

Probiotic Potential Test for Lactic Acid Bacterial Isolate from Labi Cattle Gastric Again Low Acidity and Sodium Deoxycholate

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Abstract. Lactic acid bacteria (LAB) are one of group probiotic bacteria, which have a positive impact on human and animal health. The aim of this research was to determine the potency lactic acid bacterial isolate from LABi cattle gastric as a probiotic that have resistance to low pH and sodium deoxycholate. The study was started with the cultivation test that aims to made sure uncontaminated isolates of microorganisms, including test for growth response in MRS broth medium, test on catalase production, and Gram stain. Its potency for probiotic development was tested by growing of isolate in MRS broth medium with low pH conditions (pH 2, 3, 4) and in MRS medium supplemented with various concentration of sodium deoxycholate (0,2 mM, 0,4 mM, 0,6 mM). The result showed that isolate from LABi cattle gastric was able to grow well in MRS broth medium under anaerobic condition. In addition, it also showed catalase negative and Gram-positive bacteria. In the main experiment, this isolate was found to be resistant to low pH conditions (up to pH 2) and high concentration of sodium deoxycholate (up to 0,6 mM). These results indicate that the isolate the lactic acid bacteria have resistance to low pH and the sodium deoxycholate, which is the main requirement of the probiotic bacteria.

Keywords: Lactic Acid Bacteria, Probiotic, Bali Cattle

I. INTRODUCTION

Lactic acid bacteria (LAB) is a bacterium that is safe for its host and is able to produce compounds that can inhibit the growth of pathogenic bacteria [1]. In general the LAB is defined as a group of Gram-positive bacteria, it does not produce spores, spherical or rod, catalase negative, have no cytochrome, aerotolerant anaerobic, microaerophilic bacteria until, and requires the nutrients the complex such as amino acids, vitamins (B1, B6, B12, and biotin), purin, pirimidin[2];[3].

Research on lactic acid bacteria (LAB) as a probiotic has done before, by [4] mentioning the type of LAB of rumen fluid of the origin of the genus *Lactobacillus* the superior candidate probiotics. Generally, the bacteria grow optimally at neutral pH conditions 7.0. However, generally the species LAB more tolerant of environmental conditions pH acidic [5]. This relates to the conditions of the digestive tract of animals that belong to have the condition with low pH or acid. A cow or a healthy ruminant, normally have a different pH along the digestion channel, starting from reticulum-gastric pH 5.3-6.5; abomasum pH

2.2-4.1; and the intestines with pH 5.6-6.8 [6].

II. RESEARCH METHOD

The Cultivation of Isolates

The cultivation of 5 isolates begins with planting the 5 isolates LAB (SR 1, SR 2, SR 3, SR 12, SR 13) by way of reculture isolates by taking each of the isolates LAB by using mikropipet as many as 20 μ l, then inserted into a tube containing 5 ml of MRS broth. Next tubes that already contains isolate of incubation temperature 37°C for 24 hours in an anaerobic. The success of reculture isolates characterized by the presence of turbidity on the media MRS broth.

Catalase Test

Catalase test is done to find out the presence of the enzyme catalase activity. Prepare 5 object glass for each isolate, on glass objects add 10 μ l LAB each isolate then added 10 μ l H₂O₂ 10%.

Gram Staining

Gram staining in the 5 isolates LAB do with coloring preparations that have been fixation using Crystal violet is 2%, it is left for 1 minute 30 seconds, then washed with running water, then ditetesi liquid lugol's and left for 1 minute 30 seconds. Washed again with water flowing, then dripping with acetone and alcohol left for 30 seconds, and then washed with running water. Next tinged with safranin for 1 minute 30 seconds, then washed again with water flowing. Tinting results can be seen under a microscope with 1000 magnification. Gram-positive bacteria will appear purple, while

gram-negative appear red under the microscope [7].

Test for Lactic Acid Bacteria Resistance against Low pH

Isolates LAB who has been through the test, catalase test, Gram staining potential of antimicrobial, immune-test performed next against low pH (pH 2.3, and 4). As many as 100 μ l isolates LAB which has been suspended from the stock culture of earlier research, put in a Eppendorf tube. Each of these tubes already contains 900 μ l media MRS. Broth which has adjusted its pH (pH 2.3, and 4), and then inkubasion in a waterbath temperature 37 °C for 3 hours. Furthermore, the suspension centrifuged at speeds of 3000 rpm for 5 minutes and supernatant removed. Bacterial pellet obtained was washed twice using 300 μ l of solution copy with vortex and centrifuged again. Then the pellet obtained suspended, and as many as 50 μ l suspension is inokulasi into 5 ml of medium MRS. Broth with a neutral pH for the next inkubasion at a temperature of 37°C for 24 hours in the anaerobic.

Test the Resistance of the Lactic Acid Bacteria against Sodium Deoxycholate

Test the resistance of the LAB against sodium deoxycholate is performed with the procreative LAB 5 isolates from stock germs into 5 ml of medium with a pH of liquid in MRS. 7.2. Each of as many as 50 μ l of isolates that are grown into test tubes containing 5 mL of liquid with MRS. treat each other between the control tube (media not added liquid sodiumdeoxycholate, treatment of 1, 2, and

3 each added as much as 10, 20, and 30 μ l, so that at each end of the concentration obtained treatment amounting to 0.2; 0.4; and 0.6 mM. Furthermore all the tubes are incubated at a temperature of 37 ° C for 24 hours in anaerobic. Endurance level measured by LAB isolates turbidity (OD 660 nm) using a spectrophotometer. When its absorbance (A) values < 0.1 then the strains of bacteria resistant to the is not considered, and if $A \geq 0.1$ then strains resistant to LAB [8].

Data Analysis

All data are obtained from the results of this study are analyzed in a descriptive and presented in the form of tables or images.

III. RESULTS AND ANALYSIS

In this study, the cultivation of 5 isolates grown back was able to grow well, it is characterized by the presence of sediment on the media. Furthermore, the results of the test of catalase on the 5 isolates of lactic acid bacteria when ditetetsi with the 10% H₂O₂ does not produce bubbles of oxygen gas (O₂), this shows that the 5 isolates of lactic acid bacteria react negatively to a test of catalase. Lactic acid bacteria are generally classified into a group of anaerobic bacteria, these bacteria do not have the enzyme catalase which can break down H₂O₂ into H₂O and O₂. According to

[9],[10] stated that, the oxygen in the environment of anaerobic bacteria have properties of toxins and can inhibit the growth of anaerobic bacteria. While the Results of Gram staining isolates LAB has been done using a light microscope (magnification 1000 x) as presented in Figure 1 and summarized in table 1.



Figure 1. Gram Staining test result isolates SR 3

Pay attention to the results of the ability of the LAB in the 5 isolates to inhibit the growth of bacteria *Staphylococcus aureus* ATCC 29213 indicator, then the 5 isolates can be said to be a potential for further characterized as candidate's probiotic. As is known, generally a bacterium as a probiotic bacteria must have terms include: 1) it is resistant to acids, bile salts, and anaerobic conditions, 2) is derived from the genus *Secure* consumed, 3) has the viability high so it stays alive, growing, and active in the gastrointestinal tract, 4) was able to inhibit or kill the pathogen bacteria [5].

TABLE 1. THE RESULTS OF GRAM STAINING 5 ISOLATES OF LACTIC ACID BACTERIA

Isolate	Type	Form	Arangement
SR 1	Gram positive	Bacillus	Chain
SR 2	Gram positive	Coccus	Group
SR 3	Gram positive	Bacillus	Chain
SR 12	Gram positive	Coccus	Chain
SR 13	Gram positive	Coccus	Group

Further testing of 5 lactic acid bacteria isolates on their antimicrobial activity on muellerhinton media to show the formation of a fairly large diameter killing zone (Table 2).

TABLE 2. RESULT OF ANTIMICROBIAL AKTIVITY TEST FOT 5 LACTIC ACID BACTERIA ISOLATE

Isolate	Diameter Zone (Killing Zone) (mm)
SR 1	5,4
SR 2	5,8
SR 3	6,4
SR 12	6,0
SR 13	6,0

Endurance 5 isolates LAB that have been tested with a low pH indicates that isolates possess resistance to environment with a pH of 2 – 4, despite a decrease in growth rate when presented at a lower pH (table 3). Therefore, it can be said the 5 isolates LAB has tolerance of low pH. In general, the LAB has tolerance in different

pH conditions. Tolerance high enough LAB against acid caused due to bacteria that are able to maintain a higher intracellular pH than extracellular pH [11]. This can occur through the synthesis of the enzymes and the expenditure of a proton (H⁺) from the inside of cells that occurs through the process of hydrolysis of ATP, so an increase in the pH of the cytoplasm [12].

TABLE 3. RESISTANCE OF 5 LAB ISOLATES AT LOW PH

Isolate	Growth Control	LABGrowth		
	(OD pada λ 660 nm)	(Optical Density at λ 660 nm)		
	pH 6,5	pH 2	pH 3	pH 4
SR 1	1,327	1,063 (\pm 0,005)	1,095 (\pm 0,002)	1,106 (\pm 0,002)*
SR 2	1,317	0,998 (\pm 0,003)	1,137 (\pm 0,002)	1,193 (\pm 0,001)
SR 3	1,305	0,964 (\pm 0,003)	0,998 (\pm 0,003)	1,127 (\pm 0,002)
SR 12	1,488	1,022 (\pm 0,002)	1,078 (\pm 0,005)	1,095 (\pm 0,003)
SR 13	1,300	1,008 (\pm 0,003)	1,046 (\pm 0,003)	1,088 (\pm 0,002)

Note:

OD \geq 0.1 (low pH resistant); OD \leq 0.1 (can't stand low pH), [8].

(*) The figures in the table are the result of the average of 3 repetitions (\pm standard deviation of absorbance values isolates).

TABLE 4. RESISTANCE OF 5 ISOLATES LAB AT SODIUM DEOXYCHOLATE

Isolate	Growth Control	LABGrowth		
	(OD pada λ 660 nm)	(Optical Density at λ 660 nm)		
	without Sodium Deoxycholate	0,2 mM	0,3 mM	0,4 Mm
SR 1	1,355	0,833 (\pm 0,001)	0,813 (\pm 0,002)	0,785 (\pm 0,004)*
SR 2	1,394	1,002 (\pm 0,007)	0,955 (\pm 0,001)	0,912 (\pm 0,005)
SR 3	1,307	0,990 (\pm 0,004)	0,939 (\pm 0,005)	0,895 (\pm 0,004)
SR 12	1,318	1,058 (\pm 0,005)	1,001 (\pm 0,004)	0,974 (\pm 0,003)
SR 13	1,379	1,005 (\pm 0,004)	0,978 (\pm 0,005)	0,929 (\pm 0,005)

Note:

OD \geq 0.1 (low pH resistant); OD \leq 0.1 (can't stand low pH), [8].

(*) The figures in the table are the result of the average of 3 repetitions (\pm standard deviation of absorbance values isolates).

On table 4 shows that the 5 isolates LAB has resistance to with concentrations of 0.2 mM – 0.6 mM, despite a decrease in growth rate when the concentration of the is getting improved. These results are in accordance with research conducted by [13] are testing the resilience of isolates of lactic acid bacteria on media containing bile

salts with different concentrations, after incubated for 24 hours show an increase in microbial cell death in line with increasing concentrations of bile salts that are used in the treatment.

Good tolerance against bile salt is suspected because of the role of the polysaccharide as one of the constituent

components of the cell walls of Gram-positive bacteria as LAB [14]. Research on resistance against bile salt acidity

LAB plantarium was able to withstand the acidity of bile salts with a living cell number of 1.2×10^9 CFU/ml [15]. It is accordance with the statement of [16], that some of the genus *Lactobacillus* have the enzyme bile salt hydrolase (BSH) can hydrolyze bile salts. This enzyme is able to change physical and chemical capabilities owned by bile salts, so it is not toxic for LAB. This allows to cause some isolates resistant to salt State LAB bile. However, the higher the concentration of bile salts, then the number of dead microbial cells will also increase. This is due to the increase of active

IV. CONCLUSSION

Based on the results of the research that has been conducted against the 5 isolates, LAB results rumen fluid isolation from cattle , with the ability antimicrobial activity that is quite extensive, and have a tolerance of low pH (pH conditions survival 2) and the relatively high concentration of (concentration of 0.6 mM), then it can be diimpulkan that the 5 isolates potentially developed as candidates superior probiotic bacteria.

LAB has ever done with the result that one of the genus *Lactobacillus*, namely

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