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INVESTIGATION TO TRADITIONALLY PRODUCED SOYBEAN FERMENTED FOOD (SERE KEDELE) IN GIANYAR REGION-BALI AND MOLECULAR IDENTIFICATION OF BACTERIA INVOLVED IN THEIR SPONTANEOUS FERMENTATION

Penelusuran terhadap Pangan Tradisional Hasil Fermentasi Kedelai (Sere Kedele) di Kabupaten Gianyar, Bali.dan Identifikasi Molekuler Terhadap Bakteria yang Terlibat dalam Proses Fermentasi Spontan

I Putu Suparthana[1], Agus Selamat Duniaji[1], Masayuki Hashimoto[2*]
[1] Department of Food Science and Technology, Faculty of Agricultural Technology, Udayana University
Jl. Kampus Bukit Jimbaran, Badung-Bali, Tel.: 0361-701801
[2] Institute of Molecular Medicine, College of Medicine, National Cheng Kung University
ShengLi road 367, North distinct, Tainan City, 704-56, Taiwan; Tel.+886-6-2353535 ext 3592,

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ABSTRACT

Sere kedele is a Balinese traditional soybean fermented food which involving spontaneous fermentation process in its production. It's report-ed produced by the people in Southeast area of Bali Island. Despite the importance of this product for communities health, its lack the basic scientific data. This research was aimed to figure out the existence of this product nowadays and characterize the traditional production as-pect, a survey was conducted to collect the data in the field and analyze descriptively. Furthermore, to figure out the microbial aspect, isola-tion was carried out in the laboratory and identify the bacteria by using molecular techniques. The result showed that sere kedele is still known by the most people in Gianyar region, its vended in the traditional market which supplied by four producers which locate in three districts (of seven). The way to produce sere kedele was varying in boiling time, fermentation time and the step of adding spicy. All produc-er applied spontaneous fermentation in the bamboo container, at room temperature. Nine bacterial species identified from this sere kedele, four of them belong to Bacillus species (Bacillus cereus B. flexus, B. subtilis, B. thuringiensis) and the others are Weissella confusa, W. cibaria, Acinetobacter baumannii, Proteus mirabilis and Klebsiella pneumoniae. The bacterial species of sere kedele contain non-pathogenic (B. flexus, B. subtilis, W. confusa) and pathogenic bacteria. From the result of this study, sere kedele production by using starter culture is highly recommended.

Keywords : *Sere kedele; Bacillus species; spontaneous fermentation; molecular techniques.*

INTRODUCTION

Soybean fermented food is the food product which produced from soybean and processed by using fermentation. Indonesian peoples are familiar with those products from many years ago such as tempeh, kecap, and tauco. Among those products, tempeh is con-sidered originated from Indonesia. Tempeh plays an important role to provide a low-cost high-quality protein source for Indonesian people's diet. The protein is provided by the soybean

* Corresponding author:
Email: hashmate@mail.ncku.edu.ta

which known contain in high level and quality among the others beans. This pro-tein is improved by the activity of a mold, Rhizopus, through a fermentation process which makes it easier to be absorbed by the human body.

Soybean is not only an important crop for peoples in Indonesia, for centuries the peoples in Asian countries utilize soybean for food product since it contains good nutrition. Soybean has the high level of protein, 35-40% on dry weight basis [9], its amino acid is on the top among others legumes. Besides the protein, soybean also con-tains the basic constituent of nutrients such as lipids, carbohydrates, vitamins, and minerals. Therefore Asian peoples relied on soybean for protein need of their diet since long time ago considering that the amino acid composition of soybean is close to meat protein and inexpensive than meat. Soybean also contains phytochemicals such as isoflavones which give good effects on human health.

Beside the good nutrition value of soybean, the row soybean con-tains anti-nutritional factor. Trypsin and protease inhibitor, lectins, pectin and anti-vitamin substances for instance [8], it's limiting the nutritive value of soybean. Therefore, food processing such boiling (high temperature) or fermentation prior consumption of soybean has applied by Asian peoples for a long time ago with the aim to reduce anti-nutritional factor, and also selected as a way for food preservation. The microbial population which involved in fermenta-tion process produce proteolytic enzymes, subsequently, the soy-bean proteins were hydrolyzed by those enzymes which make soy-bean proteins easy to digest.

Some fermented soybean product which famous in the Asian coun-try such as thua nao in Thailand [2], natto in Japan [6], chung-kokjan in Korea [12], and kinema in India [14]. In contrast to the Indonesian tempeh, it's reported that Bacillus strains play a role in their fermentation process resulting in a sticky soybean food. Koswara (1997) is the one who reported the existence of sticky soybean food in Indonesia. The product namely sere kedele was made traditionally as a household industry by the peoples in South-east area of Bali Island, Indonesia [5]. Sere kedele was consumed by the peoples as the snack or substitute the meat in their meal. In the production steps, after the fermentation process, the spices add-ed to give flavor to the product.

Fermentation process which applied to soybean is also resulting in the release of peptides by the activity of proteolytic enzymes pro-duced by the microbes. Bioactive peptides are considered as an alternative to synthetic drugs. Some studies showed that bioactive peptides have therapeutic properties such as antioxidant [10], anti-tumor [3], and antidiabetic [7]. Despite the importance of fermented soybean food for human health, sere kedele is lack of attention by the peoples and local scientists. This study becomes the first which report the existence of sere kedele nowadays especially in Gianyar region, the Southeast area of Bali island; describing the production process traditionally; analyze the proximate nutrition; further ana-lyzed molecularly the bacterial species from sere kedele.

METHODS

The Study Area and Survey Design

The study was conducted in Gianyar region, Southeast area of Bali Island. This region has seven districts and one public market located in each district. The existence and production process nowadays of the product sere kedele was traced from the public market in Gian-yar region. The population is sere kedele which vended in public market. Sampling was carried out by using cluster random sam-pling. The vendor was targeted in this sampling to obtain infor-mation about the producer. All producer subsequently was set as samples to obtain data about the production process. The data was collected by using questioner.

Proximate Analysis

Sere kedele was collected from each producer and brought to the laboratory for proximate analysis including water content, ash, pro-tein, fat, and carbohydrate. Water content was analyzed by drying method [12]. The ash content was analyzed by incandescent in muf-fle [12]. Protein was analyzed by semi-micro Kjeldahl [12]. The fat was analyzed by Soxhlet extraction [12]. Carbohydrate was counted by using carbohydrate by different method [14].

Microbiological Samples Preparation

Three kinds of samples, those including the soybean which added spices before fermentation (BfS), the fermented soybean without spices (AfNs), and the soybean which added spices after fermenta-tion process (AfS) were used for microbial analysis. The samples were collected from two producers locate in Blahbatuh district. The samples were collected aseptically in the sterile plastic bag and kept in box contain ice, then transported to the laboratory and stored in a refrigerator (4oC). Five grams samples were homogenized in a mor-tar and suspended with 45 ml peptone water. Meanwhile, three kinds of culture media, namely Nutrient Agar (NA), Plate Count Agar (PCA) and deMan Rogosa Sharpe Agar (MRSA) prepared according to the manufacturer's instruction. One ml sample solution was applied to a serial dilution (10-1 to 10-6) in peptone water and followed by culturing 100 µl of the diluted sample onto those three kinds of plate mediums (NA, PCA, and MRSA). The plates were incubated at 37 oC for 24 hours.

Observation and Isolation of the Colonies

The colonies which grew on those three kinds of plate mediums were observed by using magnifier glass (10x) and differentiated morphologically. A single colony respectively which shows differ-ences in morphology was selected for subculture in broth medium for 24 hours at room temperature with shaking, followed in next day by subculture (strike) onto the new plate mediums and incubat-ed at 37 oC for 24 hours. At this step, single colony bacteria was considered have isolated from sere kedele. The data was document-ed by the photograph (by using microlens camera), and isolated bacteria were preserved in nutrient broth medium containing 25% (v/v) glycerol at -70 oC for further analysis.

Colony PCR Amplification

Isolated bacteria were refreshed from glycerol stock culture solu-tions and subcultured onto NA, PCA and MRSA plate medium. The amplification of the 16S rDNA region was carried out by colo-ny PCR with the following universal primers: forward 5'-AGAGTTTGATCCTGGCTCAG-3' and reverse 5'-AAGGAGGTGATCCAGCCGCA-3' [16].

16S rDNA Sequence Analysis

The sequencing reactions were conducted by using ABI PRISM 3100 Genetic Analyzers (Applied Biosystem) in forward and re-verse direction with the same primers used for PCR amplification. The sequence obtained was matched with the published 16S rDNA sequence which available in GenBank database by using BLAST.

RESULT AND DISCUSSION

The Existence of Sere kedele in Gianyar region

Koswara (1997) was first mentioned the existence of sere kedele which found produced by the peoples in Southeast area of Bali Island, Indonesia [5]. The Bali Island which is a province of Indo-nesian country has eight regions and one capital city. One region which is located in Southeast area is Gianyar region which has seven districts under its administration. The public market which is

ruled by Gianyar government locate in each district. The infor-mation about the recent condition of sere kedele was traced from the public market since the product, sere kedele is considered as tradi-tional food which is usually vended in public market. By this strate-gy, the recent condition of sere kedele as like: do peoples still know sere kedele, what is like of the product, where to buy sere kedele, were able to be understood. Subsequently, the producer who sup-plied sere kedele in public market could be traced so that the pro-duction process could be understood.

As the result of the survey, the traditional food of sere kedele is still known to date by the peoples in Gianyar region. It's vended togeth-er with vegetables as a 50 g product which packed in a plastic pouch (Poly Ethylene) with price from Rp. 1000 to 2000 (0.2 US dollar). The form of the product is served as the whole fermented soybean (not dry) which already mixed with spices. It was not found in Gianyar region the product which formed as dry food with thin round shape as reported by Koswara (1997) [5]. Seven ven-dors were founded at 7 public markets among 14 public markets which locate in 5 districts of Gianyar region. Among the seller, 4 of them are the producer of sere kedele which locate in 3 districts namely Sukawati (1 vendor), Gianyar (1 vendor) and Blahbatuh (2 vendors). Subsequently, the information about production process was obtained from those vendors.

Characteristic of Sere kedele in Gianyar region

The production process of sere kedele in Gianyar region was varied among the four producers. The first step of the sere kedele produc-tion process is boiling after screening from unwanted material and washing of the soybean. The boiling time was varied among the producer with the range from 3 to 7 hours. The second step is fer-mentation, the longer time of fermentation which carried out spon-taneously by all producers was varied from 1 to 3 days. The third step is spicing addition, the variation showed that one producer (Blahbatuh district) added spices before fermentation process and the other 3 producers added spices after the fermentation process. All producer used container which made by the bamboo skin for the fermentation process, 2 of them closed the container tightly, howev-er, the other 2 of them let the container little bit open. The utilization of banana leaf or others leaf was not founded in the production process at all producer.

The proximate composition shows some variation as follow, the water content ranges from 56,89% to 64,65%. The ash content ranges from 1,56% to 2,42%. Protein content ranges from 14,90% to 20,93%. Fat content ranges from 5,34% to 9,50%. Carbohydrate content range from 10,81% to 12,97%.

Bacterial Identification

Bacterial identification from sere kedele was summarized in Table 1 and colony's morphology were shown in Figure 1. Bacterial isola-tion by using NA medium resulting in two colonies which showed difference morphology (Mrp 1 and Mrp 2). Gram stain

Table 1. Identified bacteria from *sere kedele* by using molecular analysis

Sample's name	Culture medium	Gram stain	Catalase production	Species
AfNs-1	NA	-	+	*Bacillus thuringiensis*
AfNs-2	NA	-	+	*Proteus mirabilis*
AfNs-3	PCA	-	+	*Acinetobacter baumannii*
AfNs-4	PCA	-	+	*Klebsiella pneumoniae*
AfS-1	PCA	-	+	*Klebsiella pneumoniae*
AfNs-5	MRS	+	-	*Bacillus cereus*
BfS	MRS	+	-	*Bacillus flexus*
AfS-2	MRS	+	-	*Bacillus subtilis*
AfNs-6	MRS	+	-	*Weissella confusa*
AfNs-7	MRS	+	-	*Weissella cibaria*
AfS-3	MRS	+	-	*Weissella confusa*

AfNs : After fermentation Non-spices
AfS : After fermentation with Spices
BfS : Before fermentation with Spices

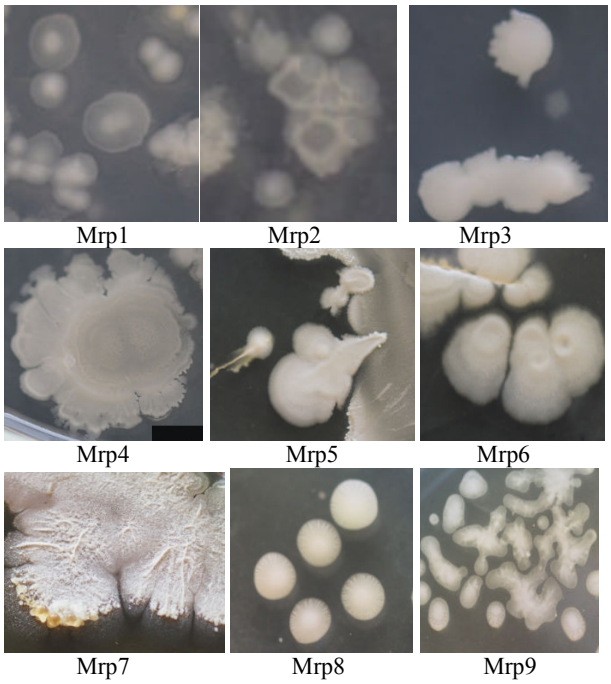

Fig. 1: Different colony morphology of the bacterial founded from *sere kedele*. Mrp: Morphology.

the result showed that both bacteria were Gram-negative bacteria. Catalase production analysis of both bacteria was positive. Further analysis by using 16S rDNA sequence showed that both species were identified as *Bacillus thuringiensis* (AfNs-1) and *Proteus mirabilis* (AfNs-2). There were also two isolates which showed difference colony morphology on PCA medium (Mrp 3 and Mrp 4). Both were Gram-negative bacteria and showed positive catalase production test. Sequence analysis of 16S rDNA showed that both species were identified as *Acinetobacter baumannii* (AfNs-3) and *Klebsiella pneumoniae* (AfNs-4). *K. pneumoniae* was founded in fermented soybean with and without spices addition (AfNs-4 and AfS-1 respectively).

Isolation by using MRS agar medium resulting in five isolates which showed difference colony morphology. All isolates belonged to Gram-positive bacteria and showed negative catalase production. Five bacterial species could be confirmed by using 16S rDNA sequence analysis. *Bacillus cereus* (AfNs-5), *Weissella confusa* (AfNs-6) and *Weissella cibaria* (AfNs-7) were founded in fermented soybean without spices addition. *Bacillus subtilis* (AfS-2) and *W. confusa* (AfS-3) were founded in fermented soybean with spices added. *Bacillus flexus* (BfS) was founded in *sere kedele* which added spices before fermentation.

Sere kedele is Balinese soybean fermented food which produced traditionally by the peoples in Southeast area of Bali Island. The production is simple, the soybean is boiled until they are tender, and then it's drained and cooled until the water is dry up well. This cooked soybean is prepared in a basket with or without leaf cover. Subsequently, it's fermented spontaneously in the particular room. This room is always used for the fermentation process, so that, the room is saturated with the spores. The fermentation process usually carried out in 1 to 2 days [5].

Sasuke (1972) mentioned that *sere kedele* has existed in Bali, Indonesia and it was relative to *natto*, *joenkuk-jang*, *thua-nao*, and *kinema* [11]. *Natto* is the Japanese soybean fermented food which studied well from the long time ago. It's produced by a single bacterial strain, *Bacillus subtillis* (*natto*), and now become a commercial product worldwide. *Joenkuk-jang* (or *Chungkokjang*) is a Korean fermented soybean paste, it's usually served as the soup with boiled rice [4]. *Thua-nao* is a traditional fermented soybean food which produced in northern Thailand. The cooked soybean is mashed and fermented in banana leaves for 2 to 3 days to produce *Thua-nao* [1]. *Kinema* is the fermented soybean food of Eastern Himalayan regions of Nepal, Darjeeling hills in India and Sikkim in Bhutan. It appears as a whole-soybean with a sticky texture and gray color.

Sere kedele as the relative of *natto*, *joenkuk-jang*, *thua-nao*, and *kinema*, has very limited data. In contrast, *tempeh* which known as Indonesian originated fermented food is well studied, however *tempeh* is considered not relative to *natto* since the soybean of *tempeh* is

fermented by mold *Rhizopus* sp. As depicted from Sasuke (1972), "Natto triangle" is a geographical area within East-, South-, and Southeast Asia which shows the indigenous of non-salted fermented soybean food in Asia. The corner of the triangles including in northeastern Japan, on the northeast of *natto*; northeastern India and Nepal, on the west of *kinema*; and in Java, Indonesia, on the south for *tempeh*. The *natto* triangle theory was proposed to hypothesize the origin of *natto* in Japan. Initially, *natto* was the presumption that came from Java, however many researchers after Sasuke's theory in 1972 suggested that "it was not correct to think that *tempeh* as a type of *natto* by any definition".

This study is the first which report the bacterial aspect of *sere kedele*. The recent condition of *sere kedele* was also investigated especially in Gianyar region which locates at the Southeast area of Bali Island, as the background information to support the analysis of bacterial aspect. The molecular analysis in this study confirmed the bacterial involvement in their spontaneous fermentation. It is also confirmed that *sere kedele* is different with *tempeh*.

CONCLUSIONS

The recent condition of a non-salted soybean fermented food namely sere kedele which produced traditionally by involving spontaneous fermentation was investigated in a region (Gianyar) of Bali island (a province of Indonesian country). The survey revealed that sere kedele is still existed nowadays as a traditional food and vend-ed especially in Gianyar region. Molecular analysis in this study is the modern approach which applied to the traditional food in Bali to get the insight view of microbial aspect. The molecular technic (16S rDNA sequence analysis) confirmed the species level of bacteria which involve the spontaneous fermentation of sere kedele. The non-pathogenic and pathogenic bacteria which founded in this product lead to the need of utilization of starter culture in sere kedele production. Future analyses of predominant bacteria which produce sere kedele is considered the key answer which leads to the starter culture composing.

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