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RESEARCH ARTICLE

The Potential Lactic Acid Bacteria from *Dadiah* Sianok Bukittinggi City, West Sumatera as Probiotic

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ABSTRACT:

Dadiah is a traditional fermented buffalo milk from West Sumatra, Indonesia. It is one of the healthiest drinks because it contains lactic acid bacteria (LAB), which has many health benefits. Lactic Acid Bacteria (LAB) are a group of bacteria that play a role in the fermentation process of food. Lactic Acid Bacteria (LAB) content in dadiah will affect the quality of dadiah in general.West Sumatra has several dadiah producing areas, one of which is the Sianok area located in the city of Bukittinggi. This research aims to find out the potential of Sianokdadiah as a probiotic food. The method used in this research is a survey method with descriptive analysis. Lactic Acid Bacteria (LAB) contained in *dadiah* was isolated using de Man RogosaSharge (MRS) media. The isolates were then identified based on their morphology and biochemical properties. The tests carried out include gramme stain, catalase test, fermentative type, retention of gastric juice and bile salts. Furthermore, testing using 16S rRNA molecular identification techniques was conducted to determine the species level. The results of the study obtained a total colony of Lactic Acid Bacteria (LAB) from dadiah $89x10^9$ CFU/g. The identification of the single colony found that the Lactic Acid Bacteria (LAB) obtained was included as Gram-positive, with the type of homofermentative fermentation, catalase negative. It has resistance to stomach acid at pH 3 with a viability of 83.7%, bile salt resistance of 0.3%, and viability of 67.3%. Identification of Lactic Acid Bacteria (LAB) using the 16S sRNA gene, the results of running PCR with a base length of 1428bp. Analysis based on phylogenetic trees showed that Lactic Acid Bacteria (LAB)dadiah from the city of SianokBukittinggi has a relationship with *Pediococcusacidilactici*. From the results of this research, it can be concluded that dadiah from the city of Sianok Bukittinggi has a good enough potential as a probiotic.

KEYWORDS: Dadiah, Lactic Acid Bacteria, Probiotics, *Pediococcus acidilactici*.

INTRODUCTION:

Indonesia has a large variety of traditional fermented food with raw materials that can be easily processed and preferred by many people. Despite the challenges, microorganisms exist and are involved throughout fermentation, and the metabolic product can be beneficial to the consumer. Therefore, further research on the advantages of the microorganisms in the traditional fermented foods of Indonesia, which will give many advantages in health, needs to be conducted. The microorganisms which will give advantages to human health are important if the superiorities and benefits of the usage of these microorganisms are known. One of the traditional fermented foods of Indonesia is *dadiah*.

Dadiah is a product of naturally fermented buffalo milk that originated from West Sumatera and has been an inseparable product of Minangkabau cuisine. West Sumatra has several dadiah producing areas, one of which is the Sianok area located in the city of

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Bukittinggi. *Dadiah* has a similar consistency to yoghurt, which is soft, creamy white, sour, and delicious. The surface of this food is soft and glistening, cleaning off air bubbles[1]. The fermentation occurred spontaneously; bamboo tubes are filled with fresh buffalo milk and each tube is covered with a banana leaf, which is then incubated at room temperature (28–30°C) for 24–48 hours[2]. Although this practise has existed for years, the number of micro flora dominated by lactic acid bacteria (LAB) can still not be exactly predicted[3]. The microorganisms are generated both from the milk or the environment, such as the bamboo tube, the banana leaf, or the buffalo milk itself. The traditional production process of *dadiah* has been passed down from one generation to the other with a low level of hygiene and sanitary. The biggest risk of this naturally milk fermentation process is the low level of hygiene during the processing, high contamination, and the absence of heating before starting the processing[4]. *Dadiah* is included as a functional food since it contains lactic acid bacteria (LAB), which has probiotic characteristics.

There are many proposed criteria as to how to choose a probiotic, such as safety, tolerance to gastrointestinal conditions, the ability to adhere to the gastrointestinal mucosa, and competitive exclusion of pathogens[5,6]. Bacterial adhesion to intestinal mucosa will enable colonisation inside the intestinal tract, albeit temporary, and has the ability to modulate the immune system, especially during its development[7]. Therefore, adhesion is one of the main criteria of selection for new probiotic strains[8]. Despite the lack of clear evidence, the relationship between in vitro adhesion and in vivo colonisation has been reported[9]. Furthermore, adhesion and colonisation on the surface of mucosa has protective mechanism property in order to combat pathogen by increasing nutrition and modulating immune system[8]. It has also been proven that certain lactobacilli has the ability to increase specificity and sharing of carbohydrate by using several enter pathogen[10], and able to block adhesion of pathogen by steric hindrance[11]. This ability serves as the function of probiotic to prevent infection by blocking adhesion of pathogen by competitive exclusion.

A probiotic is a living microbe with positive effects on health when it is consumed in a certain amount[12,13,14,15,16]. The development of functional food is in accordance with the utilisation of LAB as a probiotic microbe. A probiotic is a living microbe with positive effects on the health of its host when it is consumed in a certain amount, which makes it a GRAS (Generally Recognized as Safe) microorganism to be consumed by humans[17,18,19,20]. The characteristics of LAB from *dadiah*as probiotic in each area of production will differ from one another as well as the quality of the produced *dadiah*. This is influenced by the environmental conditions and the buffalo milk used for production. This study aimed to understand the potential benefits of lactic acid bacteria from *dadiah*originated from Sianok as a probiotic for health.

MATERIALS AND METHOD:

A sample of *dadiah was* obtained directly from the farmer of Nagari Sianok, Bukittinggi City. The sample is brought by using a cool box filled with ice gel to the Animal Product Technology Laboratory of the Faculty of Animal Science of Andalas University in order to be analyzed.

Methodology of Research:

The method of this study is the survey method with descriptive analysis.

Isolation and Identification of Lactic Acid Bacteria:

Macroscopic Identification:

Media dilution used is *de Mann Rogosa Sharpe* (MRS) broth. After incubating for 48 hours at 37^0C, BAL was planted with methods spread at inoculation and stored in an anaerobic jar. Single BAL colonies are round, smooth, white, and yellowish, and they were transferred to media *de Mann Rogosa Sharpe* (MRS) for colony purification with methods streak and incubated for 24 hours at 37^0C[21].

Microscopic Identification (Gram Staining):

Bacterial culture was taken in a Petri dish using an ose needle, then put into a glass preparation, then drops of violet crystals, waited for 1 minute, rinsed with distilled water and dried, then dipped in ethanol for 20 minutes, and dropped safranin as much as 1 drop and waited 30 seconds, rinsed and dried, and observed the shape of the bacteria on the microscope screen.

Biochemical properties:

The gas test was carried out by inserting LAB isolates in 5ml of BRS BIG MERCK. Then, invert the durham tube and incubate for 48 hours at 37°C, checking for the presence or absence of air bubbles in the durham tube. Furthermore, the catalase test is by scraping the isolate on the glass preparation and then dropping hydrogen peroxide (H_2O_2) 3%, then observed by looking at whether or not gas was formed on the bacterial review.

The catalyst test is done by taking lactic acid bacteria isolation by using an inoculating loop. The isolation is stroked on the object's glass. 3% hydrogen peroxide (H_2O_2) is dripped using a 50 l pipette. Observation is done by looking for any formation of gas on bacteria distribution[22].

Acid Resistance Test:
One millilitre of bacterial culture was inoculated into 9 ml of Broth MRS media and incubated at 37°C for 24 hours with a pH adjustment of 4(pH adjusted by addition of HCl 5N) incubated for 90 minutes. Furthermore, the dilution was carried out by the method of spread 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 Forming Unit (CFU)[23].

Test for Resistance to Bile Salt:
One ml of bacterial culture inoculated on MRS Broth medium, 9ml, was incubated at 37°C for 5 hours with oxgall settings of 0.3%, then diluted to 10-6, and planted with the spread method onto MRS media so that it was incubated at 37°C for 48 hours. The number of bacteria that are able to survive was calculated using the cup count method with the Colony Forming Unit (CFU)[24].

Antimicrobial activities:
Antimicrobial activity tests were carried out using the disc diffusion method by testing *Escherichia coli O157, Listeria monocytogenes, Listeria inocua and Staphylococcus aureus* ATCC 25923 bacteria. A 1 mL LAB culture was placed in sterile eppendorf tubes and centrifuged at a speed of 10000rpm for 5 minutes, with the supernatant used for antimicrobial resistance testing. The Nutrient Media in order to be prepared as much as 0.4grams (the general preparation is 20grams of Nutrient Agar in 1000ml of distilled water), after that 0.2% of test bacteria that have been enriched are added and then cooled to harden. made as many isolates as possible to be tested and labeled. Then 50µl of LAB supernatant was inserted into the well with a micro pipette. The antibiotics of penicillin, ampicillin and kanamycin were added with the same distance between each. After that, it was incubated for 24 hours at 37°C aerobically. The antibacterial activity of the LAB supernatant was expressed as the diameter of the clear area formed[25].

Molecular Identification:
Genomic DNA Isolation of Lactic Acid Bacteria and 16S Rrna:
A sample of single-colony LAB isolate from MRS broth is pipetted for 1,000 L and inserted into a new eppendorf tube, centrifuged at 14,000rpm for 2 minutes. Genomic DNA isolation is done by using Kit Promega (USA). The supernatant is discarded, and the pellet is retrieved and mixed with 480 L of 50mM EDTA before being mixed with 120 L of Lysozyme and incubated in a water bath at 37°C for 60 minutes before being centrifuged at 14,000rpm for 2 minutes. The supernatant is then discarded, and the pellet is retrieved before being mixed with 600L of nuclei lysis solution, homogenised with a micropipette, and incubated at 80°C for 5 minutes. It is then set aside at room temperature before being mixed with 3 L RNase solution, homogenized, and incubated in a water bath at 37°C for 60 minutes before being mixed with 200 L protein precipitation solutions, vortexed, and incubated on ice for 5 minutes before being centrifuged at 14,000 rpm for 3 minutes. The supernatant pipette is then transferred to a new eppendorf tube, the pellet is discarded, 600L of isopropanol is added, and the mixture is homogenised before being centrifuged at 14,000rpm for 2 minutes. The pellet is then removed, the supernatant is discarded, and 600L of 70% ethanol is added before being homogenised and centrifuged at 14,000rpm for 2 minutes. The pellet is retrieved, the supernatant is discarded, the eppendorf-containing pellet is aerated for 15 minutes, and the DNA pellet is rehydrated by adding 50 L of rehydration solution for 30 minutes at a temperature of 65°C. R (16S-1492R, Tm 47°C, 5'GTT TAC CTT GTT ACG ACTT-3), and F (16S-27F, Tm 54.3°C, 5'AGA GTT TGA TCC TGG CTC AG-3), primers are prepared (10pM concentration), retrieved 90 L dH_2O + 10l (R and F Primer) (Note: R and F Primer within TE buffer (100M concentrations)). Insert a DNA ladder for 5L and set the voltage at 100V for 40 minutes. Gel is then put within the container and added with TAE until submerged. Gel is observed under a UV lamp. After reading under UV, the read sample resulted from PCR is purified and ready to be sequenced.

Phylogenetic Investigation:
MEGA v7.0 tools are used for phylogenetic analysis.

RESULT:
Isolation and Identification of Lactic Acid Bacteria

Table I: Microscopy Identification of Lactic Acid Bacteria

Lactic Acid Bacteria Isolation	Color	Shape of Colony	Edge	Elevation
DS1	Yellowish white	Circular	Flat-Smooth	Glistening-Convex
DS2	Yellowish white	Circular	Flat-Smooth	Glistening-Convex
DS3	Yellowish white	Circular	Flat-Smooth	Glistening-Convex
DS4	Yellowish white	Circular	Flat-Smooth	Glistening-Convex
DS5	Yellowish white	Circular	Flat-Smooth	Glistening-Convex

From the results of this study shown in Table 1, through macroscopic observation (shape, size, and color), it is found that the lactic acid bacteria have a yellowish white colour with a circular shaped colony and glistening-convex elevation.

The Characteristics of Lactic Acid Bacteria from *Dadiah*

Biochemical testing:

Table II: Biochemical Analysis of Lactic Acid Bacteria

LAB isolation	Catalyst Test	Fermentation Type
DS1	Negative (-)	Homofermentative
DS2	Negative (-)	Homofermentative
DS3	Negative (-)	Homofermentative
DS4	Negative (-)	Homofermentative
DS5	Negative (-)	Homofermentative

The biochemical property test on a sample of lactic acid bacteria from Dairy revealed that the isolates are negative catalysts and homofermentative.

Resistance to Gastric Acid

Table III. Viability of resistance to gastric acid

LAB Isolation	(10^7CFU/ml)		Viability (%)
	pH control	pH 3	
DS1	135	113	83.70
DS2	167	109	65.27
DS3	67	44	65.67
DS4	192	158	82.29
DS5	91	62	68.13

In table 3, it can be seen that the viability to stomach acid resistance was in the range of 65.27 – 83.70%. The highest result was obtained by isolate DS1 with an 83.70%.

Resistance to Bile Salt

Table IV: Viability of resistance to bile salt

LAB Isolation	(10^7 CFU/ml)		Viability (%)
	Control	Oxgall 0.3%	
DS1	143	97	67.83
DS2	110	61	55.45
DS3	89	60	67.42
DS4	123	43	34.96
DS5	77	12	15.58

The data shown in the table above shows the viability obtained from resistance to bile salt testing from isolations of *dadiah* is around 15.58–67.83%.

Table V:Antimicrobial Activity of LAB from *Dadiah*

Source of Resistance	Clear Zone (mm)			
	E. coli O157	*Listeria innocua*	*Staphylococcus aureus*	*Listeria monocytogenes*
DS1	18.20	18.00	23.19	16.35
Ampicillin	-	-	5.05	-
Kanamycin	15.95	14.80	16.20	15.10

Based on the table above, DS1 isolation has higher inhibition than antibiotics.

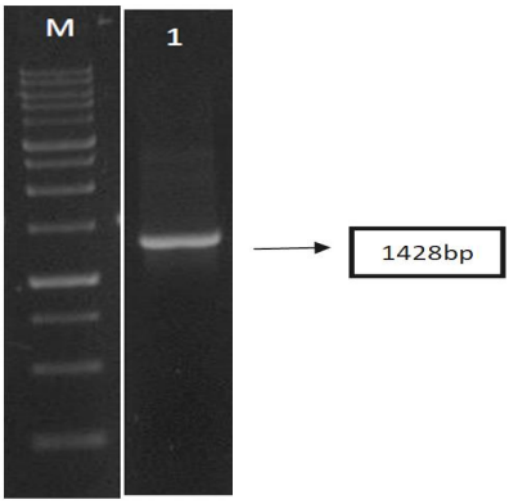

Image 1. PCR Amplification of DS1 Sample

The result obtained in this study (Image 1) shows that the result of amplification DNA on agarosa gel is 1428 bp. This indicates the specific primer used in this study can identify the bacteria up to the strain level.

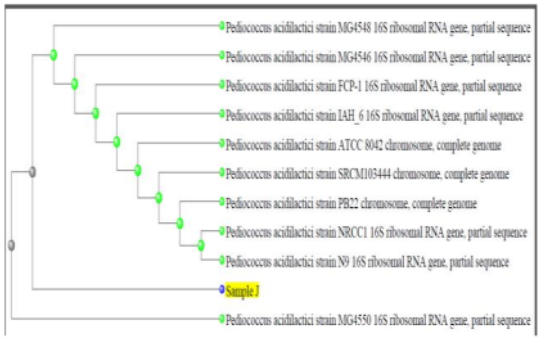

Image 2: **Phylogenic Analysis of DS1 Isolation**

Based on the results of the analysis by using BLAST on https://blast.ncbi.nlm.nih.gov/Blast.cgi, it is found that the type of bacteria in DS1 isolations from *Dah* is akin to *Pediococcus acidhilactici*.

DISCUSSION:

Isolation and Identification of Lactic Acid Bacteria:

Identification and isolation of *dadiah* obtained from Halaban is done by obtaining the total of lactic acid bacteria contained in *dadiah* is $89x10^9$ CFU/g from the result of isolation and purification of lactic acid bacteria. This result corresponds to when the result of a colony of lactic acid bacteria that has the potential as a probiotic is around 10^6-10^8 CFU/gram.

From the results of this study shown in Table 1, through macroscopic observation (shape, size, and color), it is found that the lactic acid bacteria have a yellowish white colour with a circular shaped colony and glistening-convex elevation. This is in accordance with the finding by Purwati*et al*. that the lactic acid bacteria isolation will produce a colony with a yellowish white colour in MRS agar[21].

After a single colony is obtained, the lactic acid bacteria are observed macroscopically after first going through Gram staining to see the colour and shape of the lactic acid bacteria. During the Gram staining, gram-positive bacteria are purple coloured while the gram-negative bacteria are pink colored. The colour is influenced by the thickness of the peptidoglycan wall of the bacteria. The gram-positive bacteria have a thick peptidoglycan wall, causing them to absorb more crystal violet, whereas the gram-negative bacteria have a thin peptidoglycan wall, causing them to absorb less crystal violet. This is in study[25] that found that from the gram staining of gram-positive and gram-negative bacteria isolation, the gram-positive bacteria will absorb crystal violet reagent causing it to turn purple while the gram-negative bacteria will absorb safranin reagent causing it to turn pink[26]. The result of gram staining on lactic acid bacteria isolation from *tempoyak* shows that the bacteria are categorised as gram-positive by the puple colour of the bacteria (26). The result of a study[26] also found that 63 isolates obtained from the isolation of fermented cassava are lactic acid bacteria categorised as gram-positive and negative catalysts[27]. Wasis *et al*. (2018) stated that LAB isolations from pickled bamboo shoots *tabah* (88 isolates) are gram-positive and negative catalysts, which shows that the isolation is lactic acid bacteria[28].

The Characteristics of Lactic Acid Bacteria from *Dadiah:*

The examinations of the characteristics of LAB from *Dahiah* are resistance to gastric acid test, resistance to bile salt test, and antimicrobial activity test. The result of this study is shown in the table below.

Biochemical Testing:

According to table 2 above, the results of the biochemical property test on a sample of lactic acid bacteria from *Dairy yielded the following:* the isolations are negative catalysts and homofermentative. The catalyst test is marked by the absence of gas bubble formation on lactic acid bacteria isolation stroked on cover preparate after it is dripped with 3% H_2O_2. It is that the aim of the catalyst test is to see if the presence of enzyme catalysis will be shown by the presence of gas bubbles on the cover preparate[26]. Lactic acid bacteria are categorised as gram-positive with negative catalyst bacteria and do not form spores. The LAB isolation from *tempoyak* originated by Padang-Pariaman is categorised as a negative catalyst[26]. The lactic acid bacteria isolated from shrimp intestine[29] and mango[30] are categorised as negative catalysts. The result of a study[26] also reported that 63 isolations from the fermented cassava are lactic acid bacteria, which are categorised as gram-positive and negative catalysts[27]. It is stated that LAB isolations from pickled bamboo shoots *tabah* (88 isolations) are gram-positive and negative catalyst bacteria which showed that the isolation is lactic acid bacteria[28].

There are two types of fermentation: homofermentative and heterofermentative. The homofermentative bacteria do not produce air bubbles inside the Durham tube, whereas the heterofermentative bacteria do produce air bubbles. Juliyarsi (2018) explained that the lactic acid bacteria produces lactic acid as its main fermentation product, therefore it is homofermentative, while heterofermentative bacteria produce ethanol or other acids such as acetic acid and CO_2 gases, which produce air bubbles inside the Durham tube. This study also mentioned that the lactic acid bacteria isolated from *tempoyak* originated from Padang-Pariaman are homofermentative[19]. The *Lactobacillus delbreukki* bacteria isolations from yoghurt are also homofermentative[31]. It is also found that lactic acid bacteria isolated from *bekasam* are homofermentative[32].

Resistance to Gastric Acid:

In table 3, it can be seen that the viability to stomach acid resistance was in the range of 65.27 – 83.70%. The highest result was obtained by isolate DS1 with an 83.70%. Probiotics need to be able to go through the stomach where their pH level can be as low as 3.0 and still live for 2–4 hours. This ability to withstand the gastric condition will cause the probiotic to be able to reach the ileum, colonise it, and generate benefits. The ability of microorganisms to live inside gastric juices is related to the formation of a proton motive force that releases protons from the cell so it can withstand the normal intracellular pH level. From the study conducted, LAB isolations from *dadiah* are categorised as probiotic. It is stated that the bacteria which are categorised as probiotics have the ability to influence the intestinal environment when the number of the population reaches at least 10^6–10^8 CFU/mL in acidic conditions and can withstand the increasing acidity of the cytoplasm. Therefore, the protein and enzymes inside the cell can still work at a maximum level, 7,8. The viability result of the resistance of lactic acid bacteria to *dadiah* to acid is between 48.39 and 74.71% (33). The result of this isolation is higher than the result obtained[33] which shows that the viability of lactic acid bacteria isolated from *tempoyak* originated from Padang-Pariaman is

34%. Lactic acid bacteria have the ability to withstand relatively low pH due to their system which can transport lactic acid and protons to the outer part of cells. In order for the lactic acid bacteria to be categorised as probiotics, they need to be able to withstand the very low pH level of the digestive tract and the bile salt. It was found that when an individual is fasting, the pH level of gastric condition can reach as low as 2 and the microorganisms, including *Lactobacillus*, can only live for 30 seconds to several minutes[34]. The result of the study[28] shows there are 3 LAB isolations out of 88 isolations from pickled bamboo shoots *tabah* that have good viability after being incubated for 3 hours in a low pH level condition.

Resistance to Bile Salt:

According to the data shown in table 4 above, the viability obtained from resistance to bile salt testing from isolations of *dadiah* is around 15.58–67.83%. This result is lower than the result of a study[26] on *Lactobacillus plantarum* isolations from *tempoyak,* which had a viability of 97%. The degree of resistance to bile salt is an important characteristic for lactic acid bacteria since it influences their activity inside the digestive system, especially in the upper intestinal tract where bile is being secreted. Bile salt is a surface active compound which causes the activation of a lipolysis enzyme secreted by the pancreas. This enzyme will react to fatty acids in the cytoplasmic membrane of bacteria, causing them to alter the structure and permeability characteristics of the membrane. The variety of fatty acid structures on the cytoplasmic membrane of bacteria cause them to have different permeability and characteristics, so it may also affect bacteria's resistance to bile salt[35]. The resistance to bile salt is also an important property for bacteria in order for the bacteria to be able to grow and resume their activity inside the small intestines because the bacterial cell wall consists of fat and has the possibility of lysis[36]. This is found[17] that 21 isolations from Tibetan Qula can tolerate a 0.3% bile salt concentration. The study[27] on 3 lactic acid bacteria isolations out of 88 isolations from pickled bamboo shoots, *tabah* can develop in low pH levels and 0.3% NaDC supplemented media. Therefore, the bacteria which can withstand bile salt do not undergo cellular permeability and leakage of intracellular materials due to the erosion of lipid caused by bile salt. Therefore, the bacteria are able to withstand and grow in population[37].

Based on table 5 above, DS1 isolation has higher inhibition than antibiotics. The resistance zone formed on the *E. coli* O157 test is 18.20mm, while there is no resistance zone formed on the ampicillin test and the kanamycin test is 15.95mm. The inhibition on the *Listeria innocua* test is 18.00mm, while on ampycillin it is none and on kanamycin it is 14.80mm. The inhibition of *Staphylococcus aureus*test is 23.19mm, while on ampycillin it is 5.05 mm and on kanamycin it is 16.20 mm. The inhibition on the *Listeria monocytogen* test is 16.35mm, while on ampycillin it is none and on kanamycin it is 15.10mm. The area of the clear zone formed by each bacterium is influenced by the bioactive compounds contained within DS1 lactic acid bacteria isolation.

From the results above, the DS1 lactic acid bacteria isolations have the highest inhibition of *E. coli* O157, which is 18.20mm. It was[38] explained that it is due to the ability possessed by LAB to inhibit pathogenic bacteria that can be observed from the area of clear zone formed during antimicrobial testing, which is influenced by secondary metabolites in LAB such as lactic acid and bacteriocin. It is also[39] stated that antibacterial compounds in LAB can be antagonistic toward the development of pathogenic bacteria such as *E. coli, Listeria monocytogenes*, *Staphylococcus aureus*, *Salmonella enterica*, and *Bacillus cereus*.

The result obtained from DS1 isolation is higher than the result[26] where the resistance zone formed by isolations from *tempoyak* originated from Padang-Pariaman on *Escherichia coli* is 16 mm, on *Staphylococcus aureus* is 14 mm, and on *Listeria monositogen* is 10mm.

The result obtained in this study (Image 1) shows that the result of amplification DNA on agarose gel is 1428 bp. This indicates the specific primer used in this study can identify the bacteria up to the strain level. The superiority of the identification technology of LAB based on the 16S rRNA gene can only be conducted if the nucleotide sequence information of the targeted bacteria is known beforehand.

Based on the results of the analysis by using BLAST on https://blast.ncbi.nlm.nih.gov/Blast.cgi, it is found that the type of bacteria in DS1 isolations from *Dah* is akin to *Pediococcus acidhilactici*. *Pediococus acidilactici* can produce bacteriocin[40]. It was found[41] that the identification of *Peiococcus acidilactici* from isolations from *Bekasam was difficult*. This was a study[42] on buffalo milk and identification of a sample from Agam Regency (BMA 3.3), which is classified by using BLAST as strain *Lactobacillus fermentum* (L23). This is in accordance with the result where the bacteria from *dadiah* are dominated by bacteria from *Lactococcus*, *Lactobacillus*, and *Leuconostoc* group which are grouped into lactic acid bacteria. *L. Fermentum* and *L. Plantarum* are obtained from the isolation and identification of *dadiah* originated from Lintau and Air Dingin[42,43,44].

CONCLUSION:

The result of this study shows that the total amount of LAB produced from *dadiah* is $89x10^9$ CFU/gr. Based on single colony identification, it is known that the LAB found is categorised as gram-positive with homofermentative type, negative catalyst. The bacteria have resistance to gastric acid at pH level of 3 with viability of 83.7%, resistance to 0.3% bile salt with viability of 67.3% and has antimicrobial activity against pathogenic bacteria with the best activity, namely the S. aure(1) test bacteria with a clear zone area of 23.19mm. The identification of LAB by using the 16S rRNA gene is based on the result of running PCR with a base length of 1428bp. Based on the phylogenetic analysis, it is known that LAB from *dadiah* originated from Sianok, Bukittinggi City, and is akin to *Pediococcus acidilactici*.

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