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Potential of Dadiah Kapau from Agam District, Indonesia as a source of probiotics for health

Abstract. Dadiah is a fermented product of buffalo milk prepared in a bamboo container as a traditional food in the Minangkabau tribe of West Sumatra. This study aimed to identify lactic acid bacteria that have the potential as probiotics from dadiah products. The method used in this research include an experimental survey with descriptive data analysis. The Dadiah used in this study was taken from dadiah breeders and processors in Nagari Kapau, Agam Regency, West Sumatra, Indonesia. Tests for selection of probiotic candidates included macroscopic and microscopic observations, catalase test, fermentative type, resistance to gastric acid (pH 3), resistance to bile salts, anti-microbial activity using test bacteria *Eschericia coli O157, Sthaphylococcus aureus, Listeria monocytogenes* and *Salmonella* sp. Molecular identification was carried out by using 16S rRNA to determine the relationship of species using MEGA version 7.0 application. The results of the study revealed that the total colonies of kapau dadiah lactic acid bacteria were 128 x 10^8 CFU/gr, and 12 isolates were obtained from the isolation results. Catalase negative for 12 isolates and 9 homofermentative types and 3 including heterofermentative types. Tests for resistance to gastric acid (pH 3) and bile salt resistance, the best viability was 84.61% and 86.51% respectively. The best antimicrobial activity showed clear zone against the test bacteria *E. coli O157, S. aureus, L. monocytogenes* and *Salmonella* sp. The phylogenetic analysis of the results of the sequencing shows that the Dadiah *kapau* lactic acid bacteria are closely related to *Lactobacillus plantarum.*

Keywords: Dadih, *Lactobacillus plantarum*, probiotik, West Sumatera

INTRODUCTION

Dadiah is a food product originating from West Sumatra. It is an unaltered or broken lump of buffalo milk prepared by curing milk fermented for 24-48 hours naturally in a bamboo container at room temperature (27^{o}C). The food is white like tofu, consumed with a spoon. The basic ingredient in making dadiah is buffalo milk which is fermented in a bamboo tube, covered with plastic or banana leaves without the addition of any starter. Lactic acid bacteria ferment buffalo milk contained in bamboo tubes (Surono, 2003). Clumping occurs in the dadiah due to the presence of microbes originating from bamboo and banana leaves so that it produce a form that is wrapped and yellowish white in color and has a characteristic aroma (Surono, 2004).

The fermentation process of dadiah involve Gram positive bacteria such as *Lactobacillus plantarum, Lactobacillus brevis, Streptococcus agalactiae, Bacillus cereus* and *Streptococcus uberis,* as well as Gram negative bacteria such as *Escherichia coli* and *Klebsiella* sp. Lactic acid microbes such as *Lactobacillus plantarum* involves in fermentation of Dadih is among the probiotic microbes (Usmiati et al., 2011). The bacteria in dadiah are able to inhibit intestinal pathogens and thus can help facilitate digestion (Harnentis et al., 2019). In comparison to cow and goat milk, the dadiah of Buffalo milk is highly rich in protein and fat and has the advantage of a smooth texture i.e. more compact and dense.

Figure 1. Dadiah Kapau

Nutritionally, dadiah contains water content (69-73%), protein (5.7-6.6%), fat (7.9-8.2%) (Afriani, 2010). In addition, protein content is 39.8% with an essential amino acid that is quite complete, kalsiun, vitamins B and K, which are formed during the fermentation process. The nutritional content of dadiah depends on the area of production, which may affects

variation in its nutritional contents. In general, dadiah contains high protein and fat with an average protein content of 6.75%. Setiyanto et al., (2009) and Nuraida, (2015) stated that traditional fermented foods can be used as a potential source of probiotics because they generally contain lactic acid bacteria (LAB) including species *Lactobacillus, Pediococcus, Enterococcus, Weissella,* and *Leuconostoc*.

Lactic acid bacteria (LAB) are rod or round shape gram-positive bacteria that can convert carbohydrates into lactic acid (Korhenen, 2010). These are Gram-positive bacteria that are rod or spherical, do facultative fermentation. The spores of these bacteria are anaerobic in nature, do not have cytochromes, the ability to reduce nitrate and utilize lactate, negative oxidation, negative catalase, motility. According to Syukur et al., (2011) lactic acid bacteria must have probiotic characteristics, namely having antimicrobial activity, being resistant to the selection of the digestive tract system such as gastric acid, bile and pancreatic juice, having anticarcinogenic activity, being able to colonize in the digestive tract, and being able to increase intestinal absorption.

In general, probiotic bacteria must have essential properties such as resistance to gastric and intestinal conditions, where acid and bile are factors that affect growth and survival (Miogani et al., 2015). Alonso et al., (2018) stated that probiotics are effective in inhibiting the growth of pathogenic organisms through various mechanisms, such as attachment to epithelial cells and modulation of the immune system. A strong probiotic isolate should have certain characteristics, such as viability and colonization ability under different environmental conditions (Palachum et al., 2018). Probiotic isolates must be able to withstand low gastric pH, be resistant to bile salts, and must adhere to epithelial cells (Somashekaraiah et al., 2019).

Based on research that has been done previously on traditional fermented products, many isolates of Lactic acid bacteria (LAB) have the potential as probiotics, including traditional foods from Ethiopia, namely *Teffinjera* dough, *Ergo*, and *Kocho*, obtained *Lactobacillus* strains (Mulaw et al., 2019), fermented vegetable products from China, namely Jiangshui Cai, obtained *Lactobacillus* casei (Ullah et al., 2017) and a fermented product from India, namely extract of coconut flower sap (neera) (Somashekaraiah et al., 2019). In the study Sunaryo and Marwanto (2013) found that the strains of lactic acid bacteria in *dadiah* were *Lactobacillus plantarum*, *Lactobacillus brevis, Streptococcus agalactiae, Bacillus cereus, Streptococcus uberis* and *Lactobacillus plantarum* (Usmiati et al., 2011). Surono (2004) found *Lactococcus lactis, Lactobacillus brevis, Lactococcus casei, Lactobacillus. plantarum, E. faecium*, and *Leuconostoc mesenteroides* on dadiah isolate. This study was aimed to identify lactic acid bacteria that have the potential as probiotics from kapau dadiah products.

MATERIALS AND METHODS

Sampling

The material of this research is Dadiah which was obtained from producers from Nagari Kapau, Agam Regency, West Sumatra Province, Indonesia. The research was conducted at the Laboratory of Animal Products Technology, Faculty of Animal Husbandry, Andalas University.

Isolation and identification of lactic acid bacteria

Dadiah was weighed as much as 1 gram, put in a test tube containing 9 ml of solution Demann Ragosa Sharpe (DRS) Broth MERCK and then vortexed to obtained a homogeneous solution. The resulted broth called as dilution of $10^{-1}$was then incubated for 24 hours in an incubator at 37°C. The results of the dilution were taken 100 µl and put into an Eppendorf tube containing 900 µl of solution Demann Rogosa Sharpe (DRS) Broth MERCK. Then serial dilution was carried out to 10^{-8}. From a dilution of 10^{-8}, 100 µl of the sample was taken and planted using the spread method on a petridish containing DRS Agar media, (MERCK) then leveled with a hokey stick. The inoculum was stored in an anaerobic jar and then incubated in an incubator for 48 hours at 37°C. After 48 hours, the single colony which characterized lactic acid bacteria, namely smooth round yellowish white color, was transferred to MRS Agar media (MERCK) for colony purification by streak method using a ose needle and then incubated for 24 hours at 37°C (Purwati et al., 2010).

Biochemical properties test

Total lactic acid bacteria obtained were continued for fermentative type testing by inserting the lactic acid bacteria isolate in 5 ml of inserting DRS Broth MERCK, then the Durham tube in an inverted position. Thereafter, incubated for 24 hours at 37°C. Observations were made by looking at the presence or absence of air bubbles in the Durham tube (Suryani et al., 2010). The catalase test was carried out by means of isolates of lactic acid bacteria taken using an ose needle. The isolate was scratched on the object glass. Drops of 3% Hydrogen peroxide (H_2O_2) using a 50 µl pipette. Observations were made by looking at whether or not gas is formed on bacterial reviews (Public Health England, 2014).

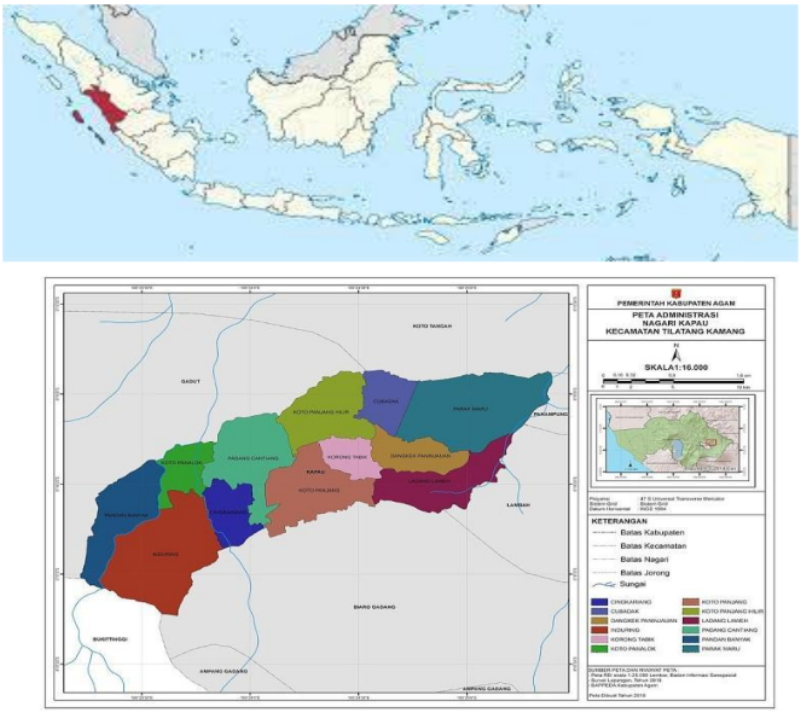

Figure 2. Maps of Kapau, Agam Regency, West Sumatera, Indonesia

Acid resistance test

Acid tolerance was determined with slight modifications in the methods used by Rashid and Hassanshahian (2014). The enrichment of MRS broth was used to assess pH tolerance. Cells from overnight cultures were collected and inoculated respectively into 1 mL of broth with pH 3 and control MRS broth (MERCK). The cultures were incubated at 37ºC for 90 minutes. 100 µl of broth was then vortexed until it became homogeneous. The resulted dilution (10^{-1}) was, then incubated for 24 hours in an incubator at 37°C. 100 µl of the dilution was put 100 µl into an Eppendorf tube containing 900 l of solution of Demann Rogosa Sharpe (DRS) Broth MERCK and serial dilution was performed. From dilution of 10^{-8}, 100 µl of the sample was taken and planted using the spread method on a petridish containing MRS Agar media (MERCK) then leveled with a hokey stick. The inoculum was stored in an anaerobic jar and then incubated in an incubator for 48 hours at 37°C. Perform the calculation of the viability of resistance to gastric acid.

Salt bile resistance

Measuring the resistance to bile salt was conducted by following the method of Zhang et al., (2016) with slight medications by using 4 hours of incubation period. DRS broth without bile salts was used as a control medium and MRS broth containing 0.3% (w/v) bile salt was inoculated overnight at 37°C with cultures of LAB, then dilute and grow bacteria. take 100 µl then vortex until homogeneous. The result is called a dilution of 10^{-1}, then incubated for 24 hours in an incubator at 37°C. The results of the dilution were taken 100 l and put into an eppendrof tube containing 900 µl of solution *Demann Rogosa Sharpe* (DRS) Broth MERCK. Then serial dilution was carried out to 10^{-8}. From a dilution of 10^{-8}, 100 µl of the sample was taken and planted using the spread method on a petridish containing MRS Agar media. (MERCK) then leveled with a hokey stick. The inoculum was stored in an anaerobic jar and then incubated in an incubator for 48 hours at 37°C. Perform resistance viability calculations for bile salts.

Antimicrobial and antibiotic activity

To study antimicrobial activity, well diffusion method was used. Cell-free supernatants were obtained from lactic acid bacteria grown in DRS Broth (MERCK) for 24 hours at 37 °C, under anaerobic conditions and centrifuged at 10,000 rpm for 5 minutes at 4 °C. The supernatant and the pellet from the centrifuge were sparated out. 50 L of the supernatant was taken and put it into a perforated well with a diameter of 6 mm . Pathogenic bacteria were grown aerobically at 37°C for 24 hours. Then 0.2% of the pathogenic bacterial culture was added to Nutrient Agar (MERCK). As a control, compared with

antibiotics (penicillin 10µg, kanamycin 30µg, ampicillin 10µg). The clear zone formed can be read after 24 hours (Yang et al., 2012).

Molecular identification of lactic acid bacteria with 16S rRNA

Lactic acid bacteria isolates were cultured in DRS broth at 37 °C for 24 hours. Isolation of genomic DNA was carried out using Extrap Soil DNA Kit Plus Ver.2. Polymerase chain reaction (PCR) amplification of the '16S rRNA isolate using a 16S rRNA gene fragment of ~1.5 KB using universal primers. Initial denaturation at 95°C for 5 min with 25 cycles followed by denaturation at 94°C for 1 min, then annealing at 56°C for 1 min, extension at 72°C for 1.5 min, and final extension at 72° C for 7 minutes. The resulting DNA was separated using electrophoresis at 100 V for 21 min, using 1% agarose in × 1 TAE buffer. Then, a gel documentation system was used to generate images of the bands in the gel. Purification was carried out using a rapid gene gel extraction kit/PCR (Nippon Genetics, Germany), and the resulting sequences were analyzed using the BLAST program in the NCBI gene bank database which can be viewed at http://blast.ncbi.nlm.nih.gov/Blast. cgi. Sequence alignment was made using the Bioedit application, and the phylogenetic tree was created using the MEGA application 7.

RESULTS AND DISCUSSION

Identification of morphology of lactic acid bacteria *Lactobacillus plantarum*

Table 1. Macroscopic identification of lactic acid bacteria

Sample	Colour	Colony	Elevation
DK1	White-Yellowish	Round	Convex
DK2	White-Yellowish	Round	Convex
DK3	White-Yellowish	Round	Convex
DK4	White-Yellowish	Round	Convex
DK5	White-Yellowish	Round	Convex
DK6	White-Yellowish	Round	Convex
DK7	White-Yellowish	Round	Convex
DK8	White-Yellowish	Round	Convex
DK9	White-Yellowish	Round	Convex
DK10	White-Yellowish	Round	Convex
DK11	White-Yellowish	Round	Convex
DK12	White-Yellowish	Round	Convex

Table 2. Total mass of lactic acid bacteria

Sample	Total Mass ($x10^7$ CFU/ml)
DK1	87
DK2	52
DK3	97
DK4	116
DK5	78
DK6	119
DK7	121
DK8	65
DK9	109
DK10	19
DK11	97
DK12	76

Biochemistry test *Lactobacillus plantarum*

Table 3. Catalase test and type of fermentative

Isolate BAL	Catalase Test	Type Fermented
DK1	Negative (-)	homofermentative
DK2	Negative (-)	homofermentative
DK3	Negative (-)	homofermentative

DK4	Negative (-)	homofermentative
DK5	Negative (-)	homofermentative
DK6	Negative (-)	homofermentative
DK7	Negative (-)	homofermentative
DK8	Negative (-)	homofermentative
DK9	Negative (-)	homofermentative
DK10	Negative (-)	homofermentative
DK11	Negative (-)	homofermentative
DK12	Negative (-)	homofermentative

Resistance on pH Acid Condition

Table 4. Resistance Isolate on pH acid

Isolate	Total Colony (CFU/gram)		Viability (%)
	pH Control	pH 3	
DK3	39 x10^7	36x10^7	84.61
DK4	39 x10^7	30x10^7	76.92
DK6	38 x10^7	27 x10^7	71.05
DK7	40 x10^7	12 x10^7	30.00
DK9	52 x10^7	22 x10^7	42.30
DK11	43 x10^7	35 x10^7	81.39

Resistance of lactic acid bacteria on bile salt

Table 5. Resistance Isolate on bile Salt 0.3%

Isolate	Total Colony's (CFU/gram)		Viability (%)
	Control	oxgall 0.3%	
DK3	76 x10^7	36x10^7	47.36
DK4	89 x10^7	77x10^7	86.51
DK6	121 x10^7	77 x10^7	73.63
DK7	35 x10^7	24 x10^7	68.57
DK9	49 x10^7	28 x10^7	57.14
DK11	38 x10^7	32 x10^7	84.21

Antimicrobial activity

Table 6. Diameter of clear zone

Sample	Clear Zone (mm)			
	E. coli 0157	*Staphylococcus aureus*	*Listeria monocytogenes*	*Salmonella* sp.
DK4	8.2	8.4	12.5	7.5
DK11	7.5	6.1	11.5	6.1
Penisilin	5.6	6.0	12.3	4.5
Ampisilin	6.7	0.6	12.5	1.5
Kanamisin	6.6	0.5	11.1	2.1

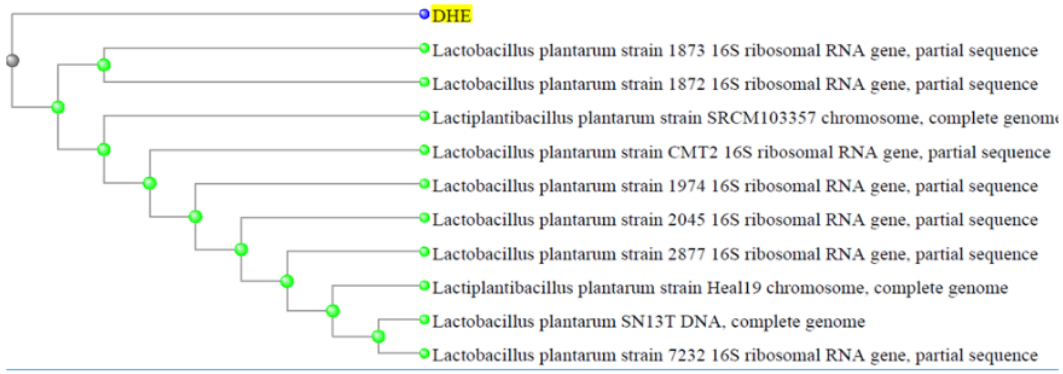

Figure 3. Analytic phylogenetic tree with MEGA application

Discussion

The results of identification of Lactic acid bacteria (LAB) based on single colonies were taken by looking at the data in table 1. Macroscopically, all single colony isolates obtained had a white-yellowish color with round shape, flat and smooth edges and smooth and slightly combed elevation . The dadiah is taken from Nagari Kapau recount total lactic acid bacteria obtained as much as 128 x 10^8 CFU / g were then carried out identification and obtained 12 isolates to do further testing.

Sample DK7 is 121x10^7 CFU/g, dadiah isolate the sample DK4 is 116x10^7 CFU/g and dadiah isolates Sample DK3 was 97 x10^7 CFU/g. This is in accordance with the criteria (Juliyarsu et al., 2018) that the results of lactic acid bacteria colonies must be in the amount of 10^6- 10^8 CFU/gram. The results of the study with macroscopic observations (shape, size, and color) of lactic acid bacteria were found to be white-yellow in color with round colonies and smooth-convex elevations. Similar characteristics of these bacterial colonies were also observed by Purwati et al., (2005) and Juliyarsi et al., (2018) on MRS Agar. This is also in accordance with research of Syukur and Purwati (2013) which states that single colonies of lactic acid bacteria have the characteristics of a round shape, cream color and smooth edges.

Furthermore, lactic acid bacteria were observed microscopically with gram staining. Based on the response to Gram staining, bacteria are divided into two types, namely Gram positive bacteria and Gram negative bacteria. These two bacteria have differences in their cell wall structure. The cell wall of Gram positive bacteria consists of a homogeneous peptidoglycan layer located outside the plasma membrane layer with a thickness of about 20-80 mm while, the cell wall of Gram negative bacteria has a peptidoglycan layer thickness between 2-7 nm and covered by an outer membrane with a thickness of 7-8 nm. So Gram positive bacteria have thicker peptidoglycan than Gram negative bacteria. Gram-positive bacteria in Gram staining looks purple while Gram-negative bacteria will produce a pink color (Willey et al., 2008). Unus (2005) stated that gram-positive bacteria treated with crystal violet remained purple even after washing with alcohol and exposed to red safranin, while gram-negative bacteria would turn red. Juliyarsi et al., (2018) stated that gram staining is carried out to see gram-positive or gram-negative bacteria isolated where gram-positive bacteria absorb crystal violet color reagent so that it turns purple while, gram-negative bacteria absorb safranin color reagent which give a red color. In the results of the study, isolates of lactic acid bacteria on tempoyak were gram-positive bacteria with a purple color (Juliyarsi et al., 2018).

Biochemical properties

Results of biochemical testing of Dadih isolates in the form of catalase test and fermentative type obtained catalase negative results and were homofermentative. On the study of Sukur et al., (2014) stated that the catalase test can be done by dripping 2 drops of H_2O_2 (3%) on a culture aged 24 hours. Syukur and Purwati (2013) stated that the breakdown of glucose by LAB is heterofermentative type via the pentose phosphate pathway. In this fermentation enzymes that work is phosphoketolase and produce 40-50% of lactic acid, ethanol, acetic acid and CO_2. Explained further that different growth conditions can produce different fermentation end products as a result of changes in the metabolism of pyruvate with the use of electron acceptor external sources such as oxygen or organic compounds. The absence of bubble formation means that there is no formation of oxygen gas as a result of the breakdown of H_2O_2 by enzymes catalase produced by these bacteria is a feature if the reaction occurs negative. The results of the study carried out by Putri et al., (2012) also reported that as many as 63 isolates isolated from cassava fermentation were lactic acid bacteria with Gram positive and catalase negative characteristics.

The fermentation type test is homofermentative, indicated by the absence of bubbles. This is in accordance with the Melia et al., (2019) that the test of biochemical properties showed that the lactic acid bacteria *Pediococcus acidilactici* strain PB22 was homofermentative as indicated by the absence of gas bubbles in the Durham tube. Based on the results of the study Desniat et al., (2013), it was generally stated that the lactic acid bacterial isolates from bisambola had a homofermentative type.

Resistance of lactic acid bacteria to acidic conditions

The viability results of the test of resistance of lactic acid bacteria to acidic conditions (pH 3) were 30.00-84.61% (table 4). DK3 had the highest viability value, which was 84.61%. Each isolate has a different viability value because each isolate has a different ability to withstand gastric pH (acid). Lactic acid bacteria that have potential as probiotics must be resistant to gastric pH, namely pH 2-3 caused by gastric fluid secretion. This is supported by Harnentis et al., (2020) which states that probiotic LAB candidates must be able to withstand extreme conditions in the digestive tract from mouth to intestine, and then be able to colonize the intestinal surface. This is also supported by Evivie et al., (2017) adding that gastric acidity serves as a prerequisite before conducting microbial selection before entering the intestine.

The results of the viability test of the resistance of lactic acid bacteria to acidic conditions in dadiah isolates ranged from 71.05-84.61%. The results in this study are in accordance with research of Harnentis et al., (2020) on isolates of Lactic Acid Bacteria (LAB) fermented dadiah, budu, and tape at pH 3 incubation for 3 hours and at 6 hours incubation the results ranged from 55.27-98.48% and 50.98-84.91%. The results of this isolate were higher than the results of the study Juliyarsi et al., (2018) where the viability of LAB isolates from tempoyak from Padang-Pariaman was 34%. And also the results of this study are higher than studies Tokatlie et al., (2015) reporting that *Lactobacillus brevis, Lactobacillus*

plantarum, and *Pediococcus ethanolidurans* isolated from traditional pickles have survival rates of 33-64%, 35-85%, and 40-76%, respectively at pH 2.5 and incubation period of 4 hours. In the study of Kwarteng et al., (2015) 80% results were obtained from testing lactic acid bacteria at pH 2.5 for 4 hours isolated from fermented milled flour. In addition, based on Melia et al., (2018) isolates of lactic acid bacteria *Lactobacillus fermentum* strain *researchL23* can survive at pH 2 with a viability of 55.64-73.94%. The yield of palm sugar isolate was even higher than that of *Lactobacillus rhamnosus* isolated from buffalo milk from Karnataka, India, which had a viability of 30% at pH 3 for 3 hours (Shafakatullah and Chandra, 2014). Lactic acid bacteria isolated from milk and cow dung samples by Jain et al., (2017) can survive at pH 3 (71.05-84.61 %).

Lactic acid bacteria are able to survive at relatively low pH because they have a system that simultaneously transports lactic acid and protons to the outside of the cell (Chavarin et al., 2013). In addition, under acidic conditions, lactic acid bacteria must be able to survive because lactic acid bacteria follow mechanisms in the regulation of LAB resistance to acids, including central pathway metabolism, proton pumps, changes in cell membrane composition and cell density, repair of DNA and protein damage, and processes neutralization (Liu et al., 2015). Melia et al., (2018) stated that any effective probiotic must survive through the gastrointestinal tract so it must be able to withstand gastric acid conditions with a pH of 1.5–3.5, which is caused by the secretion of gastric juice. It is also described by Azat et al., (2016) that the amount of lactic acid bacteria for probiotics> 10^6 cfu / g after incubation at pH 3.0 showed the survival rate and survival of the lactic acid bacteria in the digestive tract pH. Harun et al., (2020) stated that the higher the viability of lactic acid bacteria produced, the higher the resistance of lactic acid bacteria or isolates to gastric pH.

Resistance of lactic acid bacteria to bile salts

The results of testing the resistance of lactic acid bacteria to bile salts of 0.3% for 4 hours obtained viability of 47.36-86.51% with the highest isolate of lactic acid bacteria, namely Dadih isolate DK4. This indicated that the LAB isolates were able to live at 0.3-0.5%. concentrations of bile salts in the human body The isolates showed a difference in viability to bile salts of 0.3% (table 5) after 4 hours of incubation, DK4 (86.51%) was proven to be more able to withstand bile salt conditions than isolates DK7 and DK3 probiotic bacteria to bile salts is also high. Several probiotic bacteria have been shown to survive in these conditions. Testing the resistance of lactic acid bacteria to bile salts is one of the criteria for checking lactic acid bacteria as probiotics. Somashekaraiah et al., (2019) stated that tolerance to bile salts is one of the most important properties possessed by lactic acid bacteria because it can determine the ability of bacteria to survive in the small intestine and to play a functional role as a probiotic and the use of 0.3% oxgall has been widely used. for research as the threshold for bile salt tolerance of a potential probiotic.

The results of viability testing of the resistance of lactic acid bacteria to bile salts in dadiah isolates ranged from 75.00-85.63%. The results of this study were higher than the study Harnentis et al., (2020) on isolates of lactic acid bacteria from fermented dadiah, budu, and tape in bile salts of 0.3% (39.90-58.61%) and 0.5% (28.38-52.11%.). The results of dadiah isolates in this study were even higher than *Lactobacillus fermentum* strain *IMAU70167* buffalo milk isolates which survived in 0.3% bile salts for 4 hours with viability 32.23-56.13% (Melia et al., 2018). Nurnaafi et al., (2015) stated that a good probiotic candidate is an isolate that has a survival rate of more than 50% at low pH conditions and is resistant to bile salts. This is supported by the study of Bustos et al., (2018) which states that lactic acid bacteria can hold bile salts with a resistance of 20-40%. Guan et al., (2017) reported that *Lactobacillus plantarum* (HLX37) isolated from fermented milk persisted well in 0.3% bile salts at a level of 54.68% and at an incubation period of 4 h. In this study, LAB isolates from Dadih Kapau could be a good probiotic candidate because it had 50% resistance at 0.3% bile salt concentration. DK4 had the highest resistance at 85.63% in 0.3% bile salts.

According to Harun et al., (2020) the resistance of lactic acid bacteria to bile salts is related to the enzyme Bile Salt Hydrolase (BSH) which helps to hydrolyze conjugated bile salts, thereby reducing the toxic effect on cells. This is supported by the research of Harnentis et al., (2020) which states that bacterial membranes are the main target for bile acids so that bacteria can survive on bile salts, they produce BSH by conjugation to free bile acids and free bile acids can participate in various metabolic processes, including regulation of fat absorption; cholesterol metabolism; creation of homeostatic conditions on the bacterial membrane; and regulates nitrogenous base, fat, and amino acid biosynthesis, which enables the conversion of fats, resulting in the production of exopolysaccharides (EPS). Lebeer et al., (2018) added EPS function as a protective agent against bile salts (0.15-0.3%) at pH 2-3.

Antimicrobial activity of lactic acid bacteria

Based on the antimicrobial activity testing conducted, the results showed that the lactic acid bacteria from Dadih formed a clear zone against the test bacteria *E. coli* 0157, *Propionibacterium acnes*, *Acinetobacter baummannii*, and *Listeria monocytogenes*. It showed antimicrobial activity against *Escherichia coli* O157 (with zone of inhibition ranging from 5.6-8.2 mm), *Propionibacterium acnes* (between 6.0-8.4 mm), *Acinetobacter baummannii* (range 11.5-12, 5 mm), and *Listeria monocytogenes* (range 4.5–7.5 mm). Isolate sample A showed the most effective inhibition on the test bacteria *E. coli* O157.

The results of antimicrobial and acid bacteria isolates sample A with test bacteria *Listeria monocytogenes* had relatively lower inhibitory power than the study where *Lactobacillus fermentum* strain *L-23* isolates from Agam buffalo milk had an inhibitory power of 19 mm and had a higher inhibitory power than the study Desniar et al., (2013) namely

Pediococcus acidilactici strain *PB22,* isolates of tamarind which only had defense activity againsttest bacteria *Listeria monocytogenes* of 5.10mm. The test bacteria *Acinetobacter baummannii* had relatively low inhibitory power on isolate A, but it was greater than *Lactobacillus rhamnosus* GG which was only 11.76 mm (Dallal et al., 2017). Based on research Harnentis et al., (2020), dadiah isolates had inhibition zones on *Escherichia coli bacteria* ranging from 8.78-11.54 mm and bacteria *Staphyloccus aureus* ranging from 7.02-13.42 mm. The results of this study are lower when compared to antimicrobial activity on Lactobaillus brevis by Pratama et al., (2021) it was found that the clear zone area of the S. aureus test bacteria was in the range of 18-31.36 mm.

According to Morales et al., (2003) the inhibitory zone activity was grouped into four categories, namely weak activity (<5mm), moderate (5-10mm), strong (>10-20mm) and very strong (>20-30mm). Based on the above criteria, it can be seen that LAB isolates from dadiah and buffalo milk on *E. colibacteria* O157have weak activity. Bacteriocins produce clear, round and broad clear zones. Organic acids and hydrogen peroxide produced by *lactobacilli are* reported to be able to inhibit the growth of gram-positive and gram-negative bacteria, while bacteriocins are very influential on gram-positive bacteria (pan et al., 2009). Damage to the cell membrane of pathogenic bacteria that inhibits metabolic processes and prevents the growth of pathogenic bacteria is caused by lactic acid diffusion into bacterial cells and can disrupt the integrity of the cell membrane (Reuben et al., 2019).

Pan et al., (2009) stated that the diameter of the inhibition zone against pathogenic bacteria showed low antimicrobial activity when the clear zone was 0-3 mm, moderate antimicrobial activity > 3-6 mm and > 6 mm had high antimicrobial activity. Obdak et al., (2017) found that the strain identified as *Pediococcus acidilactici* was a likely producer of bacteriocins with antilisterial activity. Some LAB studied have antimicrobial activity against *L. monocytogenes* and potential probiotics. The ability to form a clear zone varies depending on the type of bacteria, the concentration of bacteriocin and the nutrient content in the media. According to Somashekaraiah et al., (2019) some types of LAB can produce bacteriocins, which are antibacterial peptides and toxins containing proteins that can prevent bacterial growth. LAB causes an acidic environment and bacteriocins increase their ability to stop the growth of harmful bacteria and pathogenic bacteria Bustos et al., (2018). The opinion Obdak et al., (2017) added that the *Lactobacillus plantarum* strain showed strong antimicrobial activity against various potential pathogens, especially *Listeria monocytogenes*. This is supported by the Harnentis et al., (2020) which states that LAB has different properties that are used to destroy pathogens where LAB can be homofermentative only to produce organic acids, while heterofermentative LAB can produce organic acids and antimicrobial compounds.

Results of 16S rRNA gene amplification by PCR

In Figure 3, you can see the amplification area of the 16S rRNA gene isolate from lactic acid bacteria from Dadih. Judging from the PCR fragment size 1542 bp using R Primer (16S-1492R, Tm 47 °C, 5'-GTT TAC CTT GTT ACT ACT-3´) and F (16S-27F, Tm 54.3 °C, 5'- AGA GTT TGA TGCC CTC AG-3´) [59]. The phylogenetic tree based on the sequence of the 16S rRNA gene can be seen in Figure 5. The results of sequencing the Dadih isolates compared with the Gene Bank data using the BLAST program on the NCBI website (http://www.ncbi.nlm.nih.gov) showed a high degree of similarity with the strains. Based on the BLAST test and the phygenetic tree, it was found that the kapau dadiah lactic acid bacteria isolate with sample code D4 had 99% similarity to Lactobacillus plantarum. The conclusion of this research is Lactic acid bacteria isolate palm sugar has a total colony of 98 x 10^7 lactic acid bacteria^7 cfu/g- 121 x 10^7 cfu/g rod-shaped, gram positive, catalase negative, and homofermentative. Based on the results of probiotic selection, isolates of lactic acid bacteria A, had viability of resistance to pH 3 of 84.61%, resistance to bile salts of 0.3% of 85.63% and had the highest antimicrobial activity against pathogenic bacteria *Acinotobacter Baumanii* with an inhibitory power of 12.50. mm. The results of sequencing the isolates of lactic acid bacteria A from the producer of Dadih Kapau Agam, West Sumatra, were *Lactobacillus plantarum*.

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