Sys Rev Pharm 2020; 11(3): 583 – 586
A multifaceted review journal in the field of pharmacy
E-ISSN 0976-2779 P-ISSN 0975-8453

Characterization of Lactic Acid Bacteria and Determination of Antimicrobial Activity in Dadih from Air Dingin Alahan Panjang District, Solok Regency-West Sumatera

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Article History: *Submitted: 09.01.2020* *Revised: 25.02.2020* *Accepted: 20.03.2020*

ABSTRACT
Traditional Indonesian fermented foods have been studied as potential probiotics, for example dadiah from West Sumatera are made by fermenting buffalo milk in bamboo tubes. But the potential of halal probiotics isolated from Air Dingin District, West Sumatra (Indonesia) has not been studied. The purpose of this study was to determine the potential of halal probiotic lactic acid bacteria (LAB) Lactobacillus plantarum strain 8m-21 isolation from dadiah in Air Dingin Alahan Panjang District, Solok Regency West Sumatera (Indonesia) against to pathogenic bacteria and antimicrobial activity. Previously lactic acid bacteria Lactobacillus plantarum had been isolationand molecular identification used I6S rRNA with Forward (27F AGAGTTTGATCCTGGCTGAG) and Reverse primer (1492R; GTTTACCTTACGACTT). In this study we analyzed the probiotics have antimicrobial activity against pathogenic bacteria Escherichia coli O157. The results showed that Lactobacillus plantarum strain 8m-21 from Air Dingin dadiah isolates have probiotic properties because they have antimicrobial properties which able to inhibit Escherichia coli, aerob (pathogenic) bacteria and have highest antimicrobial with 20.25 mm inhibition than other sintetic antibiotics.

Keywords: Antimicrobial, Dadiah, Probiotic, and Lactobacillus plantarum

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DOI: 10.31838/srp.2020.3.76

INTRODUCTION

Dadih is a fermented milk product that is traditionally stored for 1-2 days, because it uses a bamboo tube container. Dadih originated from West Sumatra made from buffalo milk and then put into bamboo tubes and covered using banana leaves or plastic and fermented at room temperature for 1-2 days to form clots (Elida, 2002). Fermentation is carried out by lactic acid bacteria (LAB), which may be present on bamboo or from a cover. From some studies it is known that dadih contains good bacteria, namely lactic acid (Lactobacillus casei) which is potential as a probiotic. Lactic acid in dadih plays a role in the formation of texture and taste.

Dadih contains LAB which is potential as a probiotic, which is a living microbe attached to the intestinal wall and is beneficial for the life and health of its host. LAB has a good effect on health because the metabolites produced can inhibit bacterial pathogens, reduce cholesterol, are antimutagenic, anti-carcinogenic and antivaginitis, improve the immune system, prevent constipation, and produce vitamin B and crystallosin bacteria (Sari 2007). LAB and its derivative products are able to prevent various diseases such as preventing enteric pathogenic bacteria, reducing blood cholesterol levels, preventing colon cancer, antimutagen, anticarcinogenic and increasing endurance. In addition, dadih is thought to be effective as an antivaginitis. According to Pato (2003), consumption of curd or products containing LAB from dadih has the potential to prevent cancer, especially colon cancer. This is probably because LAB in dadih is able to reduce and inhibit mutagenicity caused by food. The mechanism of the antimutagenic effect takes place because of the bonds between mutagens or carcinogens with peptidoglycan contained in the LAB cell wall in the dadih. Mutagens and carcinogens that are bound by these bacteria will be released through feces and urine.

According to Purwati, Arief and Rahmadi (2011) that dadih has different lactic acid bacteria in each region and can be identified using 16S rRNA. Lactic acid bacteria (LAB) are a group of bacteria that can convert carbohydrates (glucose) into lactic acid.

MATERIALS AND METHODS

LAB Isolation and Purification

The LAB was isolated purified according to the method described by Purwati et al. A tempoyak sample (1g) was diluted 9 mL of MRS broth MERCK. A 10^{-1} dilution was placed into an anaerobic jar and incubated for 24h at 37°C. The 10^{-1} dilution was then placed into a test tube containing 0,9 mL of MRS broth solution and serially diluted in 10^{-5}. Next, 100 uL samples of the 10^{-5} dilution were spread on Petri dish containing MRS agar MERCK and incubated at 37°C for 48 h.

Isolate characterization

Isolates were examined by Gram staining and catalase reaction tests and cell shape was evaluated microscopically. Gas production from glucose was tested using a Durham tube and MRS broth to determine the fermentation type.

Resistance Test against Acid

Bacterial culture of 1 mL was inoculated on MRS Broth media 9 mL and incubated at 37°C for 24 hours. Then, as much as 1 mL of bacterial culture from MRS Broth was put into a reaction tube containing 9 mL MRS Broth without pH control (control) and on MRS Broth pH 3 (pH regulated by the addition of HCl 5N) and incubated for 90 minutes. Next, pH 3 and control culture were diluted to 10^{-6} then planted using the spread method to the MRS media to be incubated at 37°C for 48 hours. The number of bacteria that can survive was calculated by the Colony FormingUnit (CFU). Comparison of cell numbers before and after incubation will be expressed in the form of viability (%). The higher

percentage of viability produced indicates the more resistant the bacteria to low pH.

Resistance Test against Bile Salts

Bacterial culture of 1 mL was inoculated on MRS Broth media 9 mL and incubated at 37°C for 24 hours. Then, as much as 1 mL of bacterial culture from MRS Broth was put into a reaction tube containing 9 mL MRS Broth without oxgall control (control) and on MRS Broth with oxgall 0.3% then incubated for 24 hours. Next, oxgall 0.3% and control culture were diluted to 10^{-6} then planted using the spread method to the MRS media to be incubated at 37°C for 48 hours. The number of survival bacteria was calculated by the Colony Forming Unit (CFU). Comparison of cell numbers before and after incubation will be expressed in the form of viability (%). The higher percentage of viability produced indicates the more resistant the bacteria to bile salts.

Antimicrobial Activity

Antimicrobial resistance test is implementing by using well diffusion method toward three testing bacteria namely *Escherichia coli* O157. 1 mL centrifuged lactic acid bacteria culture with 10.000 rpm velocity for 5 minutes length with 27°C temperature, the supernatant is used as a microbe resistance. 20 ml of Natrium Agar (NA) medium is added with 0.2% rejuvenated testing bacteria, sit still to cool down in a petri dish. Next, a ±6.5 mm diameter hole is created at NA medium. After that, antibiotics are added such as penicillin, kanamycin, and ampicillin as a positive controller in order to compare the inhibitory zone which was formed during pathogenic bacteria testing. An antibiotic was added by using paper disc containing antibiotics in order to reveal the resistance and sensitivity of pathogenic bacteria testing by using antibiotics positive control. Next, injecting 50 µl supernatant lactic acid bacteria (LAB). After that, incubated it at 37°C temperature. The observation was performed toward a transparent zone in a round shape after 24 hours.

RESULT AND DISCUSSION

LAB Isolation and Purification

The results of the calculation of the total colony of lactic acid bacteria from Dadih Air Dingin from Solok Regency can be seen in table 1 below.

Table 1: Total Colony of Lactic Acid Bacteria from Dadih Air Dingin from Solok Regency

Sample Code	Total Colony of Lactic Acid Bacteria ($x10^8$ CFU/g)
DAD	8,0

Results of research for total LAB dadih air dingin (DAD) is 8,0 x 10^8 CFU / g. This is in accordance with the FAO / WHO criteria (2002) because as LAB probiotic food the yield must be in the amount of 10^6 - 10^8 CFU / gram. This is consistent with what Nuraini, Ibrahim and Rianingsih (2014) stated that during the fermentation process, lactic acid bacteria will convert carbohydrates into lactic acid. Jannah, Legowo, Promono, Al-Baarri and Abduh (2014) added that the greater the sugar used to produce lactic acid, the greater the activity of lactic acid bacteria.

Figure 1: Colony of Lactic Acid Bacteria from Dadih Air Dingin from Solok Regency

Isolate characterization

Gram staining was carried out on Cold Water Dadih samples from Solok Regency. Gram staining test results obtained in this study were Gram positive (+) with the characteristics of each LAB isolate absorbing purple dye from crystal violet in the form of bassil (Rod). Absorption of dyes in bacteria is caused by differences in cell walls or peptidoglycan. Gram-positive bacteria have thicker peptidoglycan than Gram-negative bacteria.

The gas test results obtained by all Cold Water Dadih isolates are homofermentative bacteria types, the bacteria whose main product is lactic acid. This is indicated by the absence of gas bubbles in the durham tube which is placed in the MRS Broth MERCK media. These results are also in line with the opinion of Syukur and Purwati (2013) which states that LAB homofermentative involves the Embden Meyernof-Parnas pathway, which is glycolysis that produces lactic acid, 2 moles of ATP from 1 glucose / hexose molecule under normal conditions, does not produce CO2 and produces cell biomass twice as many as heterofermentative LAB. Ross, Morgan and Hill (2002) add that members of homofermentative groups include Leuconostoc, Weissella and some Lactobacillus.

The catalase test results were obtained on all tempoyak samples, namely negative catalase. Hidayat and Alhadi (2012) added that the catalase test was used to determine catalase activity in the bacteria tested, most bacteria produce catalase enzymes that can break down H_2O_2 into H_2O and O_2. Further explained that catalase is one of the enzymes used by microorganisms to decompose hydrogen peroxide, negative catalase bacteria do not produce bubbles, this means that H_2O_2 given is not broken down by negative catalase bacteria, so it does not produce oxygen. Syukur et al (2014), states that the positive reaction of catalase is shown by forming bubbles meaning that there is the formation of oxygen gas as a result of the breakdown of H_2O_2 by the enzyme catalase produced by these bacteria. LAB is a

negative catalase bacterium, so it does not produce air bubbles.

Resistance Test against Acid

The ability of *Lactobacillus plantarum* strain 8m-21 isolat from dadiah in Air Dingin Alahan Panjang District Solok Regency, West Sumatra (Indonesia) against acidic condition with the number of control living bacterial (without pH 3 settings) was 220 x 10^8 CFU/ml after the pH 3 settings. The number of living bacterial cell was 16 x 10^8 CFU/ml and produced 7% of the lactic acid bacteria viability. Resistance test against acid is one of the characteristics of probiotics where probiotics must be resistant and able to live in an acidic atmosphere. Viability is the ratio of the number of living cells to after being given the acidity settings expressed in the form of %. The higher the viability of LAB produced, the higher the resistance of bacteria or LAB isolates to Resistance test against acid.

According to Susanti, Kusumaningtyas and Illaningtyas (2007) stated that most lactic acid bacteria will grow more slowly at a low pH which causes decreased cell viability, this condition depends on the strain of the bacterium. Exposure to very acidic conditions can cause membrane damage and the loss of intracellular components such as Mg, K and fat from cells, which can cause death for the bacteria that are not resistant to acid.

Resistance Test against Bile Salts

The ability of *Lactobacillus plantarum* strain 8m-21 isolat from dadiah in Air Dingin Alahan Panjang District Solok Regency, West Sumatra (Indonesia) against bile salts with the number of control living bacterial (without addition of 0.3% oxgall) was 180 x 10^8 CFU/ml after oxgall addition 0.3% with the number of living bacterial cells of 11x 10^8 CFU/ml produced the highest viability of lactic acid bacteria at 6%. Viability is the ratio of the number of living cells to after being given the acidity settings expressed in the form of %. The higher the viability of LAB produced, the higher the resistance of LAB bacteria or isolates to bile salts.

Lctid acid bacteria resistance to bile salts is an important requirement in probiotics. Susanti, Retno and Fatim (2007) stated the degree of resistance to bile salts is one of the important characteristics for lactic acid bacteria, because it affects its activity in the digestive tract especially the upper intestinal tract where bile is secreted. Bile has properties as a surface active compound where this property activates the lipolytic enzymes secreted by the pancreas. This lipolytic enzyme reacts with fatty acids in the bacterial cytoplasmic membrane, causing changes in membrane structure and permiability properties that affect resistance to bile salts. The resistance of lactic acid bacteria towards bile salts is related to the Bile Salt Hydrolase (BSH) enzyme which helps to hydrolyze conjugated bile salts, thereby reducing the toxic effects on cells.

Antimicrobial Activity

Antimicrobial test was done using the diffusion well method with antibiotics as a positive control. Antibiotics were administered using a paper disc containing 2 µg/disk of ampicillin concentration, kanamycin 30 µg/disk and 3 µg/disk of penicillin. Positive antibiotic control was used to determine the resistance and sensitivity of pathogenic test bacteria.

Table 2: Diameter of Clear Zone from Antimicrobial Resistance Test with Positive Control

Sample Code	Clear zone diameter (mm)
	Escherichia Coli 0157
Lactobacillus plantarum 8m-21 (DAD)	20,25
Penicillin	2,7
Ampicillin	15,20
Kanamycin	14,19

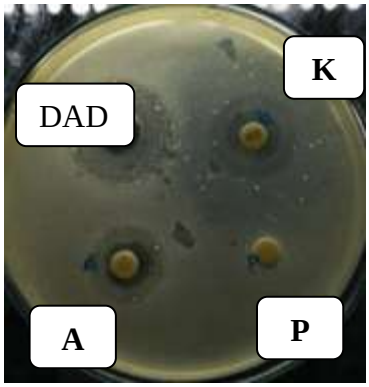

Figure 2: Annotation- Clear zone of LAB isolate against pathogenic bacteria using positive control of antibiotics (A) Ampicillin, (K) Kanamycin, (P) Penicillin Sample DAD *Lactobacillus plantarum* 8m-21 Clear zone formed against *Escherichia coli* O157

LAB isolates which have inhibitory zones on *Escherichia Coli* 0157 are 20,25 mm. The results obtained by LAB DAD isolates have greater inhibition than the antibiotics penicillin, ampicillin and kanamycin in the *Escherichia Coli* O157 test bacteria. The results of antimicrobial and LAB DAD isolate test on Escherichia Coli test bacteria were higher than Hartini research (2018) which had inhibition zones on tempoyak LAB isolates against Escherichia Coli O157 test bacteria, which was 16 mm.

Soleha (2015) states that the penicillin antibiotic is an antibiotic that is able to fight gram-positive bacteria by the mechanism of inhibiting bacterial cell wall synthesis, including the betalactam class. Amphicillin antibiotics are penicillin derivatives which have more broad spectrum able to work against gram-positive and gram-negative bacteria, also included in the betalactam class of mechanism of action to inhibit bacterial cell wall synthesis. Furthermore, kanamycin antibiotics include aminoglycosides which are bactericidal which usually often work against gram-positive bacteria that cause infections

CONCLUSION

This study shows that *Lactobacillus plantarum* strain 8m-21 lactic acid bacteria from Dadih Air Dingin isolates in Solok, West Sumatra (Indonesia) has potential as probiotics, Total aerobic bacterial colonies were 8,0 x 10^8 CFU / g ,Gram positive bacteria, bassil , homo fermentative, catalase negative, *Lactobacillus plantarum* strain 8m-21 is resistant to acidic conditions of pH 3.0 with 7% viability, 0.3% bile salt with 6% viability, having antimicrobial properties that can inhibit pathogenic bacteria with a 20,25 mm inhibition zone in *Escherichia Coli* O157.

ACKNOWLEDGMENT

This research was carried out due to the collaboration of researchers with the university through research funding by the Professor of Andalas University (HGB-UNAND) with ID number 08/UN.16.17/PP.PTP/LPPM/2019.

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