission Blocking Vaccine (TBV) (Carter, 2001).

In the last decade, new approach in vaccine development for arthropod-borne diseases is using salivary vector component. This approach based on hypothesis that

arthropod vectors saliva contains vasomodulator and immunomodulator proteins (Sack and Kamhawi, 2001; Titus et al, 2006). The vasomodulatory factors in arthropod saliva help the vector to obtain a blood meal. Many reports showed that salivary immunomodulators could enhance pathogen infection.In relation with this case, it should be possible to control pathogen transmission by vaccinating the host against the molecules in saliva that potentiate the infection, thereby blocking the enhancing effects of saliva and thus preventing the pathogen from establishing infection in the host (Belkaid et al, 1998).

Mosquito's salivary components are immunogenic i.e inducing strong immune response for example swelling and itching that accompanied mosquitoes bite (Peng and Simon, 2004). Host immune response against mosquito's saliva could decreased infectivity of transmitted pathogen (Belkaid et al, 1998). Prior exposure of mice to bites of uninfected sand flies conferred protection against Leishmania major that was associated with a strong delayed-type hypersensitivity response and with interferon-γ (IFN-γ) production at the site of parasites delivery (Kamhawi et al, 2000). Population living at endemic of leishmaniasis sites showed natural resistency against laishmania parasites (Davies and Gavgani, 1999). This has been expalined as natural immunity mediated by Th1 that has protective properties and contains antibodies against sandflies's saliva. Mosquitoes bites have shown similar effects in animal models through cytokines systemic response in host (Schneider et al, 2004). Repeated exposure to bites from uninfected mosquitoes skewed the immune response towards a T-helper 1 (Th-1) phenotype as indicated by increased levels of interleukin-12, IFN-γ, and inducible nitric oxide synthase. These data suggest that the addition of mosquito salivary components to antimalaria vaccines may be viable strategy for creating a Th1-biased environtment known to be effctive against malaria infection (Donovan et al, 2007).

However, specific components as a potential target for TBV in Anopheles mosquitoes has not yet been inditified so far. Therefore, exploring salivary components of Anopheles mosquitoes is an important step to localize new target on TBV against malaria. The objective of this research was to characterize the protein profile of *Anopheles sundaicus* salivary gland and to collect mRNA as template for RT-PCR to construct cDNA library.

MATERIALS AND METHODS

Anopheles sundaicus Salivary Gland Preparation

Anopheles sundaicus mosquitoes were collected by landing collection in coastal area of Desa Pathuk, Kecamatan Purwodadi, Purworejo, Central Java. The collection used human bite that protected by net and the mosquitoes that landing on the net aspirated using aspirator. The *A.sundaicus* female mosquitoes were identified by Reid (1966).

Salivary gland (SG) of 100 *A. sundaicus* has been isolated from the mosquitoes using fine entomological needles under a stereoscopic microscope at 4X magnification in phosphate-buffered saline [PBS;10 mM Na2SO4, 145 mM NaCl (pH 7.2)] and transferred to a microcentrifuge tube with a small volume of PBS. The glandparts were placed in a small volume of PBS and stored at -80 °C until use.

The salivary glands of adult mosquitoes were dissected in PBS and allowed to settle onto slides without drying. Photographs of the glands were taken using a digital camera (Cannon,Tokyo, Japan) attached to a light microscope.

Protein Isolation with SDS-PAGE

SDS-PAGE was carried out according to standard techniques (Hames, 1990). Salivary gland samples were thawed on ice and mixed in1:2 1X SDS gel loading buffer [50 mM Tris-HCl (pH 6.8), 100 mM DTT, 2% SDS, 0.1% Bromophenol blue, 10% glycerol]. Then, the samples were heated for five min in a boiling water bath and loaded

on 12% SDS polyacrylamide gels. Molecular weight markers (Bio-rad,USA) were applied in each gel.

mRNA Isolation from Salivary Gland

A.sundaicus salivary gland mRNA was isolated from 40 salivary gland pairs using the Micro-FastTrack mRNA isolation kit (Invitrogen, San Diego, CA, USA) following its manufacturer's protocol.

RESULTS AND DISCUSSION

Salivary gland (SG) of *A. sundaicus* has been isolated from the mosquitoes following landing collection (Fig.1). The paired salivary glands of mosquitoes are present in the thorax flanking the oesophagus. Each gland has three lobes, two lateral and one median. In the female mosquito the lateral lobes are formed by proximal, intermediate and distal regions. The median lobe on the other hand, is formed by a short neck region and a distal region (Dhar and Kumar, 2003). The proximal regions of the lateral lobes in females express and secrete salivary gland products such as amylases and a1-4 glucosidase that are involved in sugar feeding . In contrast, the medial lobe and distal-lateral lobes express genes whose products such as apyrases, anticoagulants and vasodilatory agents are involved in hematophagy (James, 2003).

Protein profile investigation showed that there were protein bands corresponding with the putative immunomodulatory proteins which are previousely published from Anopheles salivary glands. Electrophoresis was carried out to profile the protein content of saliva from *An. sundaicus*, which showed numerous bands ranking from 90 kDa to 118 kDa and 34 kDa (Fig.3). Previous study by Cornelie et al.(2007) showed the same results. The protein content profile of saliva from *An. gambiae*, which showed numerous bands ranking from 10 kDa to > 100 kDa. About twenty polypeptides with molecular masses of 103,94, 74, 62, 54, 49, 41, 39, 31, 30, 28, 26, 24, 16, 14, 13.5 and 12 kDa were clearly detected in silver-stained gel and a few others were lightly stained. The protein profile of An.dirus B studied by Jariyapan et al. (2007) showed almost similar results. There were seven major and several minor protein bands detected in the female salivary glands. The molecular masses of these major protein bands were estimated at 63, 44, 43, 35, 33, 30 and 18 kDa.

Isolation of An.sundaicus salivary gland mRNA by *Micro-FastTrack mRNA isolation kit* (Invitrogen, San Diego, CA, USA) showed negative result (Fig. 2). There were some possibilities that may cause the negative result. The amount of salivary glands (40 pairs) was not sufficient to obtain an optimum mRNA. The previous study by Francischetti et al (2002) used 80 pairs of *An.gambiae* salivary glands to isolate mRNA. Another important reason is the contammination of RNAse that caused mRNA degradation. It is most important for isolation of intact, full length mRNAs to keep an RNase-free environment. All reaction tubes, pipet tips, solutions, etc. used to handle (m)RNA should be RNase-free (Nowrousian, 2004). This research is a preliminary research to moleculary and functionally characterize the salivary components from salivary gland of *A. sundaicus* which are responsible as a immunomodulatory factor as a potential target for the development of TBV.Therefore it was suggested to use another method instead of using mRNA kit for further isolation of RNA from *A. sundaicus.*

Figure 1. *An.sundaicus* salivary gland (stereoscopic microscope at 4X magnification)

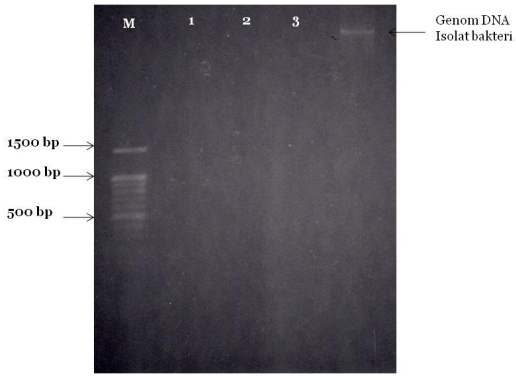

Figure 2. Negative result of isolation *An.sundaicus* salivary gland mRNA.

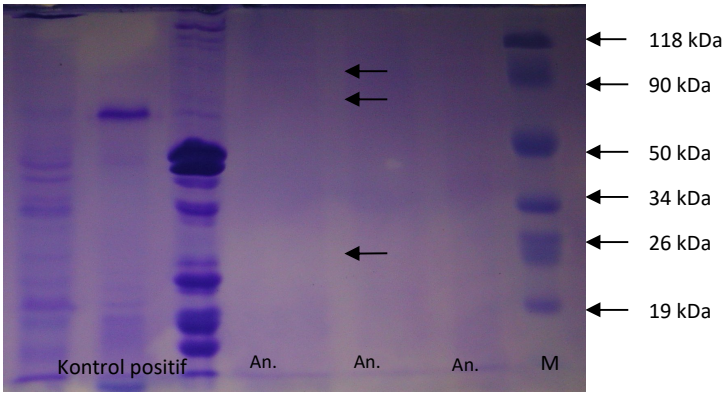

Figure 3. Protein profile of *An.sundaicus* salivary gland.

CONCLUSION

Salivary gland (SG) of *A. sundaicus* has been isolated from the mosquitoes following landing collection. Electrophoresis was carried out to profile the protein content of saliva from *An. sundaicus*, which showed numerous bands ranking from 90 kDa to 118 kDa and 34 kDa corresponding with the putative immunomodulatory proteins which are previousely published from Anopheles Salivary Glands.Using *Micro-FastTrack mRNA isolation kit* (Invitrogen, San Diego, CA, USA), the isolation of *An.sundaicus* salivary gland mRNA showed negative result. Therefore it was suggested to use another method instead of using mRNA kit for further isolation of RNA from *A. sundaicus.*

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THE COMPARISON EFFECT OF NATURAL HONEY AND SYRUP OF STORAGE ROOT BALINESE SWEET PURPLE POTATOES *(IPOMOEA BATATAS L)* LIPID PROFILE OF THE BLOOD IN RATS WITH HYPER CHOLESTEROL DIET

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ABSTRACT

High cholesterol diet in daily life can increase the total cholesterol of the blood and increase the incident of cardiovascular diseases. Flavonoid from fruits and vegetables can prevent those effects because of its antioxidant properties. The aims of this study are to examine possible hypolipidemic effect syrup of storage root Balinese purple sweet potatoes, and natural honey as a high flavonoid content of food stuff, in rats with hyper cholesterol diet. Subject of this study were 24 female adult rats divided into 3 groups with randomized pretest and post-test control group design. Before treatment blood sample were collected from retro-orbitalis sinus of all rats for examination of lipid profile as pretest data. After pre test the group 1 of 8 rats given high cholesterol diet without syrup of Balinese sweet purple potato nor natural honey. The group 2 of 8 rats given high cholesterol diet with syrup of Balinese sweet purple potato. The group 3 of 8 rats given high cholesterol diet with natural honey. After one week of treatment the blood were collected for post-test examination. The data analyzed by one way ANOVAs and t-paired test and the result showed a significant increase of total cholesterol of the blood in group 1 ($p<0.05$), significant decrease of HDL, in group 1 ($p<0.05$). In group 2 and group 3 all of that parameter after one week of treatment did not differ with pretest ($p>0.05$) but differ with control group or group 1 ($p<0.05$). From this finding it can be concluded that syrup of Balinese sweet purple potato and natural honey can prevent the lipid profile in normal value, and decrease total cholesterol of the blood in high cholesterol diet of rats.

Keyword: Balinese Sweet Purple Potato, Natural Honey, Lipid profile, Rat

INTRODUCTION

The increase of free radicals in cells and tissues, whether originating from inside or from outside the body can cause various diseases. Pathogenesis of coronary heart disease for example, is closely associated with free radical/oxidative stress and hypercholesterolemia. In developed countries like the United States mortality rate from coronary heart disease is still quite high (Zern, 2005). The role of free radicals in the pathogenesis of coronary heart disease has been widely researched (Prior, 2003). Increased of cholesterol, especially LDL and triglyceride mainly in the blood and is accompanied by oxidative stress triggers the oxidation of LDL, which would lead to atherosclerosis. Despite the known existence of hypo lipidemic drugs and the presence of endogenous antioxidant but it seems the problem of hypercholesterolemia and oxidative stress and its relation to cardiovascular disease is still a problem for the experts until now. Some results showed that oxidative stress can be prevented by various types of food. Micronutrients which are phytochemical clusters of various foodstuffs derived from plants have been widely researched, which is believed to be protective against oxidative stress (Sanchez-Moreno, 2003; Prior, 2003).

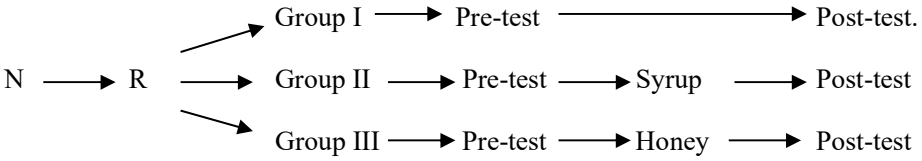
Several studies have proved that foods containing flavonoids when consumed regularly can protect the body from cardiovascular disease and some other chronic diseases (Knekt, 2002; Chepulis, 2008). It turned out that flavonoids can improve endothelial function of blood vessels (Engler, 2004), can reduce LDL susceptibility to the influence of free radicals (Stein, 1999, Ling, 2001) and can be hypo lipidemic, anti inflamation as well as a good antioxidant in some patients (Davalos, 2006; Kelley, 2006). Recently, thousands of types of flavonoids have been identified and many of them are

usefull for health (Middleton,2000). Anthocyanin pigment is one important type of flavonoid that has been widely researched and has beneficial effects on cells in mammals such as antioxidant effects, anti mutagenic, hepatoprotective and anti hypertensive. Many anthocyanin found in some foodstuffs such as vegetables, fruits, nuts, grains and tubers (Suardi, 2005).

Consumption of water of aronia melanocarpa fruits which are rich in anthocyanin can reduce hyperlipidemia in rats (Valcheva-Kuzmanova, 2006). Anthocyanin extracts from soybean (Glycine max. L) can improve the lipid profile, because it can decrease triglycerides and total cholesterol significantly and can increase HDL (Kwon, 2006). Balinese purple sweet potato tuber proved to have a high anthocyanin (Suprapta, 2004), and has been investigated have antioxidant effects in blood and various organs in mice with oxidative stress (Jawi, 2006). Purple sweet potato tuber apparently has been developed into food products such as ready-made syrup, so it needs to be investigated whether efficacious as hypo lipidemic and enhance endogenous antioxidant. Natural honey is also a food that contains various types of flavonoids, non enzymatic, enzymatic antioxidant, ascorbic acid, which is important as antioxidants. The role of honey in maintaining the health has been widely studied and shown to have various effects such as antioxidant effects, antibiotic, and probiotics. Giving honey to rat ad libitum for 54 weeks was able to increase HDL significantly (Chepulis, 2008). Most study on the honey is within a chronic period, rarely seen in the acute period. In this study we want to know whether within one week of honey or syrup purple sweet potato tuber could changes the lipid profile in the rat.

MATERIALS AND METHODS

This study is laboratory experimental with randomized pre and post-test control group design.



Legend

N	= Sample
R	= Randomization
Group I	= Rats (8 rats) which are given high-cholesterol diet for one week
Group II	= Rats (8 rats) which are given high-cholesterol foods and syrup purple sweet potato tuber during the first week.
Group III	= Rats (8 rats) which are high-cholesterol foods and natural honey for a week.

RESULTS AND DISCUSSION

Table 1. Average Total Blood Cholesterol Concentration of the Rats

No.	Average Total Blood Cholesterol (mg/dl) ± SD	
	Pre-Test	Post-Test
1	107.07 ± 3.31	174.50 ± 18.76
2	109.07 ± 7.11	116.43 ± 15.68
3	108.04 ± 3.25	114.52 ± 10.04

Table 2. Average Trygliceride, LDL, and HDL of the Rats

No.	Average Trygliceride (mg/dL) ± SD		Average HDL (mg/dl) ± SD		Average LDL (mg/dl) ± SD	
	Pre-test	Post-test	Pre-test	Post-test	Pre-test	Post-test
1	93.13 ± 3.32	119.60 ± 9.55	85.70 ± 1.59	49.30 ± 3.46	42.07 ± 2.03	88.59 ± 30.86
2	92.52 ± 2.96	103.22 ± 5.97	84.79 ± 1.30	83.27 ± 0.79	41.81 ± 1.14	43.34 ± 2.36
3	92.45 ± 10.26	75.39 ± 4.33	84.38 ± 2.24	96.57 ± 3.39	48.42 ± 4.98	28.46 ± 1.63

In this research we found that that the lipid profile changes significantly due to provision of high-cholesterol diet in rats, and this can be overcome with the provision of purple sweet potato tuber syrup or natural honey. In table 1 shows there is an increase in total cholesterol due to high cholesterol feeding during the first week. Compared to pretest the increase is statistically highly significant ($p < 0.01$). In the group given a purple sweet potato tuber syrup and high-cholesterol diet during the first week it did not occur simultaneously changes in total cholesterol ($p > 0.05$). Anthocyanin which is one type of flavonoids contained in the purple sweet potato tuber (Huang, 2004), can inhibit the absorption of cholesterol in the digestive tract or it can inhibit the synthesis of cholesterol in the liver. This research is consistent with anthocyanin extracts from soybean (Glycine max. L) which can improve the lipid profile, because it can lower triglycerides and total cholesterol significantly and can increase HDL (Kwon, 2006).

Decrease in serum cholesterol caused by provision of anthocyanin, are through inhibition of cholesterol and bile acids absorption in the intestine. This finding is supported by studies in rats which were given nasunin, anthocyanin of eggplant, nasunin can lower total serum cholesterol and increase HDL (Fumio, 1994).

Rats given a natural honey also showed similar results with mice which were given syrup. Natural honey can keep almost the same total cholesterol by pre-test. Benefits of honey in other studies have found that consumption of honey on a regular basis can improve health. Although honey contains glucose, but compared with glucose-containing foods, honey is a natural sweetener if consumed regularly, has no effect on blood sugar because of honey is food with a low glucose index. In addition honey is also a good source of antioxidants (Al-Waili, 2003), and can improve blood lipid profile in experimental animals (Chepulis, 2008). The results are consistent with Al-Waili (2004). In the study reported that consumption of honey for 15 days can reduce cholesterol levels up to 7%, lowers LDL cholesterol and decreased triglycerides 1% to 2%. In the study proved there is an increase HDL as much as 2% (Al-Waili, 2004).

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MALIGNANT TRANSFORMATION PAPILLARY THYROID CARCINOMA IN HASHIMOTO'S THYROIDITIS: A CASE REPORT

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ABSTRACT

Background: Hashimoto's thyroiditis is an inflammatory disease of the thyroid gland with autoimmune etiology. One of the complication is papillary thyroid carcinoma. Need carefulness in microscopic examination of Hashimoto's thyroiditis cases to find the possibility of preneoplastic lesion. **Aim and Objective:** To report one case coexistence of Hashimoto's thyroiditis and papillary thyroid carcinoma that suspected as malignant transformation of Hashimoto's thyroiditis. **Methods:** The specimen were thyroid gland from total thyroidectomy and neck lymph node from a patient who diagnosed as papillary carcinoma. The macroscopic examination and microscopic slides were done and stained by hematoxyllin and eosin. The diagnosis was done according to microscopic examination, supported by clinical and macroscopic examination. **Result and Discussion:** Patient was male, 13 years old, with enlargement of thyroid gland. Macroscopic examination showed one lymph node 1.5 x 1 x 0.5 cm and thyroid gland 10.5 x 5.5 x 4.5 cm in total measurement, reddish in cut section. There is one nodule, 0.5 cm in diameter, grey white, in each lobe dextra and sinistra. Microscopic examination showed small thyroid follicles, lined by epithelial with oxyphilic change. The lymph follicles were distributed within and around the lobules, apart with prominent germinal centre. Some foci showed follicles lined by epithelial with ground glass and groove appearance nuclei. The lymph node contain metastatic cells. Hashimoto's thyroiditis predominantly occur in adult woman, rare occur in children. The coexistence of papillary carcinoma and Hashimoto's thyroiditis support the malignant transformation theory of Hashimoto's thyroiditis.

Keywords: Hashimoto's thyroiditis, papillary carcinoma, malignant transformation.

INTRODUCTION

Thyroiditis refers to a group of inflammatory diseases affecting the thyroid gland. The diseases include in this group are chronic lymphocytic thyroiditis (Hashimoto's thyroiditis), subacute granulomatous thyroiditis (de Quervain thyroiditis), subacute lymphocytic thyroiditis, acute suppurative thyroiditis and fibrous thyroiditis (Riedel thyroiditis).[1] Hashimoto's thyroiditis is the most common inflammatory condition, caused by autoimmune. [1, 2, 3]

Hashimoto's thyroiditis is the most common cause of hypothyroidism,[1, 4], that affect up to 2 percent of the general population. [2] Up to 95 percent of cases of chronic lymphocytic thyroiditis occur in women, usually between 30 and 50 years of age, but also the most common cause of sporadic goiter in children. [1] Another study found the most common cases occur between 45-65 years of age and affect women 10-20 times more frequent than men. [2, 4]

Genetics play a contributory role, it is inherited as a dominant trait. This disease is an organ-specific T-cell mediated. [1, 2] Thyroid-specific autoantibodies presence in the serum. The three main targets for thyroid antibodies are thyroglobulin (a protein carrier for thyroid hormones), thyroid microsomal antigen (also called thyroid peroxidase) and the thyroid-stimulating hormone (TSH) receptor. [1] Interaction between internal (genetic) and external (environmental and endogenous) factors is required to initiate Hashimoto's disease. Environmental triggers include iodine intake, bacterial and viral infection, cytokine therapy and probably pregnancy. [2]

Clinically Hashimoto's thyroiditis usually asymptomtic, some patients may complain of a feeling of tightness or fullness in the neck and the presence of mass;

however, neck pain and tenderness are rare. At the time of diagnosis, hypothyroidism are present in 20% of patients.[1, 5] Hypothyroidism develops gradually, but it may preceded by transient thyrotoxicosis (hashitoxicosis) with increase in T3 and T4 levels caused by disruption of thyroid follicles with secondary release of thyroid hormone. As hypothyroidism supervenes, T3 and T4 levels progressively decline. [4, 5] Physical examination show goiter with firm consistency, irregular and without tenderness [1], diffuse and symmetric enlargement with intact capsule, well circumscribe. Some cases show localize enlargement, one lobe is more enlarged than the other, make it multinodular appearance. [4, 5] Hashimoto's thyroiditis has been shown to often coexist with other autoimmune diseases such as type 1 diabetes (T1D), celiac disease, rheumatoid arthritis, multiple sclerosis, vitiligo, etc. [2]

The erythrocyte sedimentation rate (ESR) and white blood cell count are normal. Clinical diagnosis support by antithyroid microsomal antibodies in titers greater than 1:6,400 or antithyroid peroxidase antibodies in excess of 200 IU per mL. Radioactive iodine uptake (RAIU) is variable, may be depressed, normal or increased, depending on the extent of follicular destruction. Ultrasonography shows an enlarged gland with a diffusely hypoechogenic pattern. [1] Papillary thyroid carcinoma with Hashimoto's thyroiditis had more irregular shapes and ill-defined edges of the borders.[6]

Patients with Hashimoto's thyroiditis have a higher risk of thyroid malignancies, [7] such as papillary carcinoma, B cell Non-Hodgkin lymphoma, leukemia and Hurthle cell neoplasm.[3,4,5] Although considered as benign condition, Hashimoto's thyroiditis almost always harbours a genetic rearrangement that is strongly associated with and is highly specific for papillary thyroid carcinoma. Careful examination is very important to find papillary carcinoma in every case of Hashimoto's thyroiditis. [3]

MATERIALS AND METHODS

The specimen origin from thyroid gland from total thyroidectomy and neck lymph node excision. This patient clinically diagnosed as papillary thyroid carcinoma. The macroscopic examination was done. Microscopic slides from right lobe, isthmus and left lobe, also from neck lymph node were made and stained by hematoxyllin and eosin. The diagnosis was done according to microscopic examination, supported by clinical and macroscopic examination.

RESULTS AND DISCUSSION

Patient was male, 13 years old, with chief complain enlargement of the mass at anterior neck, without tenderness since about one year ago. Macroscopic examination showed thyroid tissue consist of right lobe, isthmus and left lobe and one lymph node. Right lobe was 4.5 x 2.5 x 2 cm, left lobe was 3.5 x 2 x 2 cm, isthmus was 2.5 x 1 x 0.5 cm, fine outer surface, reddish in cut section, firm in consistency. There is one nodule, 0.5 cm in diameter, grey white, in lower portion in each lobe dextra and sinistra. The lymph node was 1.5 x 1.0 x 0.5 cm, solid, lobulated, and firm in consistency. Microscopic examination in right lobe, isthmus and left lobe showed similar appearance, consist of small thyroid follicles, lined by epithelial with oxyphilic change, fine eosinophylic cytoplasm. The lymph follicles were distributed within and around the lobules, part with prominent germinal centre. There were some focus of fibrotic area. Some follicle lined by epithelial with ground glass and groove appearance nuclei. The lymph node contain metastatic cells with dilated sinus. (See fig. 1-4).

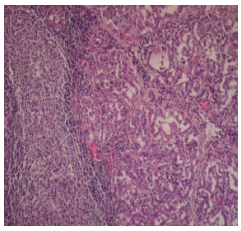

Figure 1. Papillary thyroid ca around lymphfollikel (H & E, 40 x)

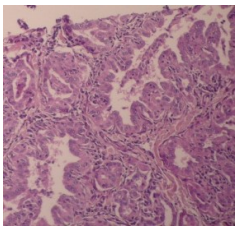

Figure 2. Epithelial with oxhyphilic change (H & E, 400x)

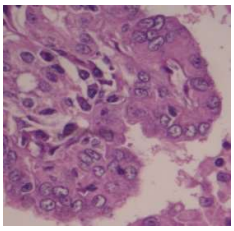

Figure 3. Ground glass dan groove nuclei (H & E, 450x)

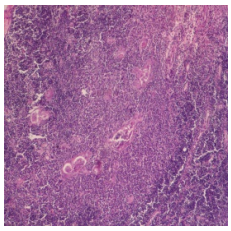

Figure 4.Metastasis in neck lymph node (H&E,200x)

In a study of 37 patients papillary carcinoma coexistent with Hashimoto's thyroiditis, mostly occur in younger women (40 years old) compare with papillary carcinoma without Hashimoto's thyroiditis. [8] This case was rare case, because occur in male, 13 years old.

Papillary carcinoma appears as an irregular solid or cystic mass in a normal thyroid parenchyma,[7] soliter or multifocal, well demarcated, encapsulated or infiltrated adjacent structure.[4] Clinically this patient diagnosed as papillary carcinoma, because occur in young patient and the tumor was multiple and mobile.

Total thyroidectomy is the surgical procedure of choice. [8] Foci of papillary carcinoma were suspected in macroscopic examination, because in cut section showed a nodule, 0.5 cm in diameter, grey white, in each lobe dextra and sinistra, without necrotic area and calcification, the capsul was intact.

Histophatologic examination support the diagnosis of Hashimoto's thyroiditis with foci of papillary carcinoma and metastasis in neck lymph node. Papillary carcinoma spreads to local lymph nodes in approximately 40-50% cases, [4, 9, 10] may infiltrative to larynx and hypopharinx.[10] Lymph node metastasis increases the morbidity of treatment and the risk of local regional relapse and may also affect cure rates and survival. Overexpression of cyclin D1, and underexpression of p27 predicted lymph node metastasis. [11] Lymph node metastasis to the neck may undergo cystic degeneration and not enlarge in long time, mimic the benign lesion, which consequently delays the diagnosis and treatment.[5, 9] Metastatic focus also possible arise from heterotopic thyroid. [12]

Moleculer examination not yet do in this case. Focal papillary thyroid carcinoma-like immunophenotypic changes in Hashimoto's thyroiditis with diffuse and strong expression of LGALS3 (galectin 3), CITED 1, KRT 19 (cytokeratin 19), FN 1 (fibronectin-1) and HBME 1 suggest the possibility of early, focal premalignant transformation in some cases of Hashimoto's thyroiditis. [13] Oncogenes RET/PTC1 and RET/PTC3 provide specific markers of the early stages of papillary carcinoma. Messenger RNA (mRNA) expression for the RET/PTC1 and RET/PTC3 oncogenes found in 95% of the Hashimoto's patients[14,15]. Several morphological, immunohistochemical, biomolecular aspects, and increased incidence of papillary thyroid in Hashimoto's thyroiditis patients might therefore indicate that Hashimoto's thyroiditis is a precursor of thyroid cancer. Hashimoto's thyroiditis patients should undergo careful clinical and technical follow-up. [16]

Until this time, this patient still do the periodically control dan the general condition is fine. In every Hashimoto's thyroiditis cases, carefulness microscopic examination was needed to find the possibility of coexistence with preneoplastic lesion. Further examination of molecular marker in this case also needed to support earlier theory of malignant transformation from Hashimoto's thyroiditis toward papillary thyroid carcinoma.

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ENHANCEMENT PHALLOPLASTY AND GIRTH ENHANCEMENT; IS IT AWAKE CRANIOTOMY FOR ELOQUENT AREA IN SANGLAH HOSPITAL–BALI: A CASE REPORT

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ABSTRACT

Awake craniotomy was performed as standard surgical approach for supratentorial intraaxial tumors, regardless of involvement of eloquent cortex. It allows for intraoperative brain mapping that helps identify and protect functional cortex. It also avoids the complications inherent in the induction of general anesthesia. We reported a case, male, 60 y.o. with brain tumor in right motor area, based on head CT Scan and MRI was suspected of high grade glioma. Patient came with headache and slight left hemiparesis. Informed concern and integrated management with anesthesia department was performed. Awake craniotomy and brain mapping were done, and near total tumor removal was done without complication. Histological pathology result was Glioblastoma Multiforme (GBM). Awake craniotomy is a practical and effective standard surgical approach for supratentorial tumors with a low complication rate, and provides an excellent alternative to craniotomy performed with the patient in the state of general anesthesia because it allows the opportunity for brain mapping and avoids general anesthesia.

Keywords: awake craniotomy, eloquent area, anesthesia

INTRODUCTION

The standard surgical approach for removal of a supratentorial intraaxial tumor is craniotomy performed with the patient in the state of general anesthesia. In the past, awake craniotomy was usually reserved for epilepsy surgery and removal of lesions (tumors and arteriovenous malformations) from areas of eloquent cortex.

Awake craniotomy was deemed necessary in these instances to allow the opportunity for brain mapping. Awake craniotomy is a useful technique for a variety of tumor locations, tumor histological characteristics, and patient presentations. The advantages of awake craniotomy over standard craniotomy performed with the patient in the state of general anesthesia include the opportunity for brain mapping, avoiding general anesthesia, and a low complication rate. Patients who undergo awake craniotomy are conscious at the end of the procedure, reducing their need for postoperative intensive care monitoring. The dysphoria seen in patients after general anesthesia wears off is absent, resulting in a short postoperative hospital stay. Monitoring devices used in patients in whom general anesthesia has been induced, such as indwelling urethral catheters, arterial lines, and central venous lines, are seldom necessary in patients undergoing awake craniotomy and the complications these monitoring devices cause are rarely seen. Brain mapping during tumor resection allows for maximum tumor resection while minimizing complications. Tumors that might be considered inoperable due to their proximity to eloquent cortex can often be completely removed when guided by brain mapping. Traditional formal awake craniotomy with brain mapping, as developed for epilepsy procedures, can be lengthy, increasing the time and cost of a procedure. We present a method of awake craniotomy and brain mapping for removal of supratentorial intraaxial brain tumors that is safe, practical, and economical.

OPERATIVE TECHNIQUE

Antibiotic, anticonvulsant, and corticosteroid medications were given immediately preoperatively. Patients were positioned in the supine to ensure patient

comfort so that the patient could remain in one position for the duration of the procedure. A patient was given rigid head fixation (Sugita headrest; Mizuho Medical Co., Tokyo, Japan) after placement of a local anesthetic agent (zylocaine) at the pin sites. Sterile draping was arranged to give the anesthesiologist constant access to the patient's face, arm, and leg. The incision was infiltrated with 0.25% marcaine as a regional field block. A patient was administered oxygen via a nasal cannula and attached to cardiac and oxygen saturation monitors. Foley catheters, radial arterial lines, or central venous lines were placed. During the opening and closing of the craniotomy, short-acting sedative agents, such as propofol, midazolam, and fentanyl were administered intravenously by the anesthesiologist to keep the patient comfortable.

With the pial surface exposed, the location of the lesion was verified. During cortical stimulation, the patient was observed by the anesthesiologist and movements of the face, arm, or leg were reported to the surgeon. A corticotomy was made, avoiding all sites identified as eloquent cortex by cortical mapping and the lesion was removed in the usual fashion. Gross-total excision was attempted; however, areas identified as functional were spared, even when grossly involved by tumor.

ILLUSTRATIVE CASES

Male 60 years old is fully alert, with chronic headache and slight weakness on the left side body. *CT Scan* and MRI were suspected of high grade glioma. Informed concern and integrated management with anesthesia department was performed, patient must thoroughly understand all the procedures to be performed, ranging from the placement position, operating procedures, and instructions for operation. The patient should willing to work together and can communicate with the surgical team, to be able to follow instructions and directions during the operation. Moment *cortical mapping*, patients must be comfortable and follow all instructions well. Patients who are not fully conscious, agitation and have a communication barrier is not suitable for this procedure. This is the flaws and limitations of this procedure.

At the time of awake surgery, brain mapping was done. Tumor successfully removed near total under a microscope. Pathology results showed a glioblastoma multiforme. After surgery the headache and weakness were improved, and medication continued by radiotherapy.

DISCUSSION

Brain mapping during the removal of intraaxial tumors allows the surgeon to choose a safe corridor to a tumor and facilitates maximum resection with decreased risk of morbidity. Tumors in areas of eloquent cortex that might otherwise be deemed unresectable can be excised with the aid of intraoperative brain mapping. In addition to mapping the cortical surface, stimulation techniques can be used to map and preserve areas of eloquent sub cortical white matter such as descending motor pathways.

Some authors have described the use of electrocorticography to detect and, subsequently, resect perilesional seizure foci to reduce seizure frequency. The traditional maxim that by debulking a tumor from within, new neurological deficits can be avoided is not valid because both high- and low-grade gliomas have been reported to contain functional tissue, including some tumors that consisted principally of functional tissue. Reliable identification of rolandic cortex by anatomical means is not possible intraoperatively. In addition, the primary motor area may extend more than 20 mm anterior to the rolandic fissure in patients with intraaxial tumors, making anatomical identification inadequate for safe resections in this area. Language cortex cannot be reliably localized on anatomical criteria alone, and there are no good methods for the determination of cortical sites essential for language in a patient in the state of general

anesthesia. Cortical sites essential for language are very different from the traditional Broca–Wernicke's model, and there is substantial variation between individuals as to the exact location of essential language cortex, making anatomical criteria inadequate for safe resection of dominant dominant hemisphere perisylvian lesions. The techniques of formal brain mapping developed for epilepsy surgery require a large cortical exposure and can add significantly to the length of the procedure. Minimum access incisions without exposure of additional cortex and quick brain mapping are sufficient to provide safety during tumor excision in a method quite distinct from the generation of functional brain maps during epilepsy surgery.

CONCLUSION

Awake craniotomy with brain mapping is an excellent, practical surgical approach to treat supratentorial intraaxial tumors regardless of the involvement of eloquent cortex. Awake craniotomy allows for brain mapping and avoids general anesthesia. It can be used for tumors in varying locations and with different histological characteristics. It is well tolerated by patients with various concurrent medical problems and various clinical presentations. Awake craniotomy carries low morbidity and mortality rates and minimizes ICU use and total hospital stay.

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TRITERPENOID SAPONIN ANTITUMOR COMPOUND OF *SAMBUNG NYAWA* (*Gynura procumbens* [Lour.] Merr) LEAVES

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ABSTRACT

This paper describes the isolation and identification of saponin compounds from sambung nyawa leaves (*Gynura procumbens* [Lour.] Merr). An amount of 41.64 g of concentrated extract was resulted from 1000 g dry powder of *sambung nyawa* leaves macerated in methanol. This extract was dissolved in the mixture of methanol-water (7:3) and then was partitioned with n-hexane and n-buthanol respectively. It was evident that only n-butanol extract contained saponin. Separation of crude saponin using column chromatography resulted in six fractions (F_A – F_F) and the fraction of F_D was observed to contain saponin. Further purification of F_D fraction using washing technique with ether resulted in a relatively pure to TLC isolate. Infra red and UV-vis spectroscopy were employed in order to identify the F_D fraction (isolate). The result of the identification showed that the isolate were of triterpenoid saponin compounds which contained functional groups such as –OH (3435.8 cm^{-1}), -CH aliphatic (2908.0 cm^{-1}), -C=C- conjugated with carbonyl (1636.3 cm^{-1}), -CH_3 bending (1384.4 cm^{-1}), and -C-O alcohol (1074.4 cm^{-1}), and these compounds absorbed at 278.2 and 324.5(nm). The isolate was found to be toxic toward Artemia salina Leach with the LC_{50} = 239.88 ppm and was able to inhibit the growth of tumor on the plant test due to *Agrobacterium tumefaciens* A-208 up to the 11th days observation at only 10 ppm concentration.

Keywords: *Gynura procumbens* [Lour.] Merr., isolation, identification, toxicity, antitumor

INTRODUCTION

Screening of bioactive compounds from plant can be conducted with the phytopharmacologic approaches and phytochemical screening approaches. One of the phytopharmacologic approaches relies on etnobotany, screening bioactive compound of plant based on its use as traditional medicine by certain society (Farnsworth, 1996). Traditional medicine represents one of Indonesia's cultural assets and has been empirically proven from generation to generation (Kardinan and Taryono, 2003).

A plant which can be exploited as traditional medicine is sambung nyawa (*Gynura procumbens* [Lour.] Merr). The leaves of this plant is applicable to heal the fever disease, bronchitis, sine, tonsil, husk (abscess, scabby, husk rash), singe, noxious animal bite, bronchitis, cough, adenoid, kidney disparity, dysentery, high cholesterol, blood-vessel acid, hypertension, diabetes, tumor, and cancer (Kardinan and Taryono, 2003; Suharmiati and Maryani, 2003; Mahendra, 2005; Anonim, 2003; Utami, 2005). This is possibly because of the chemical compounds content of the leave, especially those of secondary metabolic compounds such as saponin, flavonoid (7,3,4'-trihidroxyflavanon), alkaloides, triterpenoides, essential oils, quisertine glycoside, fenolic acid (like cafeic, penta-cumaric, penta-hydroxy benzoic, and vanilic acids), and tannin. Nevertheless there is no research explaining the correlation between the compounds in the leaves with the traditional healing, although many people have proven the effect and benefit from sambung nyawa leaf (Kardinan and Taryono, 2003; Suharmiati and Maryani, 2003; Mahendra, 2005; Anonim, 2003).

The result of phytochemical screening to methanol extract of the leaves shows that it contains saponin (major content), triterpenoid, and flavonoid compounds. Various bio-activities are related to saponin compound, for example ,antifungal, antimicrobial, antiinflamation, antisclerotic, and antitumor activities (Hostettmann, and Marston, 1995;

Mamaki and Sashida, 1996, Ohtani *et al*., 1993, Marston *et al*., 1993; Robinson, 1991). According to chemotaxonomy approach, plants from the same genus contain substances with similar chemical structures and bio-activities. It is known that leaves of tumbuhan dewa (*Gynura Pseudochina*), posses an anti-tumor activity (Turana, 2003), hence sambung nyawa leaf (*Gynura procumbens* [Lour.] Merr) is expected to contain a compound which has the same activity, since both plants fall in the same genus. Based on the traditional medicine practices and chemotaxonomy approach sambung nyawa can suppress tumor. Moreover, results of phytochemical screening tests indicate that sambung nyawa leaves mainly contain saponin compounds. Therefore it is crucial to investigate the chemical contents of the sambung nyawa leaf and their bio-activity as an antitumor agent. In this paper the isolation process of saponin compounds from the leaves and the anti-tumor activity is described.

MATERIALS AND METHODS

Materials

Materials used in this research are: sambung nyawa (*Gynura procumbens* [Lour.] Merr) leaves, obtained from Karangasem Bali and taxonomycally identified by LIPI's Kebun Raya " Eka Karya" Bali. Chemicals used are methanol (p.a and technical), technical n-hexane, n-butanol (p.a and technical), ether p.a, chloroform p.a, hydrochloric acid, concentrated sulphuric acid, anhydrous acetic acid, ethanol 70%, blood in potassium EDTA 1%, NaCl 0,9%, potassium bromide, aquadest, sea water, yeast, DMSO, larvae of prawn of *Artemia salina* Leach, bacterium of *Agrobacterium tumefaciens* A-208, cocor bebek plant (*Kalanchoe pinnata* [Lamk.], silica gel 60, and silica gel GF_{254}.

Equipments used include a set of glass wares, analytical balance, blender, knife, rotary vacuum evaporator, UVs lamp (254 and 366 nm), thin layer and column chromatographs, desiccators, test tubes, testing dishes, centrifuge, Pasteur pipettes, volumetric pipettes, glass basin/aquarium, black plastic, sterile toothpick, spectrophotometer of UV-VIS Secoman S 1000 PC and spectrophotometer of Perkin Elmer FT / IR-5300.

Methods

One kilograms dried powder of sambung nyawa leaves (*Gynura procumbens* [Lour.] Merr) was macerated using 2 L methanol (MeOH). Maceration process was conducted 4 times at 24 hours each. The methanol extract was evaporated using rotary vacuum evaporator to obtain a concentrated MeOH extract. The MeOH extract was dissolved in a mixture of MeOH-H_2O (7:3). The non polar components was fractionated using n-hexane. Furthermore MeOH-H_2O extract is fractionated using 700 mL n-butanol. Each fractions was then evaporated to obtain concentrated water extract and n-butanol extract. Each concentrated extract was tested for saponin content with the foam test. Furthermore the concentrated n-butanol extract was dissolved in 8 mL of methanol and ether was added until precipitate formed. It was tested with the foam to ascertain the content saponin. Precipitates containing saponin was fractionated by column chromatograph using 100 g of silica gel 60 and a methanol-chloroform (3:1) mixture as the mobile phase. Each fraction was tested by the foam test and those containing saponin were pooled and purified to obtain a relative pure isolate. It was once again tested against foam test, the blood hemolysis, and the sapogenin test. It was then analysed using UV-vis and Infrared spectrophotometers, and its toxicity was tested on larva of *Artemia salina* Leach. The antitumor activity was tested against *Agrobacterium tumefaciens* A-208.

RESULTS AND DISCUSSION

The result of maceration about 1000 g dried powder of *sambung nyawa* (*Gynura procumbens* [Lour.] Merr) leaf using total 8 L methanol was about 41.64 g green concentrated methanol extract containing saponin (result of foam test). After fractionation 5.10 g of concentrated n-butanol extract and 6.72 g of concentrated water extract were resulted.

Foam tests showed that both extract contain saponin, but the n-butanol extract contains more saponin than the water extract. After excessive addition of ether 3.97 g of precipitates which is suspected as crude saponin was formed. Separation of 2.00 g of the crude saponin using column chromatograph yields 6 fractions (Faction A-F). Foam test to these sixth fractions showed that only fraction D contains saponin. The fact that fraction D contain saponin is supported by the result of haemolysis blood test. Furthermore separation of fraction D which is consisted of 2 compounds was repeatedly conducted with the wash technique using ether solvent so that an isolate with single stain or relatively pure was obtained. Purity test was carried out by TLC at various mobile phases of methanol-chloroform (3:1), methanol-ether (3:1), chloroform-acetic acid-water (4:2:1), methanol-acetic acid-water (3:1:1), and n-butanol-acetic acid-water (4:1:5) mixtures.

The result of phytochemical test indicated that the isolate contained the saponin compound with sapogenin as triterpenoid which is shown by the colour change of isolate from yellowish brown to redish orange. Infrared spectra indicate that the isolate possibly contain some functional groups like -OH (3435 cm^{-1}) which is supported by the absorption at 1074,4 cm^{-1} for the C-O alcohol bond. Aliphatic C-H group with sharp absorption is appear at 2908,0 cm^{-1} and it is strengthened with the sharp absorption at 1384,4 cm^{-1}. C=C Bond of alkenes is shown by absorption at 1638 cm^{-1} which is possibly conjugated with C=O carbonyl group. Analysis using the spectrophotometer UV-vis shows that the isolate absorbed at 278.2 nm and 324.5 nm. Two absorptions at 278.2 nm and 324.5 nm are due to $\pi \rightarrow \pi^*$ transition of C=C alkenes which is conjugated with carbonyl, and $n \rightarrow \pi^*$ transition that is caused by a chromophor C=O. This suggestion is supported by the appearance of C=C bond which is conjugated with C=O bond on the infrared spectra.

Result of toxicity test shows that the isolate is toxic at 79.43 ppm, at which concentration it kills 50% of the *Artemia salina* Leach (LC_{50} = 79,43 ppm). It is categorized as very toxic and has positive correlation with antitumor, because it is smaller than 1000 ppm. Furthermore, the antitumor activity test using *Agrobacterium tumefaciens* A-208 indicates that the isolate is able to inhibit the tumor growth during 11th day at 10 ppm (lowest concentration), and at 100 and 1000 ppm are able to inhibit the tumor growth until 34 days. Therefore it can be concluded that the isolate has a great potency as antitumor agent.

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PHAEOCHROMOCYTOMA: A CASE REPORT OF A RARE ADRENAL TUMOR CAUSING HYPERTENSION

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ABSTRACT

Phaeochromocytoma is a rare fungsional tumor, arising from the chromaffin cells of the sympathoadrenal system. The tumor gives specific symptoms such as hypertension and retroperitoneal tumor. Catecholamine test can lead the diagnosis of this tumor and imaging study, CT scan or MRI, can confirm the anatomical location of the tumor. Histopathology and immunohistochemistry examination can confirm the diagnosis. We report a case of 39 year old man with abdominal tumor with paroxismal hypertension and palpitation. CT scan revealed well-defined mass in medial liver. Pathology examination of biopsy tissue was concluded as paraganglioma. According to durante operation findings and pathological examination, the tumor was diagnosed benign phaeochromocytoma. There was no metastasis found on clinical and imaging examination. In a young patient with a retroperitoneal mass and hypertension, should rise a suspicious of a functional phaeochromocytoma. Laboratory test, imaging, and pathologic examination can established the diagnosis.

Keywords: phaeochromocytoma, adrenal tumor, hypertension.

INTRODUCTION

Phaeochromocytoma is an adrenal tumor, arising from the chromaffin cells (phaeochromocytes). The tumor is rare with incidence of between 0.4-0.9 per 1,000,000.[1] Phaeochromocytoma (adrenal paraganglioma) is one of paraganglia system tumor group, which is arising from aorticosympathetic paraganglia.[2]

The tumor gives specific symptoms such as hypertension and retroperitoneal tumor. Catecholamine test can lead the diagnosis of this tumor and imaging study, CT scan or MRI, can confirm the anatomical location of the tumor. Histopathology and immunohistochemistry examination can confirm the diagnosis.

Although has a specific symptoms and laboratory and imaging test can make a clinical diagnosis, rarity of tumor can cause a missing diagnosis. This is the first case of phaeochromocytoma found and reported in Bali, with clinicopathological review.

CASE

A 39 year old man came to emergency room with abdominal discomfort. He felt an abdominal mass since 2 years ago. History of hypertention was not recognized. Physical examination revealed an upper right retroperitoneal mass and palpitation. Blood pressure was 200/160 mmHg and ECG test showed sinus tachicardia. CBC were within normal limit, BS 379 mg/dL, urine glucose +4. CT scan showed an inhomogen solid mass in adrenal area (Fig. 1).

Pathology examination of biopsy tissue was concluded as paraganglioma. Open surgery performed. Durante surgery, the blood pressure increased and then decreased suddenly after tumor removal. Tumor located in right adrenal. Macroscopically, the tumor was encapsulated, measured 15x10x6 cm, brown, with soft consistency. (Fig. 2)

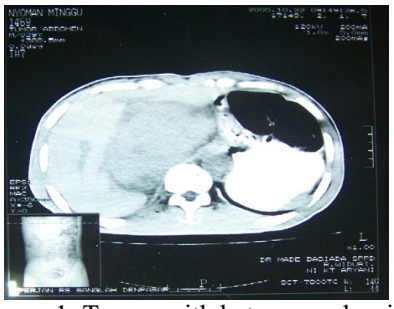

Figure 1. Tumor with heterogen density.

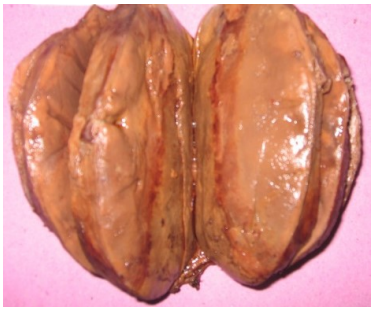

Figure 2. Encapsulated, brown, homogenous tumor.

Microscopically, tumor consist of cells with round nuclei, with mild pleomorphia. Cytoplasm was granular eosinophyl. The cells were arranged in nests seperated by thin fibrovascular tissue. Mitosis are sparse. There were also some hyaline globule found. No evidence of capsular and vascular invasion. Tumor cells were stained brown with chromogranin immunostaining. (Fig. 3) The tumor was concluded a phaeochromocytoma.

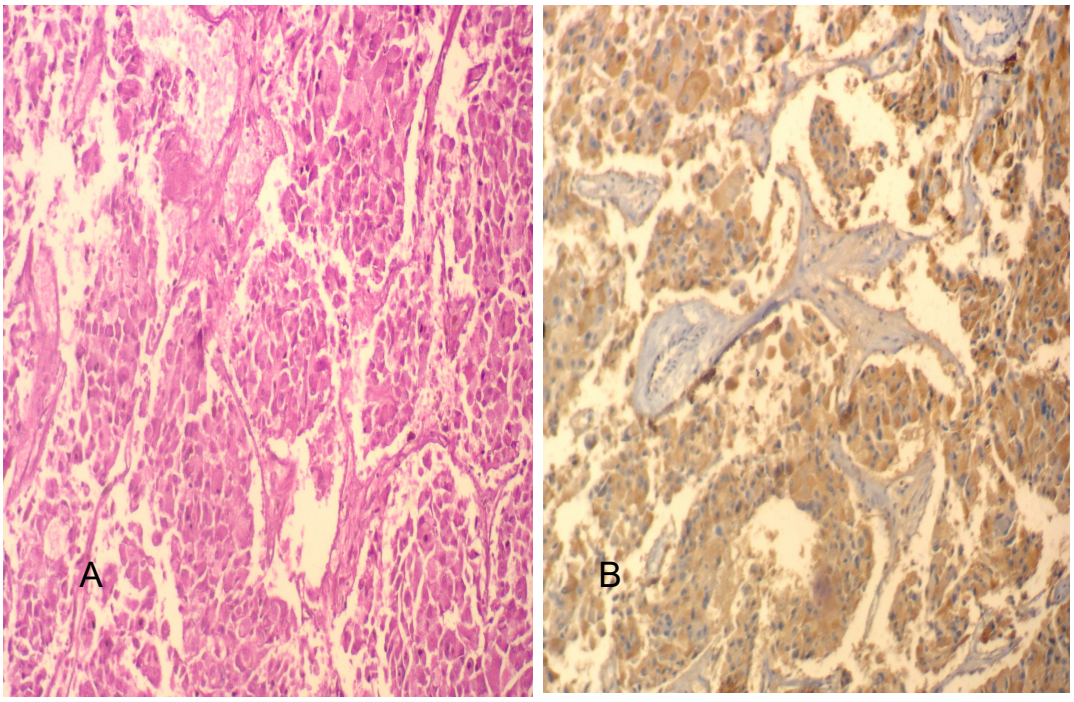

Figure 3. A. Phaeochromocytes in cell nests. B Chromogranin immunostaining

DISCUSSION

Phaeochromocytoma is a cathecolamine-secreting adrenal tumor. Hypertension or paroxysmal hypertension is a characteristic sign. Phaeochromocytomas were found in less than 1% hypertension patients.[3] Other symtoms are headache (80%), extensive perspiration (71%), and palpitation (64%). This tumor could also gives other endocrine symptom if the tumor secreting other polypeptide.[4] In this case revealed paroxismal hypertension, palpitation, hyperglicemia, and hyperglucosuria. In fungsional tumor, clinical diagnosis can be made by measuring cathecolamine and its metabolits, which was not measured in this case Tumor location can be confirmed by CT scan or MRI.[1,5,6]

Phaeochromocytomas are usually confined to the adrenal gland, and may appear encapsulated. Tumor are typically 3 cm to 5 cm in diameter but can be more in diameter.[1] The tumor in this case is large, encapsulated, confined to adrenal gland. Microscopic examination usually shows predominantly alveolar, nest-like (Zellballen) architecture, separated by a thin fibrovascular septa. Occasionally, solid or diffuse pattern, or spindle cell component can be found. The tumor cells typically resembles normal chromaffin cells. Intracytoplasmic hyaline globules occur commonly. Phaeochromocytomas are positive for chromogranin, the most reliable marker for discriminating them from adrenal cortical tumor and metastatic tumors that are not neuroendocrine.[1,6-8] Typical features of phaeochromocytoma was found in this case.

Malignancy were reported in 2-5% of the cases. Most of the cases were metastasis to regional lymphnodes, lung, vertebra, and intracranial[9]. The most reliable criteria for malignancy is metastasis. A retrospective study proposed a scoring system to evaluate malignant potency of tumor.[10] This system evaluate evidence of diffuse pattern, central necrosis or confluent, hypercellularity, monotony, spindle cell, mitosis >3/10HPF, atypical mitosis, extention to fat tissue, vascular invasion, severe pleomorphism, and nuclear hiperchromatism. The first eight feature scored 2 each and the rest scored 1. Tumor has malignant potency if total score more than 3. Another study that performed flow cytometry examination concluded aneuploidy related to metastasis potency. There was no metastasis found on clinical and imaging examination of this case. Based on scoring system, this case concluded tumor with benign potency.

CONCLUSION

Morphology and immunohistochemistry evaluation concluded this case was a benign phaeochromocytoma. Young patient with a retroperitoneal mass and hypertension, should rise a suspicious of a functional phaeochromocytoma. Laboratory test, imaging, and pathologic examination can established the diagnosis.

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ADHERENCE OF *BIFIDOBACTERIUM* ISOLATED FROM INFANT FECES TOWARDS *SALMONELLA TYPHI* ON ENTEROCYTE BALB/c MICE

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ABSTRACT

Research concerning of management, prevention, and medication of the disease have been continually improved. The aim of this research is searching *Bifidobacterium* sp isolated from infant feces and determining the adherence competition of this *Bifidobacterium* towards *S. typhi* on enterocyte BALB/c mice. Isolation of *Bifidobacterium* from infants feces was carried out following Beeren (1990) in Hadadji, et. al., (2005). Fecal suspension on NaCl solution with cystein-HCl was inoculated on broth media. The grown Bifidobacteria were separated using solid broth media. The clonal growth were then selected for identification. Nagayama (1995) method was then employed to observed inhibition adherence of *Bifidobacterium* towards adhesion of *S. typhi* on enterocyte BALB/c mice. In this experiment, it was obtained that the bacteria isolated from infants feces are *Bifidobacterium* sp. This can be seen clearly from the characteristic of the bacteria as a short rod with Y and V shape. Data show adhesion indices of *Bifidobacterium* sp on enterocyte is 1950. This means that 19.5 bacteria were adhered on 1 enterocyte-cell BALB/c mice. The adhesion model of *Bifidobacterium* sp on enterocyte is diffuse adhesion. On the other hand, adhesion indices of *S. typhi* on the present of *Bifidobacterium* sp on enterocyte is 1504. Conclusions that can be drawn from this research are the finding of *Bifidobacterium* sp isolated from infant feces and this bacteria have an ability to adhere on BALB/c mice enterocyte-cell with diffuse adhesion model. *Bifidobacterium* sp obtained has also an ability to compete towards adherence of *S. typhi*. Future work that can be carried out are further researches concerning of the use of this bacteria as an anti adhesion towards many pathogen bacteria. In addition, further study should be carried out in order to obtain whether adhesin protein can be extracted from these bacteria.

Keywords; diarrhea, *Bifidobacterium*, enterocyte, adhere, and diffuse adhesion.

INTRODUCTION

It was well established that there are much more bacteria including diarrhea bacteria are resitance towards antibacterial. This condition has endorsed researches for establishing research to obtain an alternative cure to replace antibacterial that has already used clinically.

The aim of this research is searching *Bifidobacterium* sp isolated from infant feces and determining the inhibition adherence ability of this *bifidobacterium* towards *S.typhi* on enterocyte BALB/c mice.

METHODS

This is an explorative research to elaborate isolation and characterization of *Bifidobacterium* isolated from infants feces and determined inhibition ability of this bacteria towards adherence of *S.typhi* on enterocyte BALB/c mice. Isolation was carried out following Beeren, (1990) in Hadadji (2005). Enterocyte isolation was carried out based on Weisler method. Nagayama method was employed for testing the adhesion of *Bifidobacterium* on enterocyte and inhibition of this *bifidobacterium* towards adhesion of *S.typhi*.

RESULTS AND DISCUSSION

Isolation of *Bifidobacterium* from Infants feces

Isolation of *Bifidobacterium* from infant feces was carried out following Beeren, (1990) in Hadadji (2005). Fecal suspension on NaCl solution with cystein-HCl was inoculated on broth media. The grown Bifidobacteria were separated using solid broth media. The clonal growth were then selected for identification (Figure1).

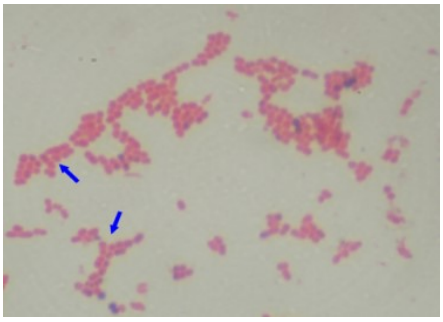

Figure 1 *Bifidobacterium* sp isolated from infants feces indicated by short rod with Y and V shape (1000 X).

Adhesion of *Bifidobacterium* sp on Enterocyte

Nagayama method was employed to observed adhesion of *Bifidobacterium* on enterocyte BALB/c mice (Figure 2 and Table 1).

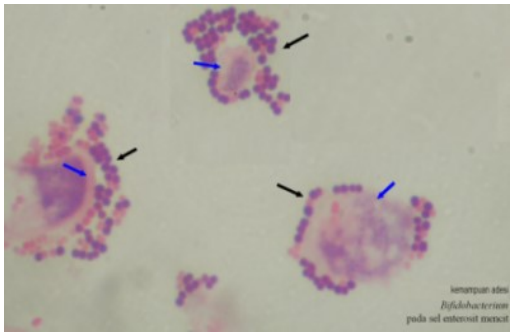

Figure 2 Adhesion of *bifidobacterium* on enterocyte,
→ *bifidobacterium* →enterocyte
(zoom of 1000 X).

Adhesion indices of *Bifidobacterium* sp on enterocyte were listed on Table 1.

Table 1 Adhesion indices of *Bifidobacterium* sp on enterocyte

No	Number of *Bifidobacterium* per 100 enterocyte	Number of *Bifidobacterium* sp per enterocyte
1	2000	20
2	1900	19
3	1800	18
4	2100	21
5	2000	20
6	1900	19
Average	1950	19.50

It can be seen from Figure 2, that the adhesion model of *Bifidobacterium* sp on enterocyte is diffuse adhesion. This model was marked by the bacteria are homogenously spread out on cell surface.

Adhesion of *S.typhi* on Enterocyte

Nagayama method was also applied to determine adhesion ability of *S. typhi* on enterocyte BALB/c mice. The adhesion model was presented on Figure 3.

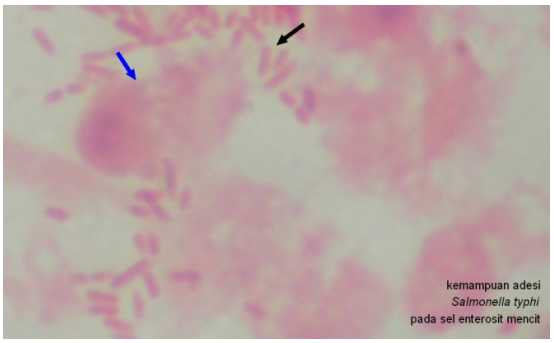

Figure 3 Adhesion of *S. typhi* on enterocyte, → *S. typhi*
→ Sel enterosit (1000 X).

Figure 3 reveals that ahesion type of *S.typhi* on enterocyte is a local adhesion indicates by *S. typhi* in rod-like shape was adhered in a group of rod soround the enterocyte receptor.

Inhibition ability of *Bifidobacterium* towards adhesion of *S. typhi* on enterocyte

Inhibition ability of *Bifidobacterium* sp towards adhesion of *S. typhi* on enterocyte BALB/c mice was tested following Nagayame method, in which all of the bacteria including *Bifidobacteria* sp and *S.typhi* were mix together on enterocyte. The result obtained is presented on Figure 4.

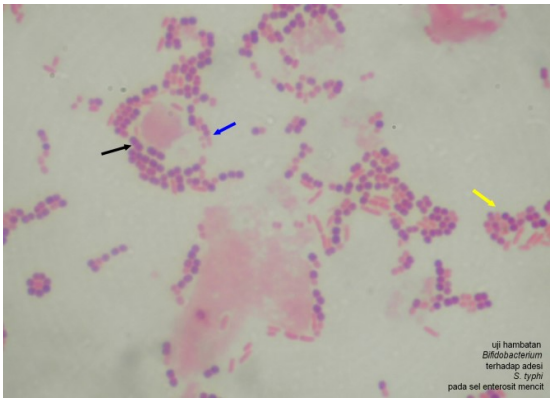

Figure 4 Inhibition ability of *Bifidobacterium* sp towards adhesion of *Salmonella typhi* on enterocyte
→ *Bifidobacterium* → *Salmonella typhi*
→ Inteaction of *Bifidobacterium* and *S. typhi* (1000 X).

CONCLUSION

1. Bacteria isolated from infants feces were exactly *Bifidobacterium* sp as indicated by their short rod like and V and Y shape.
2. Bifidobacterium sp isolated from infants feces has an ability to inhibit adhesion of Salmonella typhi on mice enterocyte.

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CRI DU CHAT SYNDROME IN A ONE YEAR AND THREE MONTHS OLD BALINESE GIRL

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ABSTRACT

A case has been reported in Denpasar; a one year and three months old girl diagnosed with cri-du-chat syndrome. Cri-du-chat syndrome is caused by haploinsufficiency of the genes on the distal part of the short arm of chromosome 5. Her chromosome study from peripheral blood showed a 46,XX,del (5) (p14p15) karyotype. She has high-pitched cat like cry as a hallmark along with other specific clinical features. From physical examination, there is a microcephaly, distinct facial dysmorphism, round face, hypertelorism, down turned corners of the mouth, micrognathia, dental malocclusion, and single palmar crease. She has mild malnutrition and a global developmental delay. During her neonatal period she experienced a cyanotic crise and failure to breastfed. She has been receiving physiotherapy and speech teherapy since and her motoric and language development (especially receptive) has been improved. We also analyzed her dietary needed and ensured that adequate nutrition was given by her parents.

INTRODUCTION

A one year and three months old Balinese girl, who first attended the Pediatric clinic with the unability to sit when she was 11 months old. The patient was unable to sit by herself and need her parents to support her back every time she wants to sit. She was able to lie on her stomach at five months old but was unable to roll back to lie on her back, at seven months she was able to raise her head. After she was eleven months old and showed a difficulty to sit then her parents started to worry about her motoric skills and decided to see an expert. At one years old she could only say two words "papa" and "yaya" without meaning, but she could turn her head to a sound and also wave her hand. Her social-personal development was also delayed because she couldn't tell her wish and sometimes lose temper if she didn't get what she wants but she never hurt herself or others. Because she had delayed achievement of three developmental milestones (e.g gross motor skills, speech or language skills, and social-personal skills) we assessed a presence of a global developmental delay.

She had a high-pitched cat like cry that first known when she was one month old, her parents describe her cry was like "a strange, small, unusual and sounds like a cat's cry", but this specific cry had eventually stopped when she got older. During her neonatal period she experienced a cyanotic crise and showed difficulty being breastfed but it stopped spontaneously when she was one month old.

During the pregnancy, her mother was in the good condition with good physical examination (e.g normal blood pressure, no hyperemesis) and she never took any other medicine except the ones that her obstetrician gave her. The patient was born after the caesarian delivery, with aterm and normal birth weight but was not immediately cry and had green-colored amniotic fluid.

Reccurent respiratoric infections appeared during her first year of life and one of them made her hospitalized when she was 10 months old with symptoms of acute respiratoric infection. In contrast with respiratoric infection, she seldom suffered from intestinal infection. She was the only child with both healthy parents and no report about this rare condition apppear in another member of extended family. She has a good appetite but her parents chose to postpone giving her adult's food because they were concerned about her difficulty in chewing foods.

From physical examination, the patient was alert and had regular pulse rate of 104 beats per minute. The respiratory rate was regular at 28 beats per minute, axillary temperature was 36,3ºC, and her head circumference was 41 cm (below 5th pecsentile with CDC curve, 2000), her weight was 8 kilogram (0-25 percentile with WHO Antro weight for age, 2006), her height was 74 cm (0-25 percentile with WHO Antro height for age, 2006), her weight for height suited to 0-25 percentile with WHO Antro 2006 weight for height, her nutritional status was between -1 SD until -2 SD (mild malnutrition according to WHO Antro, 2006).

The patient presented with microcephaly, distinct facial dysmorphism, round face, hypertelorism, down turned corners of the mouth, micrognathia, dental malocclusion. Cardiac examination didn't show any abnormality wheter from physical or chest x-ray, neither did renal examination. Examination of the upper extremities showed a single palmar crease. Laboratory investigation of her chromosome study from peripheral blood showed a 46,XX,del (5) (p14p15) karyotype, the deletion from short arm to the 5 region p14p15 caused an abnormality named Cri du Chat Syndrome.

Based on these findings, we diagnosed her with Cri du Chat Syndrome (CdCS). The patient managed with outpatient care, with routine visit to growth and development clinic to monitor her progress or any complication that may occur by maintaining close colaboration with parents. She has been receiving physiotherapy and speech therapy where her motoric and language development (especially receptive) has been improved. We also analyzed her dietary needed and ensured that adequate nutrition was given by her parents.

DISCUSSION

Cri du Chat syndrome (CdCS), also known as 5p syndrome, is a rare genetic syndrome that was first described in 1963 by Lejeune et al[1]. The French name of this syndrome translates to "cry of the cat"; the syndrome was named for the characteristic high-pitched cry that is usually heard during early infancy among affected neonates. CDC syndrome affects 1 of 15 000 to 45 000 live births and may be one of the most common deletion syndromes[2,3]. The variability of the clinical symptoms and developmental delays may be related to the size of the deletion of the 5p arm[4]. Our patient first complained with unability to sit by herself when she was 11 months old, but from our denver developmental sreening test II we assessed presence of a global developmental delay because of delayed achievement of three developmental milestones (e.g gross motor skills, speech and language skills, also social-personal skills). In general, individuals with CdCS have delayed speech and language development, and some never develop spoken language. Their receptive language is better than their expressive language, although both are delayed[5]. In our case she showed a global developmental delay including speech delay, and also social-personal delay.

In most cases the clinical features, in particular the distinct facial phenotype, in combination with the typical cat-like cry and hypotonia, allow to suspect the diagnosis since birth and confirmed by a karyotype analysis[4]. In doubtful cases it will be possible to perform the molecular cytogenetic analysis (Fluorescence in situ hybridization or FISH) in order to define the diagnosis[4]. Apparently, "the cry of the cat" didn't recognize by parents although they describe it was like "a strange, small, unusual and sounds like a cat's cry".

The characteristic cat-like cry is probably due to anomalies of the larynx (small, narrow, diamond shaped) and of the epligottis (flabby, small, hypotonic) annd to neurological organic and functional alterations[5]. Studies by Overhauser and colleagues in 1994 determined that deletions of 5p15.2 were correlated with facial dysmorphism and developmental delays and that deletion of 5p15.3 was related to the presence of the characteristic cat-like cry[6]. Additional analyses published by Zhang et al in 2005

localized the region of the cat-like cry to 5p15.31, speech delay to 5p15.32 to 5p15.33, and facial dysmorphism to 5p15.2 to 5p15.31[7].The karyotype analysis of our patient showed a 46,XX,del (5) (p14p15) karyotype, the deletion from short arm to the 5 region p15 involved the high-pitched cry which also report by the parents, it also explained her speech delay. The deletion of short arm to the 5 region p14 involved the remaining features of our patient such as with microcephaly, distinct facial dysmorphism, round face, hypertelorism, down turned corners of the mouth, micrognathia, and dental malocclusion. Malformation such as cardiac, renal, neurological abnormalities seldom find in CdCS patients[4], which is consistent with our physical examination and the normal chest x-ray of the patient. Christine Hills et al, 2006, reported that there is no clear understanding of the genomic cause of the prevalence of these defects in the population with CdCS, although congenital heart disease has been noted among patients with other deletion syndromes[8].

The CdCS patients for all ages, median head circumference and weight are near or below 2nd and 5th percentile, respectively. Difficulties in feeding and gastroesophageal reflux, frequently reported during the first year of life, can be the cause of the low weight[6]. Our patient has microcephaly and also mild malnutrition but she also has a good appetite, this fact probably because less intake than necessity according to recommended dietary allowances (RDA), despite her inability to chew. Therefore, we analyzed her dietary needed and recommended to increase the volume or the frequency of milk intake. If it can't be done, the next step is replacing the formula milk to high density formula milk. We also ensured that adequate nutrition was given by her parents.

There is no treatment in a strict sense from the word of CdCS, since the cerebral damage due to genetic mutation occurs in the early stages of the embryonal development. Nevertheless it is possible to the act on the consequences of the genetic alteration, through rehabilitative and educational interventions[4]. To provide her needs we have been colaborating with the parents to do physiotherapy and speech therapy, which made her motoric and language development (especially receptive) become improved. A study from Cornish, 2002, reported the cognitive profile of the CdCS childrens includes a relative strength in receptive language ability while moderate to severe learning disability remains a core characteristic. If developed appropriately at a developmentally critical period, enhanced communication skills could contribute to a reduction of the high rates of behavioural problems currently associated with the condition[10].

A study from Collins and Cornish, 2002, highlighted three core behaviours: headbanging, hitting the head against body parts, and self-biting, all reaching a plateau in late childhood and then remaining constant throughout early adulthood. Additionally, studies have also found a high correlation between the prevalence of self-injurious behaviour and stereotypic behaviour in this syndrome[11]. Our patient sometimes lose temper if she didn't get what she wants but she never hurt herself or others, it is important to understand the reason behind such behaviour and one possible reason because she, as well as other CdCS patients, has an absence of expressive language and substantially reduced communicative skills.

A study from Cerruti et al, 2006, about natural history of CdCS syndrome reported mortality 6.36%, has decreased from 9.67% in 1978 by Niebuhr. Mortality in patients with unbalanced translocations resulting in 5p deletions was higher than in those with isolated deletions ($P < 0.05$) as already observed by Wilkins[12].

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CORRELATION BETWEEN THE DEGREE OF DIABETIC FOOT ULCER AND THE PERCENTAGE OF $CD4^+$ CARRYING CASPASE-3*

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ABSTRACT

High prevalence of diabetic foot ulcer (DFU) and hardly healing foot ulcer indicate an ongoing process of immun cells lyses. The molecular mechanisms of these two conditions are also not clearly understood. This study aims to determine a correlation between the degree of DFU and persentage of $CD4^+$ cells bearing caspase-3 (CPS3).An observational study within cross sectional analytic study design was adopted in this study. Samples consisting of serum and tissues from diabetic were collected from patient admitted to Public and Private Hospitals in Denpasar, Badung, Tabanan and Gianyar Regencies. In this study, from 91 patients, it was observed that the average age of patients was (51.54 ± 6.51) years and HbA_{1C} percentage was (9.59 ± 3.51) %. Percentages of $CD4^+$ cells bearing CPS3 on the bases of DFU degree (2, 3, 4, and 5) obtained are 55.63, 77.77, 79.95 and 86.83%, respectively. In this study, we obtained that there are moderate positive correlation between DFU degrees and percentages of $CD4^+$ cells bearing CPS3 ($r = 0.60$, $p< 0.05$). Conclusions that can be drawn from this research are the finding of moderate positive correlation between DFU degrees and percentages of $CD4^+$ cells bearing CPS3. Future works that can be carried out are further researches concerning of the cause and effect between DFU degrees and $CD8^+$ cells bearing MDA and CPS3.

Keywords; diabetic foot ulcer, immune cell lyses, $CD4^+$ cells bearing CPS3

INTRODUCTION

Molecular mechanisms of ongoing cells lyses on diabetic foot ulcer (DFU) were not clearly understood. Many researches concerning of these mechanisms have already carried out worldwide.

The aim of this research is to prove a correlation between severity of DFU and percentage of $CD4^+$ cells bearing caspase-3 (CPS3).

METHODS

An observational study within cross sectional analytic study design was adopted in this study. Samples consisting of serum and tissues from diabetic foot were collected from 91 patients. The samples were collected from patient admitted to Public and Private Hospitals in Denpasar, Badung, Tabanan and Gianyar Regencies.

RESULTS AND DISCUSSION

Data of DFU degree and $CD4^+$ cells bearing malondialdehyde were presented on Table 1 and Figure 1.

Table 1 Data of Percentage of $CD4^+$ marked CPS3 and DFU**

DFU degree	$CD4^+$ CPS3 (%)	*P*		
2	55,63 *	*$vs^{\#}$ <0.05	* vs^{+}<0.05	* $vs^{\wedge}$ <0.05
3	77,77 $^{\#}$	$^{\#}$ vs^{+}<0.05	$^{\#}$ $vs^{\wedge}$ <0.05	
4	79,95 $^{+}$	$^{+}$ $vs^{\wedge}$ >0.05$^{@}$		
5	86,83 $^{\wedge}$			

** Resume of comparison test *Mann-Whitney U*
The mean difference is significant at the .05 level.
Not significant $^{\$}$p=0, 32; &p = 0, 56; and @p = 0, 79

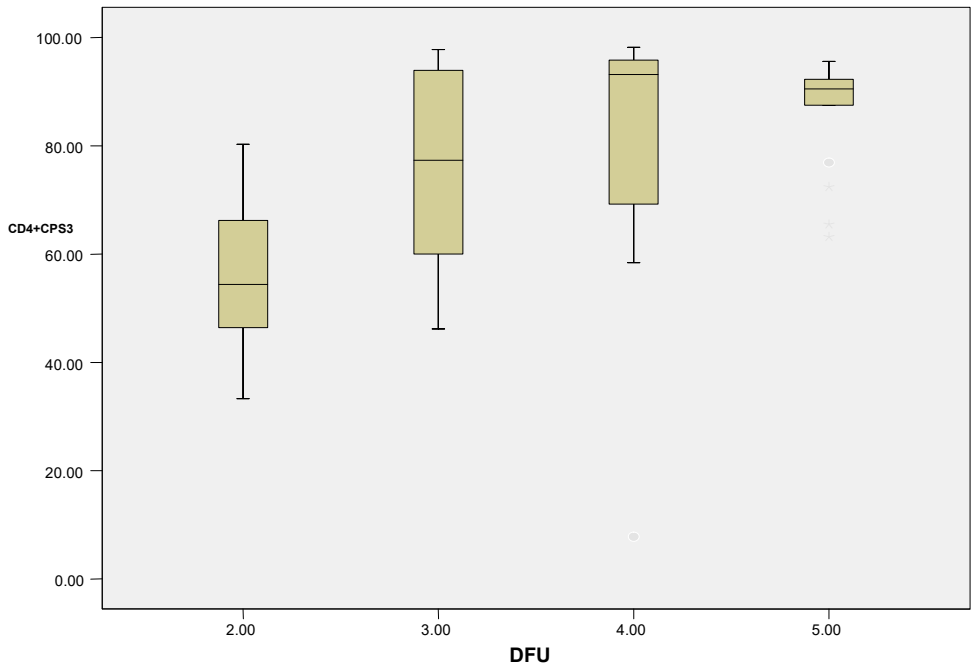
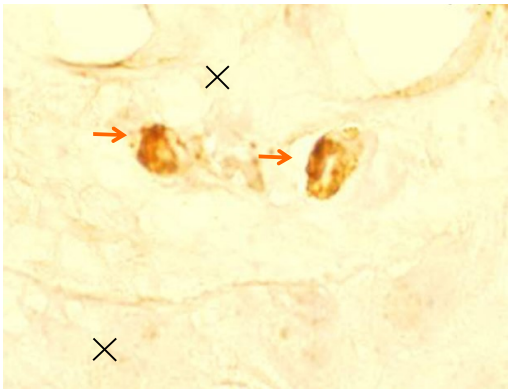


Figure 1 Box plot of Percentage $CD4^+$ Cells Bearing CPS3 and DFU Degree

Based on Spearman Correlations it was obtained that there is a moderate positive correlation between percentage $CD4^+$ bearing CPS3 and DFU degree ($r = 0.60$, $p < 0.05$). Inflammation and lyses cells were strongly influence by the lyses of $CD4^+$ immune cells through formation of CPS3 (Figure 2 and 3).



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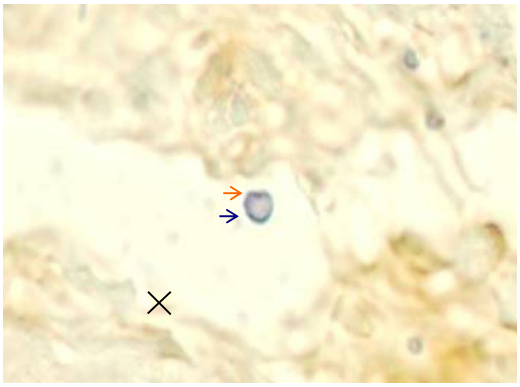
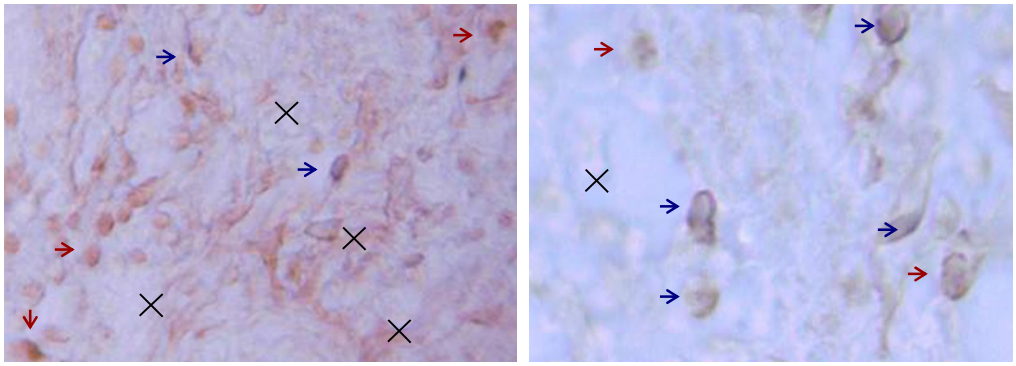


B

Figure 2 $CD4^+$ Marked CPS3 of DFU Tissue:
A) non diabetic foot (1000 zoom),
(x) tissue structure, → $CD4^+$ cells without CPS3

B) DFU level 2 (1000 zoom),
Start damaging of tissue structure, → many $CD4^+$ cells, → CPS3 carrier, sign of immune cells apoptosis



A **B**

Figure 3 $CD4^+$ cells bearing CPS3 of DFU tissue

A) DFU level 3 (1000 zoom), (x) indicates tissue structure demaged, (→) clear $CD4^+$ cells, (→) indicates CPS3 carrier, present of immune cells apoptosis

B) DFU level 5 (1000 zoom), (x) indicates most tissue structure demage, (→) clear $CD4^+$ cells with feeding brown colour, (→) indicates CPS3 carrier, a sign of immune cells apoptosis.

CONCLUSION

There is a moderate positive correlation between DFU degree and percentage $CD4^+$ bearing CPS3.

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AMMONIA GAS (NH_3) ELIMINATION USING BIOFILTRATION UNDER ANAEROBIC CONDITION

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ABSTRACT

Ammonia is one of gas pollutant that generated from organic substance degradation by microorganism such as through composting, manure, landfill as well as from anthropogenic source such as fertilizer industries, nitrate acid production and from oil refinery. Standard of ammonia concentration in atmosphere based on Kep-50/MENLH/1996 is 2 ppm. Biofiltration is one of method that is developed to treat Ammonia. Ammonia gas that flow through a filter media will be absorbed by biofilm that is formed around the surface particle media. The ammonia then will be degraded by microorganism living in the biofilm. Anaerobe condition is easier to be achieved because ammonia gas usually released from an anaerobic process. The study has been able to isolate Bacillus spp from septic tank sludge which was able to live in environment with high concentration of ammonia. Application of the isolated bacteria into biofiltration reactor (media thickness was 70 cm) has shown good performance that reach 95.63% efficiency with ammonia loading 5.152 mg/m^2/min, debit of 500 ml/min, retention time of 5.857 min, while efficiency of 93.27% was reached by ammonia loading of 20.48 mg/m^2/min, debit of 1700 ml/min, retention time of 1.7 min. However the mass balance calculation showed only small amount of ammonia that has been used by bacteria. The amount was ranged from 21.5-36.4% of total ammonia that entered the reactor. In this study the highest elimination rate achieved was 45 mg/m^3/min with ammonia loading of 38.69 mg/m^2/min. The pH value in anaerobic filtration did not decrease as much as in aerobic filtration.

Keywords: ammonia, anaerobic, biofiltration, gas

INTRODUCTION

Ammonia is one of gas pollutant that generated from organic substance degradation by microorganism such as through composting, manure, landfill as well as from anthropogenic source such as fertilizer industries, nitrate acid production and from oil refinery. Standard of ammonia concentration in atmosphere based on Kep-50/MENLH/1996 is 2 ppm. Ammonia is one nitrogen compound from seven of possible oxidation levels that are NH_3, N_2, N_2O, NO, N_2O_3, NO_2, N_2O_5. The oxidation level change on nitrogen could be done by living creature, where for example bacteria could bring the oxidation process into positive or negative based on aerobic or anaerobic environment of the process (Sawyer and Mc Carty, 1994).

Beside odor pollution caused by ammonia gas, ammonia in the form of aerosol particulate could be transported to a long distance that may result international pollution as consequences. Moreover, not very long ago it has been found out that acid disposition (e.g. $(NH_4)_2SO_4$) has resulted more acid impact to soil compared to strong acid such as H_2SO_4. On the other hand, Nitrogen in the form of ammonia was generated more than nitrogen in the form of NO_x in Europe (Apsimon and Kruse-Plass, 1991).

The use of biofilter for gas pollutant elimination has been started since 1920, however it has been started to be used more intensive in industries since 1980 with good performance and more efficient in cost (Devinny et al., 1999). Aerobic treatment that mainly utilizing nitrification process that produce nitrate at the end has been used in biofiltration of Ammonia gas. However this method has caused reduction of pH value of media dramatically (Soedomo et al, 1994).

Biofiltration is one of methods that are developed to treat Ammonia as pollutant gas. Ammonia gas that flow through a filter media will be absorbed by biofilm that is

formed around the surface particle media. The ammonia then will be degraded by microorganism living in the biofilm. The filter media is generally rich of organic nutrients (compost, soil, top soil etc.) that provide large contact surface area with enough nutrient supplements for the microorganism. The biofilter is not purely filtration unit, but it is combination of absorption, adsorption, degradatioin, desorption of contaminant in gas phase.

The study was designed to learn the biofiltration process of ammonia gas under anaerobic condition by using bacteria from bio-augmentation process. Anaerobe condition is easier to be achieved because ammonia gas usually released from an anaerobic process.The elimination efficiency and the relationship between factors that may contribute in the process also examined.

MATERIALS AND METHODS

The study began with isolation of bacteria from septic tank sludge that exposed with ammonia gas from vent of operating septic tank. The medium used as biofilter was sterilized top soil. Before the operation of biofilter with isolated bacteria, the medium was operated without bacteria to examine the medium absorption capacity rate. The medium then inoculated with the isolated bacteria, and bifilter was operated with various concentration of ammonia gas in two different flow rate that is 500 ml/min and 1700ml/min. The maximum medium height used was 70 cm. The conditions maintained were anaerobic, 60-70% humidity and 24-27 degree of Celsius in temperature. Gas concentration was measured at inlet and outlet from various height of medium filter as well the pH value of medium.

RESULTS AND DISCUSSION

The study has been able to isolate Bacillus spp from septic tank sludge which was able to live in anaerobic environment with high concentration of ammonia. Application of the isolated bacteria into biofiltration reactor has shown good performance that reach 95.63% efficiency with ammonia loading 5.152 mg/m2/min, debit of 500 ml/min, retention time of 5.857 min, while efficiency of 93.27% was reached by ammonia loading of 20.48 mg/m2/min, debit of 1700 ml/min, retention time of 1.7 min.

According to the assumption that taken by Ottengraf (1986 and 1987) and Usman et al. (1996) that the degradation process that occur in biofilm is first order level reaction, thus the process does not depend on the substrat concentration, but has limited inlet maximum concentration. Therefore in this biofilter, the elimination capacity that calculated was the combination of physical, chemical and biological conversion process.

The sorption process in the reactor is a serial sorption along the loaded gas transportation line, thus the gas elimination in the susequent media is the elimination of gas that is not absorbed in the previous media thickness. As the result the elimination capacity that is showed is tend to be smaller compared to the performance of the previous thickness of the media.

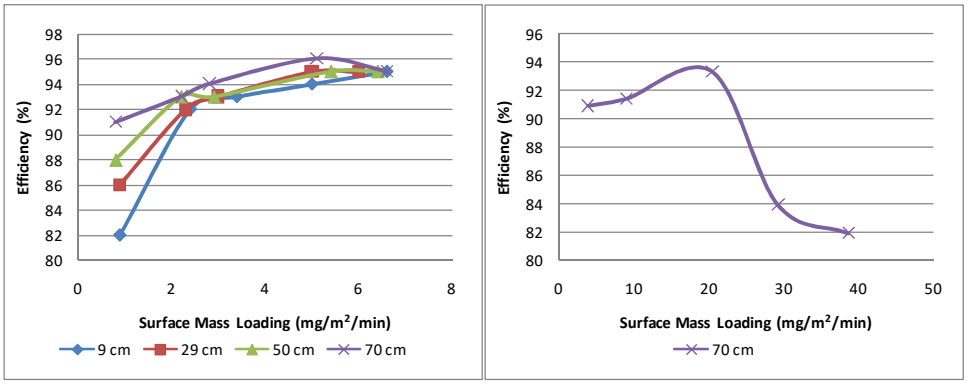

Figure 1. Correlation of Surface Mass Loading and Elimination Efficiency with flow rate 500 ml/min (left) and flow rate 1700 ml/min (right)

The pH value within the media showed that in low ammonia loading rate, the pH value tend to be lower that the pH value reached by the high ammonia loading rate at the begining of the reactor (at the bottom layer of reactor). However the pH value then reduced into the lowest value of 5.65 at the 29 cm media thickness, and started to increase at the following media thickness reach to 6.5 in average at the 70 cm media thickness.

However the mass balance calculation showed only small amount of ammonia that has been used by bacteria. The amount was ranged from 21.5-36.4% of total ammonia that entered the reactor. In this study the highest elimination rate achieved was 45 mg/m3/min with ammonia loading of 38.69 mg/m2/min.

The pressure loss in this research is ranged from 1.3 to 5 cm H_2O, while in general the pressure loss in biofilter is 0.2 – 1.0 cm H_2O with maximum loss reach 10 cm H_2O (Devinny et al., 1999). In the biofilter that using top soil as media showed average pressure loss at 250 Pa (2.55 cm H_2O) (Scholtens and Demmers, 1991). In term of contact time of gas in the reactor, in this research the time (1.7 – 5.85 minutes) is longer than the usual contact time used in biofilter (0.25-1 minutes) (Devinny et al., 1999).

The elimination capacity reached in this study of anaerobic biofiltration is far lower that the elimination capacity that reached by aerobic biofiltration that is 6.76 gr/m^3/min(Soedomo et al., 1994). This is because the loading rate in this study has not reached the maximum value, where the elimination value reaches the optimum results.

CONCLUSION

The experiment in this study only reach a small surface mass loading compare with the ammonia concentration generated by oil refinery in the field. Moreover the isolated bacteria in this study performed very low capacity in eliminating ammonia gas that shown through a mass balance calculation. High efficiency achieve in this study was mainly because the absorption of ammonia gas by biofilm surrounding the filter media. Therefore, it is recommended to increase the surface mass loading for future studies to reach actual loading and to put the bacteria in the high ammonium concentration in their environment.

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LESS HEALTHY FAMILY FUNCTION IN CHILDREN WITH ATTENTION DEFICIT HYPERACTIVITY DISORDER

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ABSTRACT

BACKGROUND: Child behavior patterns are influenced by genetic factors, physical and environmental factors. Parents are the first social environment which is known by the child. If the child has ADHD, so the child can not be understood apart, separated from their parents, because children and their parents is a family. Child behavior patterns are determined by the structure, organization and patterns of transactions within the family. The presence ADHD function is said to be a sign of a disturbed family. OBJECTIVES: To know this family function in families who have children with ADHD compared with families who have children without ADHD and support the prevention and treatment of ADHD, especially in Family Therapy. METHODS: Study was conducted at Sanglah Hospital in Denpasar subject of this study is the family of out patients with ADHD children. Parents has been filling questionnaires SPPAHI and FAD. The child ADHD diagnosis is established according to DSM-IV TR criteria by psychiatrist. From June 1, 2010 until August 31, 2010 is obtained 27 samples of ADHD and 27 subjects as control
RESULTS AND CONCLUTION: The Study sample was composed of 27 cases ADHD and 27 cases of ADHD patients as a control. There were significant differences in family functioning (FAD) between groups of ADHD cases with a control group with p = 0.006. In the group children with ADHD, 77.8% have function of less healthy family and only 22.2% who have a good family function. This study found that families with ADHD have a risk estimate " function of less healthy family " is 2.4 x compared with non-ADHD family. No correlation was found between high score ADHD with a family function with p = 0.7.

Keywords: ADHD, Family Assessment

INTRODUCTION

Child behavior patterns are influenced by genetic factors, physical and environmental factors. Parents are the first social environment which is known by the child. If the child has Attention Deficit Hyperactivity Disorder (ADHD), so the child can not be understood apart, separated from their parents, because children and their parents is a family. Child behavior patterns are determined by the structure, organization and patterns of transactions within the family. The presence ADHD function is said to be a sign of a disturbed family.[1,2]

Prevalence ADHD of up in the Indonesia (Saputra D, 2009), 12.6 – 22.5%. Male: female ratio ranges from 4: 1 to 9: 1.[3] DSM-IV TR (*Diagnostic and Statistical Manual of Mental Disorders-IV Text Revision*) criteria for ADHD, the cluster of age-inappropriate behavioural abnormalities of the triad: inattention, hyperactivity, impulsivity have persisted for at least 6 months, begins before age 7 years and may persist into adulthood and must be apparent in more than one setting (e.g.school and home). Impaired attention leads to frequent changes from one activity to another and to unfinished activities. Overactivity manifests as excessive restlessness, e.g. running and jumping arround, noisiness and excessive talkativeness.[1,2,3,4]

The function family is a complex phenomenon, which can be determined using a screening instrument developed by Epstein et al, and is called "The Master Family assessment device (FAD). FAD is a questionnaire consisting of 53 items and is divided in 7 scale.

Scale problem solving, communication, roles, affective, responsiveness affective, involvement behavior control, general functioning. Scoring: each item is scored on a 1

to 4 basis using the following key: SA = 1, A = 2, D = 3, SD = 4. Items describing unhealthy functioning are reverse-scored. Lower scores indicate healthier functioning. Scored responses to the items are averaged to provide seven scale scores, each having a possible range from 1.0 (healthy) to 4.0 (unhealthy). [5]

The results of reliability, the FAD re-test showed high reliability values (alpha > 0.70). FSD validity studies in clinical and non clinical groups showed significant difference ($p < 0.02$). This study aims to find relations with ADHD on family functioning.[5] The objectives of this research is to know this family function in families who have children with ADHD compared with families who have children without ADHD and support the prevention and treatment of ADHD, especially in Family Therapy.

Hypothesis

In families that have children with ADHD will be found there are less healthy family functioning.

METHODS

Study was conducted at Sanglah Hospital in Denpasar subject of this study is the family of out patients with ADHD children. Parents has been filling questionnaires SPPAHI and FAD. The child ADHD diagnosis is established according to DSM-IV TR criteria by psychiatrist. From June 1, 2010 until August 31, 2010 is obtained 27 samples of ADHD and 27 subjects as control.

- Criteria inclusi.
  1. Families with children diagnosed with ADHD
  2. Children age> 3 -10 years
  3. Family (biological parents) willing to participate in research
- Criteria exlusi
  1. Adoptive or foster parents.
  2. Single parents.
  3. One of the parents suffered from chronic physical or mental disorder

How to study : cross sectional analytic
Tool : "The Mc Master Family Assessment Device (FAD) [5]
Processing data:

The SPSS Program in computer. Normality test used Kolmogorov - Smirnov test. To assess the respective values in FAD score performed by Kruskal-Wallis showed significance while FAD was assessed by the Mann-Whitney test.

RESULTS AND CONCLUSION

The results of normality tests showed no normal distribution.The Study sample was composed of 27 cases of ADHD and 27 patients as a control (non ADHD). Characteristic data in two groups are shown in Table 1.

Table 1. Characteristic data in two groups

Variable	Male	Female	Mean age child (Year)	Mean Parents age (yr)	Mean Score ADHD	Mean Score FAD
control	18	9	8,3	38,4	40,22	153,89
case	15	12	8,33	37	71,30	167,67

Based on education, employment and economic condition of parents between ADHD group without ADHD (control) can be seen in Figure 1 and Table 2.

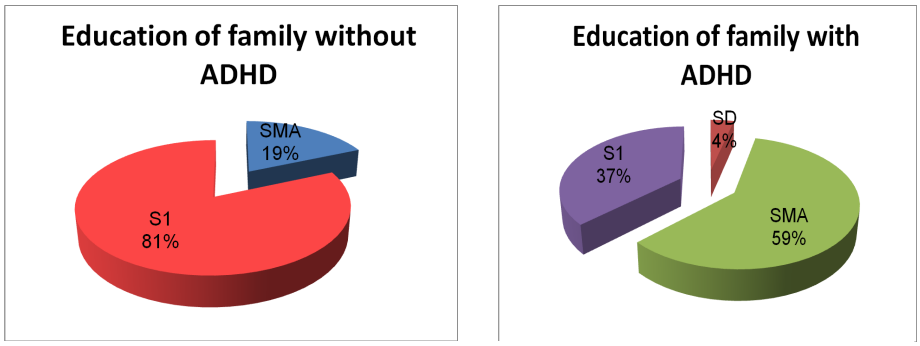

Figure 1. Education of family between family with ADHD and without ADHD

Table 2. Work on the cases and control parents

		ADHD		Control	
		Frequency	Percent	Frequency	Percent
education	PNS	3	11.1	12	44.4
	Swasta	24	88.9	15	55.6
Economy	Low	1	3.7	-	-
	moderate	22	81.5	23	18.5
	high	4	14.8	4	14.8

In the group children with ADHD, 77.8% have function of less healthy family and only 22.2% who have a good family function (Table 3).

Table 3. Family function with category ADHD case and control

			Group		
			Case	Control	Total
Family Functioning	Less	Total	21	11	32
		%	77.8%	40.7%	59.3%
	good	Total	6	16	22
		%	22.2%	59.3%	40.7%
		Total	27	27	54

There were significant differences in family functioning (FAD) between groups of ADHD cases with a control group with p = 0.006. This study founded that families who have children with ADHD had 5.1 times likelihood (RO = 5.091, 95% Cl 1.55 to 16.7) for having a "function of less healthy family" than the families that have children

without ADHD, but did not find any relationship between education of parents with Family Function in this study (p = 0.69). There families with ADHD have a risk estimate " function of less healthy family " is 2.4 x compared.

No correlation was found between high score ADHD with unhealthy family function with p = 0.7.

Tabel 4. **Test Statistics[a,b] Family Assessment Device (FAD)**

	Problem solving	Communication	Roles	Affective	Responiveness affective	Involvement behavior control	General functioning
Chi-Square	4.475	2.027	9.758	7.690	6.167	6.613	10.178
Df	1	1	1	1	1	1	1
Asymp. Sig.	.034	.155	.002	.006	.013	.010	.001

a. Kruskal Wallis Test
b. Grouping Variable: Family functioning

There were significant differences between ADHD with problem resolution, roles, affective responses, affective involvement, behavior control, general functions in family function, but not significantly with communication within the family (Table 4). Communication in families with ADHD children was not significant, this can be caused the study was conducted retrospectively so that there have been improvements, but there are still residual symptoms.

CONCLUSION

In families that have children with ADHD, found a lack of the ability of families to establish patterns of behavior in the form of division of tasks and responsibilities, family functions as a source of supplies, care and support for the development of an individual. Also seem less patient and less family show affection.

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EFFECT OF *CENTELLA ASIATICA* EXTRACT ON THE LEVEL OF INTERLEUKIN 6 (IL-6) IN MICE

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ABSTRACT

A study was conducted to find out the ability of *Centella asiatica* (*C. asiatica)* in Interleukin 6 (IL-6) of *C. asiatica* treated mice after *Salmonella typhi* (*S. typhi*) infections. It is therefore expected that herbal drug such as *C. asiatica* can be used as an alternative medicine to prevent and cure salmonellosis in both animals and human. Experimental laboratory studies were conducted using Completely Factorial Randomized Design. Mice were divided into four groups and they were treated respectively with destilated water (negative control), 125, 250, and 500 mg/kg BW/day of *C. asiatica* extract. The treatment was conducted daily for two weeks and the mice were inoculated with 10^5 cells/ml of *S. typhi.* The IL-6 response were examined by enzyme-linked immunosorbent assay (ELISA) on first day, second week and fourth week after *S. typhi* infections. The result showed that treatment of mice with *Centella asiatica* extract significantly ($p<0,05$) enhaced IL-6 level of Balb/C mice following inoculation with *S. typhi*. The highest IL-6 level were observed in mice treated *Centella asiatica* extract at the dose of 500 mg/kg bw (385,93 ± 125,43 pg/ml serum). And the highest IL-6 level were observed at 2 weeks following inoculation with *S. typhi* (232,22 ± 21.82 pg/ml). A further study is recomended to examine the celluler immune response and more detailed study on the humoral immune response of animals or human before this herbal is used as alternatif medicine to prevent and cure typhoid fever. It is also important to study the best preparation, the half life, and the side effect of *Centella asiatica* in human and animals.

Keywords: pegagan, *Centella asiatica*, IL-6, antibody, *S. typhi*

INTRODUCTION

Salmonellosis still remains a public health problem in developing countries, in Indonesia for example. The disease is often known as typhoid fever or typhus disease. The incidence rate of the disease increases in the long dry season and the beginning of the rainy season. Incidence of the disease in children is mainly occurred at the age of 5 years or older with mild clinical manifestations. The younger the child, the more unspecific the clinical signs of the disease. In addition, the mortality rate of the disease in child is lower than the adult (Supali, 2002).

There are many constrains in order to prevent and control of *salmonelosis*. Incorrect antibiotic treatment on *salmonellosis* can cause resistance. In addition, the cost for maintenance of the infection is relatively expensive, and the restoration from the infection needs quite long time. Therefore, it needs to find alternatives for prevention of *salmonellosis* in the future that may tackle these constrains in more easier, cheaper and more effective ways. An alternative that can be done is to increase the body defence so that, in the same way, it can prevent the occurrence of infection. The body defence can be increased through activated fagositic cell, such as: macrophage and neutrophil. Both of theses cells play an important role to eliminate infectious agents that entered the body (Tizard, 2000).

Activated macrophages also have capability to produce interleukin. This interleukin is a substance that helps interacting between the cells. There are several kinds of interleukin (IL) that are released by macrophages, such as: IL-1, IL-4, IL-6, and TNF. Basically, these interleukins have important roles in inflammation reaction and immunity management system. Specifically, interleukin can increase or even halt the cell' growth and also increase the activity of cell chemo-taxis.

Centella asiatica (*C. asiatica*), or more commonly called pegagan, has been famous for traditional medicine for years. Pegagan is also used for wound healing and improving memory span. It can alsoincrease hyperplasia cell activity and the existence of collagen in wound tissue (Sagrawat and Khan, 2007). Jayathirta and Mishra (2004) believed that *C asiatica* extract from 100 to 500 mg/kg bw in mice could significantly increase the total of white blood cells and macrophage phagocyte ability against carbon molecules in those mice. Further more, there was a significant linear relation between *C. asiatica* doses and the total of white blood cells, as well as the macrophage ability to phagocyte carbon molecules. While, Rao, et all (2006) found that giving pegagan to the mice could significantly affect the length of dendrite hippocampus cells. The scientific benefits of *C. asiatica* in humans and animals have been evoked these days. However, research on *C. asiatica* ability to induce IL-6 related to infection had not been reported.

MATERIALS AND METHODS

A total of 48 mice, with at least 8 weeks of age, was weighted and adapted to the environment for 2 weeks. The all mice were randomly divided into 4 groups, and each group consisted of 12 mice. Group I was a control group that only given 1 ml / day sterile aquades; while, group II, group III and group IV were given 125 mg / kg bw / ml, 250 mg/kg bw / ml, and 500 mg / kg bw / ml of extract pegagan respectively. Those treatments were given daily for 14 days.

As many as 10^5 lethal dose 50 (LD 50) of *S. typhi* in 1 ml PBS was infected intraperitoneally to each of the mice on day 15. The blood from as many as 4 mice from each group was also collected to examine the IL-6 levels and antibody titers. Blood sampling was continued to examine 2 weeks and 4 weeks after *S. typhi* infection.

Levels of IL-6 in mice serum was performed by capture ELISA (BMS 603, Vienna, Austria). The result was then analyzed from the color intensity that generated by the ELISA DAX 800 (Automatic Diagnostic, USA) on a filter with a wavelength of 450 nm. Concentrations of IL-6 serum result were determined based on comparison of the optical density (OD) value from each serum samples with standard IL-6 OD values of IL-6 using logistic regression parameters.

Levene's Test at 5% significance level was used to evaluate data homogeneity. While, the group differences were analyzed by using 5% significance level in analysis of variants. Further more, the homogeneous data were analyzed by Least Significant Difference (LSD) at 5% significance level. All of data were analyzed by using version 15 of SPSS.

RESULTS

A total of 48 mice was used for sera collection in this study. The sera were tested for IL-6 on ELISA test. The result of IL-6 level can be seen in figure 1.

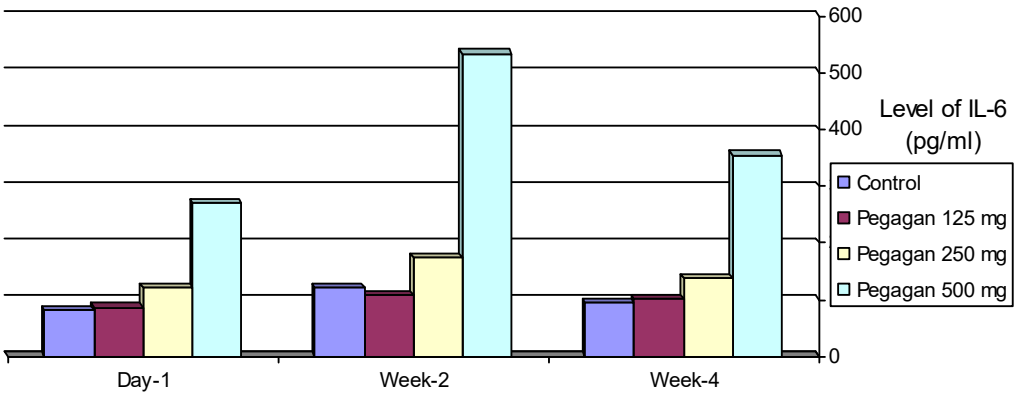

Figure 1. IL-6 Level from Each of Mice Group Treatments

The level of IL-6 fluctuated depending on the dose of *C.asiatica* given. The highest IL-6 level was observed in mice given by *C. asiatica* extract 2 weeks after *S. typhi* inoculation (533,426475± 81,7184 pg/ml) where the control group had the lowest level (82,1337±2,9464 pg/ml). In general, IL-6 level in all groups increased in week 2, but gradually decreased in week four. The analysis result between *C. asiatica* doses and interval observation period can been seen in table1.

Table 1. Variety of IL-6 Levels in Mice that Treated with *C. asiatica* Extract in Dose Variatio and Interval Observation Period.

Source	Sum of square	Df	Mean Square	F	Sig.
Correction model	839025.051	11	76275.005	92.025	0,000
Intersep	1584090.83	1	1584090.83	1911.18	0,000
Dose	685667.622	3	228555.874	275.749	0,000
Time	69667.422	2	34833.711	42.026	0,000
Group* Time	83690.008	6	13948.335	16.828	0,000
Error	29838.740	36	828.854		
Total	2452954.62	48			
Correction	868863.791	47			

Table 1. shows there was a significant difference ($p<0.01$) between dose of *C. asiatica* extract and interval observation period. There was an interaction between dose of *C. asiatica* extract and interval observation period to the IL-6 level in the treated mice. The difference between them can be shown in Table 2.

Table 2. The Difference Doses in *C. asiatica* Extract to the IL-6 Level in Mice

Group	Group	Mean difference	Sig.
Control	125 mg/kg bw	-2.17116	.854
	250 mg/kg bw	-48.6191(*)	.000
	500 mg/kg bw	-289.2787(*)	.000
125 mg/kg bw	250 mg/kg bw	-46.4479(*)	.000
	500 mg/kg bw	-287.1075(*)	.000
250 mg/kg bw	500 mg/kg bw	-240.660(*)	.000

The highest IL-6 level was at dose 500 mg/kg bw of *C. asiatica* extract and it also had a significant difference ($p<0.05$) compared to dose 250 mg/kg bw or 125 mg/kg bw. The *C. asiatica* extract at dose 250 mg/kg bw had significant difference ($p<0.05$) compared to dose 125 mg/kg bw. Nevertheless, no significant difference ($p>0.05$) was observed between dose 125 mg/kg bw of *C. asiatica* extract and control group.

A significant difference ($p<0.05$) was also found in observation period of IL-6 level in mice that given *C. asiatica* extract. Details of the difference can be seen in Table 3.

Table 3. Interval Observation Period IL-6 in Mice that Given *C. asiatica* Extract

Observation period	Observation period	Mean difference	Sig
Day-1	2nd week	-91.9657(*)	.000
	4th week	-32.269(*)	.003
Week-2	4th week	59.6963(*)	.000

Level of IL-6 in mice that given *C. asiatica* extract and observed in week 2 showed higher significant different ($P<0.01$) compared to the mice observed in day 1 or week 4. In 4th week, there was a higher significance ($P<0.01$) different compared to day one.

DISCUSSION

IL-6 levels in mice given the highest seen at doses of pegagan 500 mg / kg bw was observed in the second week (533.4262±81.7184 pg). Statistical analysis showed that administration of pegagan can enhanced levels of IL-6 significant difference ($p<0.05$), with highest levels found in pegagan dosage of 500 mg/kg bw (385.9257±125.4315 pg), which is significant difference ($p<0,05$) than doses of 250 mg/kg bw (145.2661±24.4383 pg), 125 mg/kg bw (98.81813±9.5618 pg) or control (96.6470±15,2474 pg). Levels of IL-6 at doses of 250 mg / kg was significant difference ($p<0,05$) compared with a dose of 125 mg / kg, but between the dose of 125 mg with controls showed no significant difference ($p>0.05$).

The same research results obtained by Hongzong (2009), medecassoside content on pegagan may increase levels of IL-6 in mice suffering from artristis. In mice that are experiencing arthritis will experience increased levels of IL-6, then will be decreased along with wound healing. Kwon et al. (2008) found that ethanol extract of *C. asiatica*

ultrasofication increased levels of TNFα and IL-6 is secreted by T cells Further found that with the addition of extract of *C. asiatica* causes activation of NK cells increased by 10%.

These results prove that pegagan can enhanced levels of IL-6 in the body. Increased levels of IL-6 is due to the stimulation caused by germs *C. asiatica* and *S. typhi*. *C. asiatica* stimulate macrophages to increase their activities, thus becoming more responsive to antigens that enter the body. Furthermore, the germ *S. typhi* also provide signals captured by the macrophage to migration and phagocytosis. Macrophages that perform phagocytosis will issue a cell mediators such as IL-1 and IL-6, which stimulates other macrophage cells to respond and approached the source of stimulation. Expenditures of IL-6 in this chain will increase the number of IL-6 in the circulation of the body.

Levels of IL-6 has begun to rise on the first day of bacterial infection. The highest levels found in week two, and finally declined in the fourth week. Production of IL-6 caused by the activation of macrophages and the presence of antigen stimulation. Activation of macrophages without the stimulation of infection will not result in increased levels of IL-6. In this study, antigen stimulation in the form of the infection of *S. typhi* given only once on the first day. Thus, IL-6 production will be increased beginning the first day until the second week and gradually began to decline.

CONCLUSION

. *C. asiatica* extract increased the levels of IL-6 in mice Balb/c infected with *S. typhi* significantly ($p<0.05$)., The highest levels found at a dose of 500 mg / kg body weight two weeks after infection S. typhi (533.4262 ± 81.7184 pg / ml).

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COMPARISON ON EFFECTIVENESS OF *Chrysomyia rufifacies* AND *Musca domestica larvae* IN EXTRACT TEST IN VITRO, EXTRACT TEST IN VIVO AND MAGGOT DEBRIDEMENT THERAPHY ON METHICILLIN-RESISTANT *Staphylococcus aureus* (MRSA) INFECTED WOUNDS.

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ABSTRACT

Maggot has an important medical benefit. Some species of maggot can be used for wound debridement. This method is known as Maggot Debridement Therapy. In addition to its ability to feed on necrotic tissue, maggot has been proved to be able to kill bacteria in wounds. Of the usable maggots, two of them are the maggots of Chrysomyia rufifacies and Musca domestica. Usage of maggot has difficulties in its application on human. Therefore, it is considered beneficial to research whether maggot extract also has antibacterial effect. The research starts on the search of the appropriate maggots, drying and grinding the maggots, and extraction of the pulverized maggots by means of percolation. The design of the research is experimental with post-test and control group. The research divides MRSA samples into 2 groups: treatment group, which is treated with maggot extract, and positive control group, which is treated with mupirocin. Tube dilution test is used to determine the Minimum Inhibitory Concentration (MIC) of maggot extract, after which samples are grown in solid media by means of plate-streaking method to detect any growth of bacteria, thus determine the Minimum Bactericidal Concentration (MBC). The result of in vitro-extract test indicates that the MIC from both maggot extracts remains undetermined. The MBC from Chrysomyia rufifacies's maggot extract is 0,25 mg/ml, while the MBC Musca domestica's maggot extract is greater than 6,25 mg/ml. Thus, it can be concluded that the maggot extract of Chrysomyia rufifacies has a more potent anti-bacterial activity to MRSA than the maggot extract of Musca domestica.

Keywords: maggot, Chrysomyia rufifacies, Musca domestica, extract, MRSA.

DIAGNOSTIC TOOLS FOR THE DETECTION OF RABIES VIRUS IN HUMAN

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Rabies is a zoonotic disease (a disease that is transmitted to humans from animals) that is caused by the Rhabdovirus of the genus Lyssavirus. Rabies infects domestic and wild animals, and is spread to people through close contact with infected saliva (via bites or scratches). Rabies is almost always a fatal infection. A progressive illness of approximately two to 21 days follows an incubation period of usually three to eight weeks. Several tests are necessary to diagnose rabies antemortem (before death) in humans; no single test is sufficient. Specimens for rabies testing should be collected only after more common etiologies of encephalitis or myelitis have been ruled out. Tests are performed on samples of saliva, serum, spinal fluid, and skin biopsies of hair follicles at the nape of the neck. Saliva can be tested by virus isolation or reverse transcription followed by polymerase chain reaction (RT-PCR). Serum and spinal fluid are tested for antibodies to rabies virus. Skin biopsy specimens are examined for rabies antigen in the cutaneous nerves at the base of hair follicles.

Keyword: Diagnostic Tools, Rabies Virus, Human

SURVEY THE NUMBER OF COLIFORM AND IDENTIFICATION OF *ESCHERICHIA COLI* IN *SIOMAY* VENDORS`S RINSE WATER IN SUB-DISTRICT TEMBALANG, SEMARANG

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ABSTRACT

Water has potential for spreading disease through water washed disease. In water washed disease, infection can be caused person to other person through water supply. Coliform bacteria is a bacteria group that used as indicator of contamination on the water. Coliform that caused diarhea is *Escherichia coli*. The aim of this research are to know the number of coliform and identify the existence of *E.coli* in *siomay* vendor`s rinse water in Sub-districtTembalang, District Tembalang, Semarang. This research is descriptive with cross sectional design. Population in this research are all *siomay* vendor`s rinse water in Sub-district Tembalang, District Tembalang, Semarang. The samples is rinse water from 17 Siomay vendor which taken by using purposive sampling technique. Data was analyzed by descriptive. The result of this research indicated that all rinse water contain the number of Coliform more than 2.400/ml. While from identification result *of E. coli* known that 11 samples shows positive result to the existence of *E coli*. A conclusion of this research are all *siomay* vendor`s rinse waters aren`t fulfill microbiologies quality standard so that improper to be used for washing eating utensils. Therefore, it was suggested that *siomay* vendor should pay more attention to rinse water sanitation.

Keywords: Coliform, *E.coli*, rinse water, *siomay*

COLONIZATION OF LACTOBACILLUS SP. F2 IN THE INTESTINAL TRACT AND ITS FUNCTIONAL EFFECT TO REDUCE BLOOD CHOLESTEROL CONTENT OF RATS (*Rattus norvegicus*)

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ABSTRACT

Lactobacillus sp F is an endogenous strain of Indonesia that have been tested for its survival under intestinal tract condition (in vitro) and its ability to hydrolyze bile salt and this strain is potential to reduce blood cholesterol content. For the development of this strain as a potential probiotic its ability to colonize intestinal tract and to r educe blood cholesterol content in vivo needs to be investigated. Rats were administrated with 108 cells/day of Lactobacillus sp. F2 for 3 week by oral gavage. Following this, the total lactic acid bacteria (LAB) population, anaerobic bacteria in the ceccal content and blood cholesterol content were analysed. Using MRS Agar, anaerobic agar and cholesterol kit, respectively. For the confirmation of the colonization of Lactobacillus sp. F2, RAPD method with specific primer (M13R) was applied. The results showed that the total LAB in the intestine of rats administrated with Lactobacillus sp.F2 was about 1.99x109 CFU/g while is the ceccal rat found 9.36x107CFU/g . About 48.99% of the total LAB consisted of Lactobacillus sp. F2, indicating that Lactobacillus sp. F2 had the ability to colonize the rats intestinal tract. High total number of LAB resulted in lower pH in the intestinal tract of rats treated with Lactobacillus sp. F2 when compare to the control (5.68 in the treated rats and 6.02 in the control). A decrease in blood cholesterol content by 33% in the treated rats was also observed in this experiment when compare to the control. The latest tendency was suspected to be due to fermentation process and hydrolysis of bile salts by Lactobacillus sp.F2. The above results showed that Lactobacillus sp. F2 is potential to be developed as an endogenous probiotic, although some intensive research, especially on the development of delivery methods of this probiotic candidate along intestinal tract need to be conducted in the future.

Keywords: Lactobacillus sp. F2, Colonization, Cholesterol, RAPD, Probiotic

PHACOEMULSIFICATION FOR BETTER VISION

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ABTRACT

Cataract is prevalent throughout the world and Cataract surgery is a successful and increasingly frequently performed ophthalmic surgical procedure to reverse the visual and functional disability caused by cataract. Over the years, the surgical technique has evolved from intracapsular extraction to modern phacoemulsification. The advantages of phacoemulsification ranging from small incision and sutureless that could reduced the post operative astigmatism, closed anterior chamber to help the operator broken and removed the nucleus of the lens easily with ultrasound waves and better visual outcome than the other methods of cataract extraction. There are also choices of intraocular lens to be inserted to the patient according to the visual needs.

Keywords: Cataract, Phacoemulsification, visual acuity, intraocular lens

SMOKING HABIT AT SCHIZOPHRENIC PATIENT TO SEE FROM LEVEL OF MILD/SEVERE AND MOTIVATION FOR STOPING

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ABSTRACT

Background. According to research amount of schizophrenia at population estmeted 1%.
Indonesia with 238 million population there are 2,4 million people with diagnostic shizophrenia and 75% of shizophrenia have smoking habit. Difficult to stoping. Nicotine at tobacco cigarette increase metabolism anti psychotic in the liver. Anti psychotic can not block dopamine in the brain. Cause anti psychotic drug in the brain become weak. That way symptom action schizophrenia did not recovery. Beside that nicotine cause cardiovascular desease, heart desease, stroke and Buerger's desease. Hazard of smoking there are much tar can cause pulmonary obstructif desease and cancer. Schizophrenic patient and family have high stress physically, mentaly and financial. That way smoking habit in schizophrenia patient really must be stoping. Aim. To know the level of nicotine dependence, mild/severe of smoking and motivation to stoping from smoking. Method. The research mike in the privat practice, sample all patient at diagnostic schizophrenia. Time for research three monts. All patient interviewing by doctor. Diagnostic made by PPDGIII, level devendence of nicotine to assess by Fagerstrom Test, motivation to stop smoking assess by Prochasca Test. Result. Amount of sample are 38 samples. 33 patients are male and 5 patients are female. Did not smoking 8 patients, 3 male and 5 female. Conclusion. From 38 patients, 30 patients (78,9%) was smoking.

THE DUALLY DIAGNOSA PATIENT SCHIZOPHRENIA AND SUBTANSTANCE USE DISORDERS AT PSYCHIATRIC DEPARTMENT SANGLAH HOSPITAL DENPASAR – BALI

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ABSTRACT

Background. Approximately 1% of the population has schizophrenia. The ravages of the disease are felt throughout the lifetime of the patient. Men, who are diagnosed with the disorder significant thy more often than women, may have an onset of the disease at a younger age. As with substance dependence, schizophrenia is a chronic, relapsing, and progressive disease estimates are that from one quarter to as may of two thirds of individuals with schizophrenia have a comorbid substance use disorder. The dually diagnosed tend to be hospitalized more often as well. Interestingly, no clear evidence shows that the severity of schizophrenia symptoms or long-term functioning is substantially worsend by the presence of substance misuse, based on the above explaination, the author is interested in to learn. Based on the above explaination the author is interest in to learn the picture schizophrenia and substance inisuse at Psychiatric Department Sanglah Hospital Denpasar – Bali. Goal. This study has a purpose how to approach the patient, how to really initiate, titrate, stop, or combine medications. Method. This study is retrospective quantitative descriptive study, since February 2003 to February 2010. Results. Number of subject study totaly 2500 participant. About 2000 participant (80%) substance misuse nicotine 1000 participant (40%) misuse alcohol, schizophrenia and marijuana (0,4%), misuse ecstasy 72 participant (2,4%) schizophrenia and opioids misuses in this participant in common. Conclusion. Detecting the presince of a substance use disorder in the face of schizophrenia can be difficult. Instruments commonly used to make the diagnosis of substance abuse or dependence.

APOPTOSIS STUDY OF RED FRUIT OIL ETHANOL EXTRACTS (*Pandanus conoideus* Lam) ON CERVIX CANCER CELL LINE SiHa

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ABSTRACT

Background: Antioxidant has been shown to prevent cancer and can serve as an adjuvant therapy for cancer. Red fruit oil (Pandanus conoideus Lam) contains antioxidant carotenoid and tocoferol, therefore may potentially be used in cancer prevention and treatment. Objective: This study was aimed to investigate the effect of P. conoideus Lam on apoptosis of cervix cancer cell line SiHa. Method: This experimental study was done by in vitro culture of cervic cancer cell line SiHa. In this study, the red fruit oil ethanol extract was divided into four different doses, 0.0156, 0.0078, 0.0039, and 0.0019 µL/mL, respectively and replicated three times for each dose. Doxorubicin was used as control positive. The examination of apoptosis effect was evaluated by direct counting after staining with TUNEL method. Results: The apoptosis of cervix cancer cell line SiHa were increased by 69.33±2.08 %, 53.33±3.06 %, 41.33±1.53 %, 33.00±2.65 % following induction of 0.0156, 0.0078, 0.0039, and 0.0019 µL/mL red fruit oil, respectively. The control positive (doxorubicin) increased apoptosis by 77.67±3.05%, 70.00±3.00%, 60.67±2.52%, 49.33±2.52% with dose 3.75, 1.875, 0.9375, 0.4688 µL/mL, respectively. Conclusion: In conclusion, red fruit oil ethanol extract could increase apoptosis of cervix cancer cell line SiHa.

Keywords: Red fruit oil ethanol extract - Apoptosis - Cell culture - Cervix cancer cell line SiHa

NUTRITION IN PREGNANCY RELATED FERRO DEFISIENCY ANEMIA

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ABSTRACT

Physiologic changes will seen in pregnancy to sustain fetal growth and development. Nutritional status before pregnancy is a key factor that influence maternal health. Normal fetal birth weight is influenced by increase of maternal body weight during pregnancy. To keep maternal health and fetal growth stable, there are requared adequate macronutrient and micronutrient during pregnancy. Supplementation of vitmin and mineral are given in deficiency state, but until now supplementation are still given for adequate micronutrient requariment such as ferro. Ferro are needed to perform ferro-sulfida complex and heme. Ferro from food staff are consist of heme and non heme ferro. Ferro intake from food are 10-15 mg, but there are only 1-2 mg will be absorped. Absorption of ferro take place in duodenum and jejenum proximal. The absorption will influenced by gastrointestinal tract condition and content of subtance in food. Based on Indonesian recommended daily allowence 2004, ferro dietary in female dependent of age and physiologic condition such as pregnancy. In 13-49 years old female, ferro dietary are recommended 26 mg daily. In pregnancy, there is added ferro dietary 9 mg daily on second semester and 13 mg daily on third semester. Dietary ferro heme are needed in ferro deficiency and intake of vitamin C as ferro absorption activator. Ferro supplementation in pregnancy is done during first semester, but supplementation before pregnancy has positif effect to decrease ferro deficiency anemia.

Keywords: nutrition, pregnancy, anemia, ferro

FUNCTION OF T-CELL-MEDIATED IMMUNITY DURING TOXOPLASMA GONDII INFECTION

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ABSTRACT

One of the most distinctive immunologic features of Toxoplasma gondii infection is strong and persistent Cell Mediated Immunity (CMI) elicited by parasite, resulting in host protection against rapid tachyzoite growth and consequent pathologic changes. Studies of the importance of T cells in resistance against T. gondii are nonequivocal. Athymic nude mice, which lack functional T cells, are extremely susceptible to both virulent and avirulent parasite strains. Adoptive transfer of immune T cells to naive mice protect animals against challenge with virulent T. gondii strains. Immunogenetic studies also point to a major influence of major histocompatibility complex (MHC) class I and II on resistance and susceptibility to the parasite, consistent with the idea that T lymphocytes are crucial in determining the outcome of infection. Virtually, all mouse strains that develop a strong Th1 immune response to T. gondii has possess resistant. Cytokines such as IFN-γ and TNF-α, which activate macrophage functions are important for controlling tachyzoite replication during both acute and chronic phases of infection. Interleukin-10 (IL-10) and IL-12 appear to be crucial at the initial phase of infection and less important during chronic toxoplasmosis. While IL-12 is clearly important in initiating astrong and effective CMI against T. gondii tachyzoites, IL-10 appears to modulate both IL-12 and IFN-γ synthesis, avoiding an excessive immune response that could cause extensive inflammation and host tissue damage. Thus, IL-10 and IL-12 are two major antagonists involved in regulating IFN-γ synthesis during the initial phase of infection. Whereas NK cells, CD41 and CD81 T lymphocytes appear to be major sources of IFN-γ at the early stages of infection. αβ T lymphocytes are the dominant source of this cytokine during the chronic phase.

Keyword: *Toxoplasma gondii*, T cells, Cytokine

NUTRITION IN CARDIOVASCULAR DISEASE RELATED HOMOCYSTEINE AND VITAMIN B6 CYSTATHIONINE BETA SYNTHASE GENE POLYMORPHISM

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ABSTRACT

Cardiovascular disease is a major cause of mortality in Indonesia. Homocysteine is a compound produced by methionine metabolism. Methyonine is a sulfure essensial amino acid and it will degraded to S-Adenosile-Metionine (SAM) and S-Adenosine-Homocysteine (SAH). High methyonine intake in long period increase plasma homocysteine level. Plasma hyperhomocysteine can remetabolic to form methyonine trought remethylation and to form cystationine trought transulfuration. This metabolism is importance because homocysteine is very reactive and has high risk on vascular. Hyperhomocysteinemia is a vascular risk factor which is not influenced by other major risk factor and its autosomal recesive disorders. Its couse by Cystathionine Beta Synthase (CBS) gene deficiency/mutation/polymorphism which function transform homosysteine to cystathionine (transulfuration). Vitamin B6 is needed to activate CBS enzyme in homocysteine metabolism. Several studies found that CBS gene mutation/polymorphism in Italian is G374A which cause changes arginine amino acid to glutamate, C770T cause changes threonine amino acid to methionine, and T833T cause changes isoleusine amino acid to threonine. Today, there are 17 location of mutation have found in CBS gene. Increase of vitamin B6 intake is importance to sustain the expression of Ce BS gene and expected decrease of cardiovascular disease risk factor.

Keyword: CBS gene, Polymorphism, Homocysteinemia, Cardiovascular disease

CYTOTOXICITY AND ANTIPROLIFERATIF EFFECT OF ETHANOL EXTRACT PURPLE SWEET POTATOES (*IPOMOEA BATATAS* L) ON CELL LINE CERVIC CANCER SIHA

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ABSTRACT

Antioxidants is a reducing agent chemical that reduces the rate of particular oxidation reactions or by breaking the oxidative chain reactions. Antioxidants usually scavenged reactive oxygen species in the body before they can cause damages to the cells. Antioxidant react by releasing their electron to the free radicals in order to stabilize them and hence, preventing cell damages in the body. Cancer is still one of the leading death causes. By consuming antioxidants can reduce the cancer incident. In order to prove the activity of purple sweet potatoes on cancer cell, it is necessary to have some direct research on cancer cell line. This study will be using a simple experimental research method. The activities of the anticancer will be evaluated from cytotoxic and antiproliferatif effects on cell line cervic cancer SiHa. Cytotoxic effects will be examined with cultured cell stained with tryphan blue exlusion. Each well will be containing 36.000 cells, which is given ethanol extract purple sweet potatoes with 10 dose variation starting from 500 µg/mL up to 10.000 µg/mL replicated three times, then the inhibitory percentage is calculated. Antiproliferatif activity will be evaluated by incubating cancer cells that have been given ethanol extract purple sweet potatoes in 3 dose variation; 4,000, 5,000 and 6,000 µg/ml in 24, 48 and 72 hours. Result of this study showed that ethanol extract purple sweet potatoes had cytotoxic activities on SiHa cervic cancer cell line in these dose variations, 500; 1,000; 2,000; 3,000; 4,000; 5,000; 6,000; 7,000; 8,000; 9,000 and 10,000 µg/ml. The cytotoxic activity on each of dose variations above were 31.19; 35.78 ; 42.20 ; 48.62 ; 51.38 ; 57.34 ; 64.22 ; 68.35 ; 73.39 ; 79.81 and 97.61%. Ethanol extract purple sweet potatoes showed antiproliferatif activities on SiHa cervic cancer cells in 24, 48, 72 hours incubation in concentrations 4,000; 5,000; and 6,000 µg/ml; those were 31,500; 27,333; 23,833 cells (24 hours) ; 64,600 ; 55,500; 48,800 cells (48 hours) ; 139,600; 112,300 ; 100,000 cells (72 hours). In conclusion, ethanol extract purple sweet potatoes have cytotoxic and antiproliferatif activities on cell line cervic cancer SiHa.

Keywords: ethanol extract purple sweet potatoes – cell line cervic cancer SiHa
-cytotoxicity and antiproliferatif activities - in vitro

MOLECULAR EPIDEMIOLOGY OF HEPATITIS C VIRUS IN KEDUNG PANE PRISON SEMARANG, INDONESIA

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ABSTRACT

Introduction: Prisoners are being associated with high risk of human blood borne virus infection, including that of Hepatitis C virus (HCV). However, at present there is no molecular epidemiological data about HCV in prisoners in Indonesia especially that of imprisoned in Kedung Pane Prison Semarang, Indonesia. Material and methods: All prisoners willing to sign the informed consent in Kedung Pane Prison (110 persons) were enrolled in this study. Plasma were collected and addressed for serological assay. The nucleic acid was extracted from the anti-HCV positive samples. The RT-PCR nested was performed to detect part of NS5B region of the HCV genome. The positive PCR products were directly sequenced and phylogenetic analysed. Results: The data presents preliminary data results from on going molecular epidemiology study of human blood borne viruses in Central of Java. Anti-HCV positive was found in 32.7 % (36/110) of total samples. The HCV RNA was detected in 12 out of 36 anti-HCV positive samples. Based on 366 bases of the NS5B sequences, the HCV strains were classified into genotypes 1 and 3. The HCV 1a (50 %) was the most prevalent, followed by subtypes 3a (16.7 %), 3k (16.7 %), and 1c (8.3 %), respectively. These results were quite different to all previous reports about HCV molecular epidemiology data in hepatitis patients in Indonesia. Conclusions: Results indicate the discrepancy molecular epidemiology data of HCV found in hepatitis comparing to that of the non hepatitis patients community.

Keywords: HCV, Prison, Prisoner, Indonesia

HEPATOPROTECTIVE POTENTIAL OF VITAMIN C AND VITAMIN E ON THE SWISS-WEBSTER MICE (MUS MUSCULLUS) THAT EXPOSED BY AFLATOXIN

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ABSTRACT

The research was carried out to know the ability of vitamin C and vitamin E to reduce toxic effect of aflatoxin in Swiss-Webster mice. Mice were treated with of 375 µg/kg body weight and 750 µg/kg body weight as a liver destruction inducer then vitamin C and vitamin E at 2 mg/kg body weight were given every day for 15 days. Liver was isolated and fixated in Bouin solution and made histologis preparate by paraffin method and Haematoxylin-Eosin staining. From the preparate was observed the liver tissues and cells destruction of the liver were analysed by analysis of variance (Anova) and continued by Duncan Multiple Range Testif obviously different. The result showed that liver relative weight were not different compare to negative control. Vitamin C and vitamin E at doses 2 mg/body weight effective to decrease the liver tissues and cells destruction such as necrosis, apoptosis, steatosis, steatosis + necrosis. Vitamin E have hepatoprotective potency better than vitamin C to reduce aflatoxin toxic effect liver tissues and cells destruction Swiss-Webster mice.

Keyword: Vitamin C, vitamin E, aflatoxin, liver tissues destruction, liver cell destruction, Swiss-Webster mice.

SCREENING OF PENICILLIN G ACYLASE PRODUCING BACILLUS STRAINS AND CLONING OF THE PAC GENE

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ABSTRACT

Penicillin G Acylase (PAC) is the key enzyme used in the industrial production of betha lactam antibiotics. It hydrolyses the side chain of Penicillin G releasing 6 Amino Penicillanic Acid (6 – APA) and Phenyl Acetic Acid (PAA). 6-APA is the betha lactam nucleus and is the key intermediate in the synthesis of semi-synthetic penicillins such as ampicillin and amoxicillin. PAC activity is present in various organisms including gram positive and negative bacteria, filamentous fungi and yeast. This poster presents screening of the pac gene by using PCR as well as microbiological approach. There were no positive signals revealed by PCR screening using gene specific primer of B. megaterium ATCC 14945 pac gene on genomic DNA of some strains of Bacillus megaterium. PCR screening by using gene specific primer of B. thuringiensis resulted in positives signals on all the tested genomic DNA of B. thuringiensis strains. However further analysis by using microbiological method showed that positive signal was only produced by two of the four positive screened B. thuringiensis. The gene was cloned from the strain giving positive signals in PCR as well as microbiological screening.

CLONING AND EXPRESSION OF *Bacillus subtilis* AQ1 ENDOXYLANASE GENES IN *Bacillus megaterium* USING CONJUGATIONAL TRANSFORMATION METHOD

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ABSTRACT

Endoxylanase is an important enzyme in many industrial applications. We have isolated several potential xylanase-producing bacterial strains from local habitats. One of them was Bacillus subtilis AQ1. An endoxylanase gene from this strain was subcloned into conjugational shuttle plasmid vector pBBRE194 between Xho I and Kpn I site. The mutant endoxylanase gene which experienced silent mutation in the Catabolite Repression Element (CRE) region was also subcloned in the exactly similar method. The endoxylanase genes could be expressed well in E. coli. Using conjugation mechanism between E. coli and Bacillus, these two recombinant plasmids were transformed into Bacillus megaterium. The two kinds recombinant B. megaterium expressing endoxylanase and the mutated one were obtained. The comparison of these recombinant endoxylanase activity in LB, in LB-xylan medium, LB-glucose and LB-glucose-xylan medium were analyzed and compared.

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