Isolation and Identification of Lactic Acid Bacteria from Fermented Buffalo Milk (Dadih) Originated from Kerinci District, Jambi Province of Sumatera, Indonesia

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DOI: 10.29322/IJSRP.11.02.2021.p11005
http://dx.doi.org/10.29322/IJSRP.11.02.2021.p11005

Abstract- *Dadih* is a traditional fermented buffalo milk that originated from the region of Sumatra, particularly in the province of West Sumatera, Riau, and Jambi. This popular Indonesian beverage contains Lactic Acid Bacteria (LAB), which has been proven to lower cholesterol levels and prevent cancer. In this study, LAB in dadih was isolated using the Man Rogosa Sharge (MRS) medium. The isolates were then identified based on their morphology, physiology, and biochemical properties. The tests conducted include; gram staining, spore staining, growth in different temperatures, growth in different salt concentrations, catalase test, ammonia production from arginine, diacetyl and acetoin production, reaction in milk litmus, carbon dioxide production from glucose, dextran production from sucrose, and carbohydrates fermentation. Furthermore, confirmatory testing was carried out using the 16S rRNA molecular identification technique, to determine the species level. Out of the 29 identified species, 5 isolates were identified as *Lactobacillus plantarum* and 1 isolate, *Lactobacillus delbrueckii.*

Keywords: Dadih, Lactobacillus plantarum, Lactobacillus delbrueckii, 16S rRNA.

I. Introduction

Dadih is a traditional Indonesian fermented milk product that is quite popular with local communities in Sumatra islands, particularly in the province of West Sumatra, Riau and Jambi (Surono et al., 1984). In Jambi Province, dadih could be found in the Kerinci district, which includes the regions of Gunung Kerinci, Kayu Aro, and Air Hangat. Dadih produced in Kerinci district is similar to dadih produced in West Sumatra province and other regions.

In Kerinci district, the process of making dadih from buffalo milk has long been known by the community and it has been passed down through many generations while retaining its traditional processing techniques without being altered. The process is traditionally done by not giving heat to buffalo milk and not using starter culture. Raw and fresh buffalo milk is stored in a plastic bucket, filtered with a cloth,then placed in a bamboo tube. A quantity of $\pm$ 250 ml is covered with banana leaves and withered over on fire.The milk is naturally fermented at room temperature for 24-48 hours until the clump formed resembles milky-white colored pasta with smooth heterogeneous texture, sour taste, and a distinctive aroma (Surono and Hosono, 1995; Surono and Hosono, 2000).

Nutrition content of buffalo dadih varies depending on the area of production and type of buffalo milk used. Kerinci dadih has a water content of about 69.08-73.12%, protein of 5.65-5.78%, fat of 7.94-8.27%, and acid content of 0.96-1.03 % (Afriani, 2008). Dadih contains 16 amino acids (13 essential amino acids and 3 non-essential amino acids) and 1.70-7.22 IU/g of vitamin A (Yudoamijoyo et al., 1983), thus it has high nutritional value that is easily absorbed by the body.

Bambu gombong (*Giganto chloaverticillata*), bamboo ampel (*Bambusa vulgaris*), bamboo talang (*Schizo stachyumbrachycladum*), and bambu betung (*Dendrocalamus asper*) are different types of bamboo commonly used to make dadih. Bamboo, serving as the processing container, contains a relatively low water content which helps to produce high-qualitydadih. Bamboo is cut and shaped into a container with a height of 20-30 cm and a diameter of 5-8 cm. At the top,there is a perforated hole, approximately the size of a

finger. Bamboo is further cleaned to remove potential dirt contained in the tube (Zulbardi, 2003). The type of bamboo used is hygroscopic and has a bitter taste preventing products from ants and other insects. It also contains several types of microbes that naturally ferment milk into dadih (Usmiati et al.,2011).

The fermentation process is natural, and it involves different types of microbes found on the inner surface of bamboo tubes, the leaf surface, and buffalo milk itself (Usmiati and Risfaheri, 2013). The fermentation process involves an array of 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 most importantly (Pato, 2003).

Lactic Acid Bacteria, especially the genus *Lactobacillus* and *Bifidobacteriaceae*, are the most widely used microorganism in the food and pharmaceutical industry (Piano et al., 2006). Multiple studies have reported that Lactic Acid Bacteria in dadih has beneficial effects. Dadih has a positive effect on anti cholesterol-activity, stimulatory effects of the immune system, lactose absorption improvement of the body and diarrhea prevention as well as increasing antimutagenic activity that could prevent cancer-especially colon cancer and is thought to be effective as an anti-vaginitis (Hosono et al.,1990; Pato, 2003; Surono 2004a). *Lactobacillus plantarum* from dadih is reported to eliminate microcystin-LR, heptapeptide hepatotoxin cyclic produced by *cyanobacteria* (Nybom et al., 2008), in addition to its ability to β glucosidase (Suhartatik et al., 2014). This study aims to isolate, screen, phenotypically identify, and genotype Lactic Acid Bacteria from fermented milk buffalo (dadih).

II. METHOD AND MATERIAL

Samples

Fermented buffalo milk (dadih) was obtained from conventional buffalo farms in Kerinci District, Jambi Province-Sumatera, Indonesia as a local source of lactic acid bacterial strain. Testing was carried out in the form of isolation and identification of lactic acid bacteria derived from dadih. Isolation was done using de-ManRogosa Sharpe media (MRS, Oxoid UK) and identification of lactic acid bacteria included morphological, physiological and biochemical characteristics as well as molecular identification.

Isolation of lactic acid bacteria

Isolation of lactic acid bacteria derived from the Kerinci dadih was done according to the methods used by Hayakawa 1992; Adnan and Tan, 2007 and Khedid et al., 2009. The isolation of lactic acid bacteria was carried out by suspending 10 grams of dadih (48 hours fermentation) in 90 mL of 0.85% NaCl (dilution 10^{-1}) and serially diluting the solution until a concentration of 10^{-8}. Then, 0.1 mL from dilution 10^{-2} to 10^{-8} was pipetted and inoculated into the MRS agar plate containing Purple Bromo Cresol (BCP) 0.01%. The agar plate was anaerobically incubated for 48 hours at 37°C for bacterial growth. Colonies observed with flat, shining, irregular edge and yellow or gray to brown color. Colonies of different colors and sizes were subcultured into the same media as quadrant streaking and incubation were performed under the same conditions as above. Streaking was done until a single and uniform colony was obtained, and pure colonies were chosen and wound up on the MRS agar for further identification. For stock culture, a pure colony was grown for 2 days in the semi-solid MRS agar (0.7% agar) containing 0.2% $CaCO_3$ in the form of agar and stored at 4°C. Alternative pure colonies were grown in MRS broth containing 20% glycerol stored at -80°C.

Identification and characterization of isolates of LAB

Identification of lactic acid bacteria isolates included phenotype and genotype characteristics. Phenotype identification included morphology, physiology and biochemistry, and genotype identification based on molecular analysis using 16S rRNA.

Morphological characteristic for LAB identification

Identification and characteristic of colonies included size, pigmentation, shape, edge, and elevation, while cell morphological characteristics included gram staining and spore staining. Colonies observed with flat sightings and yellow or gray to brown around the colony. Colony observations included categories dependent on (1) size (small, medium and large), (2) pigmentation, (3) edge formation (circular, irregular and rhizoid) and (4) elevation (flat, raised, convex and umbonate) (Cappuccino and Sherman, 2001; Sunatmo, 2007).

Gram Staining

Gram staining began with dropping the main dyes (crystal violet), evenly above the culture on the glass object and leaving it for 1 minute. Then the glass was tilted to remove any additional crystal violet and rinsed with aquadest carefully.Furthermore, it was bathed inlugol for 2 minutes and tilted and rinsed as described above. The remaining color was cleaned with 95% ethanol for 10-20 seconds until the color of the crystal was no longer flowing from the glass of the object. The glass was then washed with the flowing aquadest, drained and dripped with safranin solution for 10-20 seconds. The glass was tilted and rinsed as mentioned previously, drained and the remaining water was absorbed by absorbing paper. Preparations were now ready to be observed with a microscope. Observation with a microscope was performed using an objective lens of immersion oil (1000x), starting with the lowest magnification and gradually being increased for clarity. Observation was done on size, form, and method of grouping (single, couple, chain, clustered, etc). The positive gram reaction was characterized by purple cell color or blue cell colors and negative pink **(**Hadioetomo, 1993; Cappuccino and Sherman, 2001; Sunatmo, 2007).

Spore staining
Similar to gram staining, as much as one loop sterile aquadest was placed on a clean glass object. A small amount of isolate was then transferred to the glass by using a sterile ose needle to mix and spread the isolate evenly on the glass to air dry. The sample was saturated with malachite green stain solution and heated for 2-3 minutes making sure to prevent any despoil or boil. Counter staining with safranin solution for 30 seconds begins after the sample has been rinsed with aquadest flows carefully and drained. At last, the object glass was tilted, rinsed with flowing aquadest, and drained. The remaining waste water was absorbed by absorbing paper. Preparations were ready to be observed with a microscope. Observation with a microscope was performed using an objective lens of immersion oil (1000x), starting with the lowest magnification and gradually being replaced (Sunatmo, 2007).

Physiological characteristic for LAB identification
The physiological characteristic test consisted of LAB survivability based on temperature and salt concentrations done according to the method used by Hayakawa, 1992 and Aziz et al., 2009.

Temperature survivability test
Temperature survivability was performed by inoculating one loop of colony into a tube containing MRS broth (each duplo) and incubating it for 7-14 days by adjusting the temperature of the tube series at 10ºC, 15ºC, and 45ºC, while the controlwas at the temperature of 30ºC. The presence of growth was seen by the turbidity of the tube (Hayakawa, 1992; Aziz et al., 2009).

Salt concentration survivability test
The survivability of salt concentration was carried out by inoculating 1 loop of colony into a tube of MRS broth containing NaCl with a concentration of 4% and 6.5% (each duplo). The control tube was incubated for 7-14 days at 37ºC. Growth was confirmed by comparing control and treated colonies turbidity. (Hayakawa, 1992; Aziz et al., 2009).

Biochemical characteristic for LAB identification
The biochemical characteristic tests performed include the catalase test, production of ammonia from arginine, diacetyl production and acetoin, reaction on litmus milk, production of carbon dioxide from glucose, production of dextrose from sucrose, and carbohydrate fermentation.

Catalase test
2 drops of 3 percent hydrogen peroxide (3% H_2O_2)were placed on a clean glass object, sterilized and transferred to one ose of bacterial isolate culture and were then evenly mixed. A positive test was characterized by the formation of oxygen bubbles proving that the organism produced the catalase enzyme that converts hydrogen peroxide into water and oxygen (Collins and Lyne, 1980; Hadioetomo, 1993; Sunatmo, 2007).

Ammonia production from Arginine
This test aims to distinguish homofermentative and heterofermentative *Lactobacillus*. The same test was also used to distinguish *Streptococcus* and *Leuconostoc* from other heterofermentative lactic acid bacteria. MRS arginine broth was used to differentiate *Lactobacillus* spp by adding 3 grams of L-arginine monohydrochloride into 1 liter of MRS broth. The arginine broth was used to distinguish *Streptoccus* spp, which was prepared by dissolving 5 grams of tryptone, 2.5 grams of yeast extract, 0.5 grams of D-glucose, 2.0grams of monopotassium phosphate (KH_2PO_4), and 3 grams of L-arginine monochloride in 1 liter of water (pH 7) and then sterilized at 121ºC for 15 minutes. One loop of culture was inoculated into the MRS broth media and incubated at 37ºC for 2-5 days. Afterward, 2 mL of the grown culturewasinserted into the tube with added Nessler's reagent of the same volume. The formation of ammonia was characterized by the formation of brownish-orange color after the addition of Nessler's solution (Nuraida, 1988; Cogan, 1996).

Diacetyl and acetoin production
One full loop of culture was inoculated in Methyl Red Voges Proskauer (MR-VP) media and incubated at 37ºC for 2 days. As much as 1-2 mL of growth from MR-VP medium was transferred into the tube containing 3 drops of 5% alpha-naphthol solution and 40% potassium hydroxide (KOH) solution. MR-VP medium culture residue was incubated for 1 week, and then pressed with a red methyl indicator solution. The formation of a dark red color indicatesthe formation of acetoin. Thus, the color change indicated the formation of acids (Fardiaz, 1993).

Reaction to litmus milk
One loop of culture isolate was inoculated into a tube containing Litmus Milk medium within an inverted Durham tube and then incubated at 30ºC for one week. Observations made revealed color change (into pink) and blob formation, indicating a positive reaction to Litmus Milk **(**Nuraida, 1988; Sunatmo, 2007).

Carbon dioxide production from glucose

This test aims to distinguish homofermentative and heterofermentative lactic acid bacteria. The medium used was Gibson's semi-solid liquid tomato juice. Prior to use, the medium's temperature was lowered to 45ºC. 0.5 mL isolate cultures grown in MRS broth media was added to the liquid above, 2-3 cm to create an anaerobic condition, and incubated at 37ºC for 2-5 days. Fermentative lactic acid bacteria will procedure gases marked with rupture of agar, whereas the homofermentative will not produce any gas **(**Nuraida, 1988; Nuraida et al., 1995).

Dextrose production of sucrose

This test was to distinguish species of the genus *Leucosnostoc*. The composition of sucrose agar mediumof 1-liter volume consists of 10 of grams tryptone, 5 grams of yeast extract, 5 grams of KH_2PO_4, 5 grams of Triamonium citrate, 50 gram of sucrose, and 15 grams of Bacto agar. Sterilization was done at 121ºC for 15 minutes. 0.1 mL of the isolate was inoculated into a sterile Petri dish. Sucrose agar was then poured into the dish, shaken evenly, and incubated at 37ºC for 5 days. Production of dextran from sucrose was characterized by growth in Petri dish **(**Harrigan and McCance, 1976).

Carbohydrate Fermentation

The liquid medium used in this part of the study was MRS broth without sugar and meat extract, with added 0.004% BCP (Bromo Cresol Purple) as an indicator and sterilized at 121ºC for 15 minutes. 10% solution of the sugar used for carbohydrate fermentation wasprepared and sterilized by the filtration method. Aseptic was added up to 2%.The freshened culture was inoculated and incubated at 37ºC for 24-48 hours. Growth was indicated when the medium color was transformed from purple to yellow, indicating the formation of acid (meaning positive fermentation test) and if the gas-formed tubes indicate the gas-producing bacteria **(**Harrigan and McCance, 1976, Sunatmo, 2007**).**

Identification of *Lactobacillus* isolates using 16S rRNA, sequencing and analysis

The 16S rRNA molecular DNA sequencing technique was performed on the isolates (Sambrook and Russel, 2001), including genomic DNA extraction, DNA electrophoresis, gene amplification and analysis of 16s rRNA gene base sequence.

Genomic DNA extraction

DNA genomic isolates of selected lactic acid bacteria were extracted using Ref. 740952.50 NucleoSpin® Tissue Lot number 1397/009 (Macherey-Nagel, German) following the manufacturer's instructions. Bacterial isolates were cultured in 10 ml MRS broth, shaken in the incubator shaker at 150 rpm, at 37ºC overnight. 1.5 mL of bacterial culture was centrifuged at 8,000 xg, at 4ºC for 5 minutes. The supernatant was then removed. For cell lysis, the bacterial pellet was suspended in 180 µl of T1 buffer. By pipetting up and down we ensured no clumps remained. 25 µl of proteinase K was added to the pellet, which then was fast vortexed, and shaken in the incubator shaker at 56ºC for 3 hours until the lysis was formed. After sample was again vortexed, 200 µl of buffer B5 was added to it. The sample was vortexed for the last time, incubated at 70ºC for 10 minutes, centrifuged for 10 minutes at 11,000 xg speed, and the supernatant was transferred to a new sterile micro tube. For DNA binding, 210 µl of ethanol (96-100%) was added to the sample and vortexed to obtain a homogenous mixture. The sample was then carefully placed into NucleoSpin@ Tissue Column sterile collection tubes and centrifuged for 1 minute at 11, 000 xg. The incoming stream was discarded, and the column was placed back into a sterile collection tube (2 mL). To wash the cell, buffer BW was heated up to 50ºC, from which 500 µl was added to the column and centrifuge for 1 minute at 11, 000 xg. The incoming stream was discarded, and the column was placed back into a sterile collection tube, followed by the laundering step with B5 buffer. 600 µl of B5 buffer was added and centrifuged for 1 minute at 11, 000 xg. The incoming stream was discarded at last, and the column was placed back into a sterile collection tube. The dry silica columns were centrifuged for 1 minute at 11,000 x g and the collection tube was removed. For DNA elution, the NucleoSpin@Tissue Column was placed on a sterile1.5 mL micro centrifuge tube. 100 µl of BE buffer was added to the sample, incubated at room temperature for 1 minute, and then centrifuged for 1 minute at 11,000 xg. All purified DNA samples were stored at -20ºC or 70ºC for future subsequent use.

Electrophoresis

Genome DNA was confirmed using Major Science Mini Horizontal Gel Electrophoresis (USA). 1 µl of loading dye and 5 µl of DNA sample were mixed homogeneously on parafilm, and the mixture was loaded into wells of 0.7 % agarose gel for electrophoresis using a micropipette, while the marker was made using as much as 3 µl of sample mixed homogeneously with 1 µl of loading dye, placed into different wells. Electrophoresis was performed for 60 minutes under 85volts of electrical current in 1X TBE buffer. To visualize the DNA fragments, the gel was stained with fluorescent red gel staining dye for 5 to 10 minutes and rinsed with aquades for 10 minutes. Bands were visualized using UV trans-illuminators and gel was stored at 4ºC.

Gene amplification

The selected 16S rRNA universal primers, 27Forwad (5'-AGAGTTTGATCCTGGCTCAG-3') and 1492Reverse (5'-GGTTACCTTGTTACGACTT -3') were used to amplify the 16S rRNA region (Marchesi et al., 1998; Zhang, et al., 2007). Amplification process was done by Applied Biosystem PCR utilizing Veriti 96 Thermal Cycler machine (Fisher Scientific, USA).

The PCR reaction was made in a total volume of 25 µl with a final concentration of 1X, consisting of 12.5 of Econotaq Plus Green 2x Master Mix, 0.25 µl of Forward primer (100 µM), 0.25 µl of Reverse primer (100 µM), 1.0 µl of DNA Template (10 ng/µL), and 11.0

µl of nuclease-free water. All constituents of the mixture were placed into a thin wall PCR tube and vortexed briefly with micro centrifuge. At last, the mixture was amplified using PCR machine. PCR conditions were performed as follows, thermocycler was preheated to 94ºC and initial denaturation of DNA was carried out at an initial temperature of 94ºC for 2 minutes. PCR was proceeded with 30 cycles of denaturation at 94ºC for 30 seconds, annealing at 50ºC for 30 seconds, extension at 72ºC for 30 seconds, and a final extension at 72ºC for 10 minutes before cooling down to 4ºC (EconoTaq® PLUS GREEN 2X Master Mix – Lucigen). All the positive colonies which amplified via colony PCR were analyzed by 0.7% agarose gel electrophoresis. Furthermore, the gel was stained with red gel staining dye and bands were visualized using UV trans-illuminators. Purification of PCR products and sequencing was done by a sequencing service company. The complete 16S rRNA DNA sequence was built using contig assembly by VectorNTi (Invitrogen, USA) and compared to other DNA sequences in the Genbank database using the BLASTN program (http://www.ncbi.nlm.nih.gov/BLAST/). Homology sequences over 97% were considered to have the same species. To determine the phylogenetic relationship, the 16S rRNA sequence was aligned using Clustal W (Thompson et al., 1994). A phylogenetic tree was created using the MEGA program (Molecular Evolutionary Genetics Analysis) version 4 (Tamura et al., 2007).

III. RESULTS

Isolation of lactic acid bacteria

There are twenty-nine colonies obtained from dadihkerinci have different characteristics consisting of size, pigmentation, shape, edge and elevation are presented in Table 1 and Figure 1.

Table 1. Characteristic morphology of colony of lactic acid bacteria origin of dadih

No.	Isolate Code	Size	Pigmentation	Shape	Edge	Elevation
1.	B1.1,B1.2,B2.1.A,B2.1.B,B2.1.C,B4.1.1,B4. 2.1,B4.3.1,B4.3.2,B7.B,B8.1.1,B8.2.1,B8.3.1 ,B8.3.3A,B9. A, B9. B, B11.B	small	milky white or cream	circular	entire	convex
2.	B6.1.1. B,B7. A,B8.1.2, B8.3.2,B10.1,B11.A	medium	milky white or cream	circular	entire	convex
3.	B2.2,B3. A,B4.3.3,B5.2,B6.1.1. A,B6.2	big	milky white or cream	circular	entire	convex

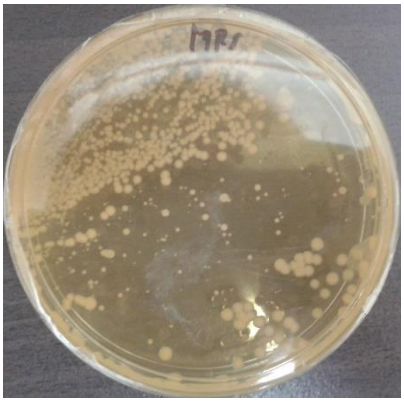

Fig. 1
Figure 1. Colonies of LAB origin from *dadih*

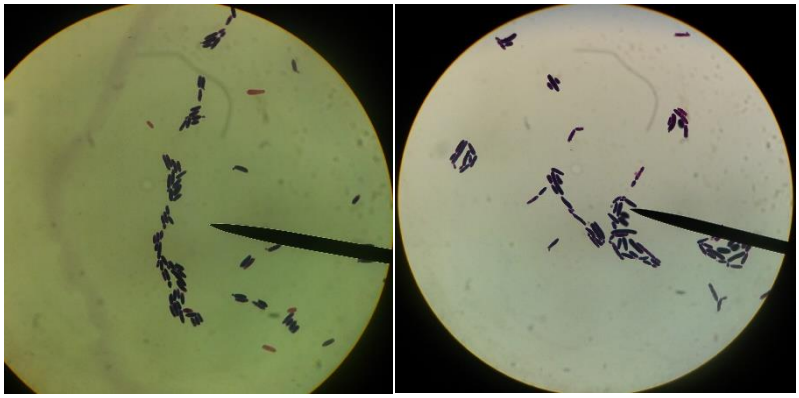

Figure 2. Cell morphology of LAB of dadih (1000 X)

Morphological characteristic for LAB identification

Bacterial staining is performed to distinguish morphological characteristics and cell structure of lactic acid bacteria. The morphological characteristic of isolate cells of lactic acid bacteria are presented in Table 2 and Figure 2 (a and b).

Table 2. Cell morphological characteristic isolates of lactic acid bacteria origin dadih

No	Isolate Code	Staining		Cell morphology	Genus
		Gram	Spore		
1.	B1.1, B2.1.C, B4.3.2, B8.1.1, B8.3.1, B9. A, B9. B	+	-	Bacilli, short, smalll	Lactobacillus
	B1.2, B2.1.A, B2.1.B, B2.2, B3.A, B4.1.1, B4.2.1,				

2.	B4.3.1, B4.3.3, B5.2, B6.1.1.A, B6.1.1.B, B6.2, B7.A, B7.B, B8.1.2, B8.2.1, B8.3.2, B8.3.3A, B10.1, B11.A, B11.B	+	-	Bacilli, long, large	Lactobacillus

Physiological and Biochemical characteristic for LAB identification

The physiological characteristics test of lactic acid bacteria from dadih include survivability test against temperature and salt concentration. Physiological characteristic test results are presented in table 3.

Table 3. Physiological characteristic isolates of lactic acid bacteria origin of dadih

No	Isolate code	Growth at temperature (°C)				Growth at salt (%)	
		10	15	30	45	4	6.5
1.	B1.1	+	+	+	-	+	+
2.	B1.2	+	+	+	-	+	+
3.	B2.1. A	+	+	+	-	+	+
4.	B2.1. B	+	+	+	-	+	+
5.	B2.1.C	+	+	+	-	+	+
6.	B2.2	+	+	+	-	+	+
7.	B3. A	+	+	+	-	+	+
8.	B4.1.1	+	+	+	-	+	+
9.	B4.2.1	+	+	+	-	+	+
10.	B4.3.1	+	+	+	-	+	+
11.	B4.3.2	+	+	+	-	+	+
12.	B4.3.3	+	+	+	-	+	+
13.	B5.2	+	+	+	-	+	+
14.	B6.1.1. A	+	+	+	-	+	+
15.	B6.1.1. B	-	-	+	+	+	+
16.	B6.2	+	+	+	-	+	+
17.	B7. A	+	+	+	-	+	+
18.	B7. B	+	+	+	-	+	+
19.	B8.1.1	-	+	+	-	+	+
20.	B8.1.2	+	+	+	-	+	+
21.	B8.2.1	+	+	+	-	+	+
22.	B8.3.1	-	-	+	-	+	+
23.	B8.3.2	+	+	+	-	+	+
24.	B8.3.3A	-	-	+	-	+	+
25.	B9. A	+	+	+	+	+	+
26.	B9. B	+	+	+	+	+	+
27.	B10.1	+	+	+	+	+	+
28.	B11.A	+	+	+	-	+	+
29.	B11.B	+	+	+	+	+	+

Characteristic test biochemistry of lactic acid bacteria from dadih includes catalase test, the production of ammonia from arginine, diacetyl production and asetoin, reaction on litmus milk, production of carbon dioxide from glucose, production of dextrose from sucrose and carbohydrate fermentation. The biochemical characteristics of lactic acid bacteria are presented in Tables 4 and 5.

Table 4. Biochemical characteristics isolates of lactic acid bacteria origin of dadih

No.	Isolate code	Catalase test	The production of ammonia from arginine		Production of carbon dioxide from glucose	Diacetyl production and asetoin	Production of dextrose from sucrose	Reaction on litmus milk
			MAB	SAB				

1.	B1.1	-	+	+	+	-	-	+
2.	B1.2	-	-	-	-	-	-	+
3.	B2.1. A	-	-	-	+	-	-	+
4.	B2.1. B	-	-	-	+	-	-	+
5.	B2.1.C	-	+	+	+	-	-	+
6.	B2.2	-	+	+	+	+	+	+
7.	B3. A	-	-	-	-	-	+	+
8.	B4.1.1	-	-	-	+	-	-	+
9.	B4.2.1	-	-	-	+	-	-	+
10.	B4.3.1	-	-	-	-	-	-	+
11.	B4.3.2	-	+	+	-	+	-	+
12.	B4.3.3	-	-	-	-	-	+	+
13.	B5.2	-	+	+	-	-	+	+
14.	B6.1.1. A	-	+	+	-	-	+	+
15.	B6.1.1. B	-	+	+	-	-	-	+
16.	B6.2	-	-	-	+	-	-	+
17.	B7. A	-	-	-	-	-	+	+
18.	B7. B	-	-	-	-	-	-	+
19.	B8.1.1	-	+	-	-	-	+	+
20.	B8.1.2	-	+	+	-	-	+	+
21.	B8.2.1	-	+	+	-	-	+	+
22.	B8.3.1	-	-	-	+	-	-	+
23.	B8.3.2	-	+	+	-	-	+	+
24.	B8.3.3A	-	+	+	-	+	+	+
25.	B9. A	-	+	+	+	+	+	+
26.	B9. B	-	+	+	-	+	+	+
27.	B10.1	-	+	+	-	-	+	+
28.	B11.A	-	+	+	+	+	+	+
29.	B11.B	-	+	-	+	+	+	+

Table 5. The test result of carbohydrate fermentation isolates of lactic acid bacteria origin of dadih

No.	Isolate code	Type of carbohydrate																				Lactobacilllus ssp
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	
1.	B1.1	d	-	+	+	+	-	+	+	-	d	+	+	+	+	+	-	+	d	+	+	+	*L.plantarum*
2.	B1.2	d	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	-	+	+	-	+	*L. plantarum*
3.	B2.1. A	d	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	*L. plantarum*
4.	B2.1. B	d	+	+	-	+	-	+	+	+	+	-	d	+	+	+	+	+	+	+	+	+	*S. faecium*
5.	B2.1.C	d	d	+	+	+	d	+	d	+	+	-	d	+	+	+	+	+	+	+	+	+	*L. plantarum*
6.	B2.2	-	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	-	+	*L. lactis*
7.	B3. A	-	+	+	-	+	d	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	*L. plantarum*
8.	B4.1.1	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	*L. plantarum*
9.	B4.2.1	d	d	+	d	+	+	-	d	-	+	-	d	+	+	+	-	-	-	-	+	+	*L. curvatus*
10.	B4.3.1	+	+	+	+	+	+	d	+	+	+	+	+	+	+	+	+	+	+	+	+	+	*L. plantarum*
11.	B4.3.2	+	+	+	+	+	+	-	+	-	d	d	+	+	+	+	-	-	-	d	+	d	*L. mesentroides*
12.	B4.3.3	+	d	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	-	+	+	*L. brevis*
13.	B5.2	-	+	+	+	+	6	+	+	+	+	+	+	+	+	+	+	+	d	+	+	+	*L.brevis*
14.	B5.1.1 A	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	*L. brevis*
15.	B5.1.1 B	+	+	+	+	+	+	+	+	+	d	-	+	+	+	+	d	+	d	+	+	+	*L. brevis*
16.	B6.2	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	d	*L.brevis*
17.	B7. A	d	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	*L.plantarum*
18.	B7. B	d	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	*L.plantarum*
19.	B8.1.1	d	+	+	+	+	d	+	+	+	+	+	+	+	+	+	+	+	+	d	+	+	*L.plantarum*
20.	B8.1.2	d	+	+	+	+	d	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	*L.plantarum*
21.	B8.2.1	+	+	+	+	+	d	+	+	+	+	+	d	+	+	+	+	+	+	d	+	+	*L.plantarum*

22.	B8.3.1	d	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	*L.plantarum*
23.	B8.3.2	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	*L.plantarum*
24.	B8.3.3 A	+	+	+	d	+	+	+	d	d	d	-	+	+	+	+	+	+	+	+	d	+	*L. lactis*
25.	B9. A	+	+	+	+	+	+	+	+	+	d	+	+	+	+	+	+	+	+	+	d	+	*L.plantarum*
26.	B9. B	d	+	d	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	d	+	*L.plantarum*
27.	B10.1	d	+	+	+	+	+	+	d	+	d	+	+	d	+	+	+	+	+	+	+	+	*L.plantarum*
28.	B11.A	d	d	+	+	+	+	+	+	+	d	+	+	+	+	+	+	+	+	+	+	+	*L.plantarum*
29.	B11.B	+	+	+	+	+	+	+	+	+	d	-	+	+	+	+	+	+	+	+	+	+	*L.lactis*

(+): can ferment; (-): can not ferment; (d): dubius 1. Amygladin; 2. Arabinose; 3. Cellobiose; 4. Fructose; 5. Galactose; 6.Glucose; 7. Lactose; 8. Maltose; 9. Mannitol; 10. Mannose; 11. Mellizitose; 12. Mellibiose; 13. Raffinose; 14. Rhamnose; 15. Ribose; 16.Salicin; 17. Sorbitol; 18. Sucrose; 19.Trehalose; 20. Xylose; 21. Esculin

Identification of *Lactobacillus* isolates using 16S rRNA, sequencing and analysis.

Based on 16S rRNA gene sequence analysis result of lactic acid bacteria isolates origin dadih using BLAST program two species of Lactobacillus were obtained among them *Lactobacillus plantarum* and *Lactobacillus delbrueckii* (Table 6).

Table 6. The analysis result BLAST of the isolate of lactic acid bacteria origin of dadih

No.	Isolate code	Description	GenBank Accession Nomor	Query coverage	Max Ident
1.	B.1.1	*Enterococcus faecalis*	KJ725203.1	100%	100%
2.	B.1.2	*Klebsiella pnuemoniae*	CP006656.1	100%	99%
3.	B.2.1A	*Lactobacillus plantarum*	KJ725205.1	100%	100%
4.	B.2.1B	*Lactobacillus plantarum*	KR816164.1	100%	100%
5.	B.2.1C	*Enterococcus faecalis*	CP004081.1	100%	100%
6.	B.2.2)	*Enterococcus faecalis*	CP004081.1	100%	99%
7.	B.3A	*Enterococcus faecalis*	KT260534.1	100%	100%
8.	B.4.1.1	*Enterococcus faecalis*	KT260534.1	100%	99%
9.	B.4.2.1	*Lactoacillus plantarum*	KR816164.1	100%	99%
10.	B.4.3.1	*Lactoacillus plantarum*	KR816164.1	100%	100%
11.	B.4.3.2	*Klebsiella pnuemoniae*	CP013322.1	100%	100%
12.	B.4.3.3	*Enterococcus faecalis*	CP004081.1	100%	99%
13.	B.5.2	*Klebsiella pnuemoniae*	CP006656.1	100%	99%
14.	B.6.1.1A	*Enterococcus faecalis*	KJ725203.1	100%	100%
15.	B.6.1.1B	*Lactobacillus delbrueckii*	KJ725217.1	100%	100%
16.	B.6.2	*Lactoacillus plantarum*	KR816164.1	100%	100%
17.	B.7A	*Enterococcus faecalis*	CP004081.1	100%	100%
18.	B.7B	*Enterococcus faecalis*	KT260534.1	100%	100%
19.	B.8.1.1	*Enterococcus faecalis*	KT260534.1	100%	99%
20.	B.8.1.2	*Klebsiella pnuemoniae*	CP006656.1	100%	99%
21.	B.8.2.1	*Klebsiella pnuemoniae*	CP013322.1	100%	99%
22.	B.8.3.1	*Enterococcus faecalis*	CP004081.1	100%	99%
23.	B.8.3.2	*Enterococcus faecalis*	CP004081.1	100%	99%
24.	B.8.3.3A	*Bacillus thuringiensies*	CP010088.1	100%	97%
25.	B.9A	*Klebsiella pnuemoniae*	CP006656.1	100%	99%
26.	B.9B	*Klebsiella pnuemoniae*	CP006656.1	100%	99%
27.	B.10.1	*Enterococcus faecalis*	CP004081.1	100%	100%
28.	B.11A	*Enterococcus durans*	KJ725230.1	100%	100%
29.	B.11B	*Bacillus antrachis*	CP008853.1	100%	100%

Furthermore, the preparation of phylogenetic trees of lactic acid bacterial isolates was made to determine the relative connection of the species based on genetic resemblance and the difference. The phylogenetic tree analysis results are shown in Figure 3.

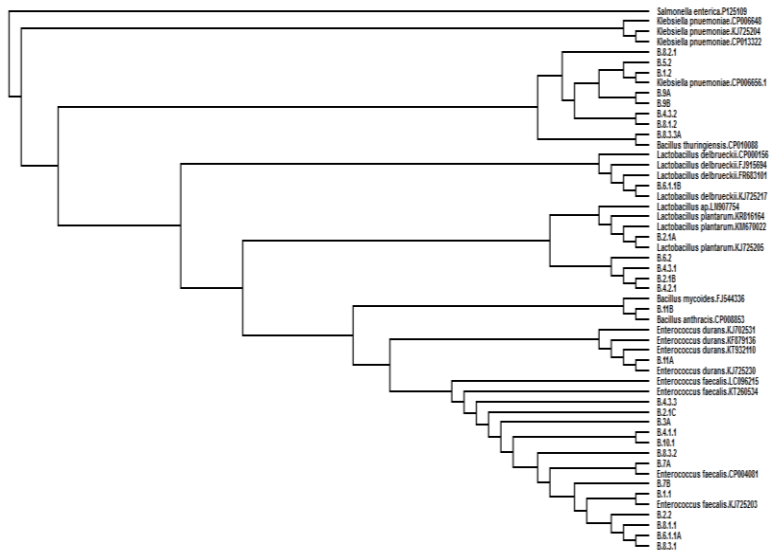

Figure 3. The phylogenetic tree of the lactic acid bacteria isolate originated from dadih is aligned with Genbank isolates

IV. DISCUSSION

Isolation and identification of Lactic Acid Bacteria (LAB)

Twenty-nine colonies that were obtained from the isolation, selection, and identification of the Kerincidadih, Jambi have different characteristics. Overall, colonies were milky white or creamy, circular-shaped, with an entire edge, and convex elevation (Figure 1). Based on the size, colonies could be distinguished into 3 types; consisting of 17small isolates, 6 medium-sized, and 6 large-sized isolates (Table 1). Hayakawa (1992), reported that colony characteristics of lactic acid bacteria that grew on agar plates were different.For example, the color of the colony depends on the origin of the isolate. *Lactobacillus* sp. colonies come from milk and were usually yellow, with gray to brown areas surrounding the colony, flat-shaped to bulging, and colonies weresometimes irregularly colored. The brown color seen in some colonieswasdue to the production of acids by isolates of lactic acid bacteria.

Some researchers have reported the characteristics of lactic acid bacteria coloniesfrom both fermented milk and non-fermented milk. Sunaryanto and Marwoto (2013) reported that colonies of lactic acid bacteria from dadih had round edges and milky white color. Purwati et al., (2014) reported that colonies of lactic acid bacteria from the Dadih Air Dingin, Solok District grown in MRSagar medium had a milky white color,round shape, slippery edges, and convex elevation. Nur et al., (2015) reported the morphology of lactic acid bacteria colonies from Dangke as having a white or milky color, with a round shape anda flat edge, in addition tothe sparkling surface of the colony. Also, a clear zone forms surrounding the colony with different sizes (small to medium and medium to large).

Syukur et al., (2015) reported the morphology of lactic acid bacteria colonies from dadih grown in a solid medium have white or a milky white colorand weresmall-sized with a smooth surface and organized surrounding colonies. Syukur et al., (2016) added that colonies of lactic acid bacteria from dadih were rounded with soft and smooth surfaces. Marlida et al., (2016) reported that colonies of lactic acid bacteria grown in MRSagar medium containing 2% CaCO3 were white and form a clear zone around the colony. Maged S. Bin Masalam et al., (2018) reported the morphology of lactic acid bacterial colonies grown on the MRS agar have varying colors ranging from white to pale cream, with a circular shape and a size range of 0.5 to 4 mm. In

addition, the morphology of lactic acid bacteria colonies from buffalo milk was reported by Rizqiati et al., (2015) and Rizqiati et al., (2016) as having an oval or round shape with a white color and the formation of a yellow zone around the colony.

Morphological characteristic for LAB identification

Lactic acid bacterial isolates from Kerinci dadihwere included in the genus *Lactobacillus*, because they appear to be a non-spore forming gram-positive bacteria (Figure 2a and b). Based on the observed cell size, they were differentiated into 2 types one group consisting of 7 isolates in the form of bacilli, short, and small, and the other group consisting of 22 isolates in the form of bacilli, lengthy, and large (Table 2).

Some researchers reported the morphological characteristics of lactic acid bacterial cells from fermented dairy products. Shi et al., (2012), reported that isolates of lactic acid bacteria from traditional fermented mare milk were bacillary and include Gram-positive bacteria. Sunaryanto and Marwoto (2013) reported that isolates of lactic acid bacteria from dadih include Gram-positive bacteria with bacillary cellular shape and no spores. Purwati et al., (2014), reported that 11 isolates of lactic acid bacteria from the dadih Air DinginSolok District had a bacillary form and included Gram-positive bacteria. Syukur et al., (2014a) and Syukur et al., (2016) reported that lactic acid bacteria isolates from dadih included Gram-positive bacteria.Syukur et al., (2015) reported that the isolates of lactic acid bacteria of dadih were included in Gram-positive bacteria with cellular coccus shaped cells in pairs or in chains. Nur et al., (2015) reported that isolates of lactic acid bacteria from Dangke were Gram-positive bacillus shaped bacteria. Marlida et al., (2016) reported that isolates of lactic acid bacteria of dadih were Gram-positive bacteria, bacillus shaped with no spores. Dekumpitiya et al., (2016) reported that the isolates of lactic acid bacteria from Sri Lankan Buffalo Milk Curd consisted of 69%*Lactobacillus* sp and 21%*Streptococcus* spp. *Lactobacillus* sp in curd wasa catalase-negative gram-positive bacteria, and *Streptococcus* spp in curd was non-spore forming oval-shaped catalase-negative Gram-positive bacteria, that was not motile. Maged S. Bin Masalam et al., (2018) reported isolates of lactic acid bacteria from raw and fermented milk (68%) were included in Gram-positive bacteria.

Axelsson, (2004) reported that lactic acid bacteria were Gram-positive bacteria, bacillus shaped or rounded, with no spores, that were carbohydrate fermenting, catalase-negative, and microaerophilic. Salminen and Wright (1998), also reported that lactic acid bacteria were Gram-positive bacillus or round-shaped with catalase-negative characteristics. The morphological characteristic of the lactic acid bacterial cells of the raw camel milk was reported by Khedid et al., (2009) as a Gram-positive bacterium, consisting of bacilli (37.5%) and coccus (62.5%). Setyawardani et al., (2011) reported that 33 isolates of lactic acid bacteria from Ettawa crossbreed and Saanen crossbreed were Gram-positive bacteria consisting of 26 rod-shaped isolates; 1 short rod-shaped isolate; 3 round, oval-shaped isolates; and 3 round-shaped isolates. Mithun et al., (2015) reported that lactic acid bacteria from raw milk of Aarey Colony of India was a rod-shaped, non-spore forming gram-positive bacteria. Rizqiati et al., (2015) reported that 84 of the 96 isolates of the original lactic acid bacteria from North Sumatera River buffalo milk included Gram-positive bacteria, consisting of 19 isolates of round-shaped and 65 isolates of rod-shaped. Rizqiati et al., (2016) reported that 21 of the 30 lactic acid bacteria isolated from Pampangan buffalo milk, South Sumatra Indonesia included Gram-positive bacteria, consisting of 15 rod-shaped isolates and 6 round-shaped isolates.

Physiological and biochemical characteristic for LAB identification

The physiological characteristic test against lactic acid bacterial isolates includes a survivability test for temperature and salt concentration. Growth of lactic acid bacterial isolates from Kerinci dadih at different temperatures was more varied. Overall, the isolates were able to grow well at a temperature of 30 ^{o}C. 26 of the isolates could grow at 10 ^{o}C and 15 ^{o}C, but there were only 5 isolates that have survivability at 45 ^{o}C (Table 3).

Surono (2004b) reported that temperature was one of the factors that affected bacterial growth. El Soda et al., (2003) reported that from the *Lactobacillus* genus, thermophilic and cocci groups were bacterial groups that

could grow at 45ºC and could not grow at a temperature of 10 ºC. The mesophilic group was a group of bacteria that was not able to grow at a temperature of 45 ºC, but could grow at a temperature of 10ºC. In contrast, Lactococci mesophilic was a bacterial group capable of growing at 10ºC which could not grow at a temperature of 45ºC. The Enterococci group was a group of bacteria that grows at both temperatures of 45 ºC and 10 ºC. Elgadi et al., (2008) reported that 14 isolates from raw milk have the ability to grow at a temperature of 45 ºC, and could not grow at a temperature of 15 ºC.

Setyawardani et al., (2011) reported that all isolates of original goat's milk could grow at 37ºC, 24 isolates were able to survive at 45ºC and only 18 isolates were able to survive at 10ºC. Rizqiati et al., (2015) stated that 78 of the 84 original isolates from North Sumatera river buffalo milk were able to survive high temperatures, and only 6 isolates were not resistant to high temperature. Further, Rizqiati et al., (2016) added that 15 of the 21 Pampangan buffalo milk isolates from South Sumatra Indonesia were able to survive at high temperatures and only 6 isolates were not resistant to high temperature.

The tolerance test of lactic acid bacteria to salt concentration revealed that all isolates from dadih of Kerinci were able to survive at a salt concentration of 4% and 6.5% (Table 3). Setyawardani et al., (2011) obtained the same results, showing that all isolates of goat's milk have survivability at a salt concentration of 4% and 6.5% for 7 days. Rizqiati et al., (2016) added that all Pampangan buffalo milk isolates could grow well at a salt concentration of 4% and 6.5%. Biochemical characteristic tests were performed on lactic acid bacterial isolates from dadih. Based on catalase testing, all isolates of lactic acid bacteria from dadih did not produce O_2 vesicles (Table 4), thus they were grouped with the catalase-negative bacteria. This was in line with the opinion of Surono (2004b),stating the bacteria which does not produce O_2 vesicles possesses peroxidase enzymes that could prevent the production of O_2 and were classified as catalase-negative bacteria.

Some researchers have reported the characteristic catalase test of lactic acid bacterial cell isolated from fermented dairy products. Shi et al., (2012), reported that isolates of lactic acid bacteria from the traditional fermented mare milk have a negative catalase activity. Sunaryanto and Marwoto (2013), reported that the isolates of lactic acid bacteria from dadih were non-motile, catalase and oxidase-negative. Syukur et al., (2014a) reported that isolate of lactic acid bacteria from dadih includes a catalase-negative bacterial group that was resistance to low pH. Syukur et al., (2014b; 2015 & 2016) also reported that isolate of lactic acid bacteria from dadih includes catalase-negative bacteria. Nur et al., (2015), reported that two isolates of lactic acid bacteria selected from Dangke were capable of surviving at the pH of 2. The same strain of bacteria were catalase, KIA, urea, citrate, and LIA-negative and were not motile. Marlida et al., (2016) reported that lactic acid bacteria of dadih have a negative catalase and oxidase activity and produce lactic acid as their major metabolite of carbohydrate fermentation. Maged S. Bin Masalam et al., (2018) reported that 71% of lactic acid bacterial isolates from raw and fermented milk were catalase-negative, 72% were oxidase-negative, and 96% were non-motile.

In addition, in regard to the catalase characterization of lactic acid bacteria from milk, Setyawardani et al., (2011) reported that all isolates of goat's milk had negative catalase activity. Mithun et al., (2015) reported that lactic acid bacteria from raw milk of Aarey, India have a negative catalase activity with anaerobic properties. Rizqiati et al., (2015) reported 52 of 70 lactic acid bacterial isolates from North Sumatera river buffalo milk were catalase-negative. Moreover, Rizqiati et al., (2016) added, that 20 of the 21 lactic acid bacterial isolates from Pampangan buffalo milk were catalase-negative. The Biochemical characteristic test of CO_2 production from glucose was performed on lactic acid bacterial isolates from dadih. Consequently, 12 isolates of lactic acid bacteria were able to produce CO_2 (heterofermentative) and 17 isolates were not able to produce CO_2 (homofermentative), data presented in Table 4. Similar results were obtained by Abdulah and Osman, (2010) stating that homofermentative lactic acid bacteria were found in cow's milk, cheese, and fermented milk. Rizqiati et al., (2015) added that 21 of the 70 isolates of North Sumatera river buffalo milk produce CO_2

(heterofermentative) and 49 isolates do not produce CO_2 (homofermentative). Rizqiati et al., (2016) confirmed that 6 of the 21 Pampangan buffalo milk isolates produce CO_2 (heterofermentative) and 15isolates do not (homofermentative). Based on fermentation, Axelsson (2004) explained, that lactic acid bacteria consisted of three groups; obligatory homofermentative, facultative heterofermentative, and obligatory heterofermentative.

According to the ammonia production from arginine test results of the lactic acid bacterial isolates from dadih, it was found that 14 of the 29 lactic acid bacterial isolates do not produce ammonia but 15 isolates do produce ammonia (Table 4). Ammonia producing isolates were not selected because they were feared to affect the product's aroma. Setyawardani et al., (2011), reported that all isolates of lactic acid bacteria from goat's milk do not produce ammonia from arginine. Rizqiati et al., (2015) noted that 41 of the 48 isolates did not produce ammonia from arginine and only 7 isolates produced ammonia. Rizqiati et al., (2016) reported that 19 of the 21 isolates did not produce ammonia from arginine and only 2 isolates produced ammonia. Tserovska et al., (2002) reported that 60% of lactic acid bacteria from cheese and milk were capable of producing ammonia from arginine.

Testing of diacetyl and acetoin production, dextran production of sucrose, and litmus milk reactions were also performed on lactic acid bacterial isolates from dadih. The results revealed that 22 of the 29 lactic acid bacterial isolates from dadih did not produce diacetyl and acetoin in contrast to only 7 isolates that did. Overall, lactic acid bacterial isolates from dadih showed a positive reaction to litmus milk, while the results of dextrose production from sucrose pointed that that 13 of the 29 isolates of lactic acid bacteria from dadih did not produce dextran but 16 isolates did (Table 4). Setyawardani et al., (2011) reported that all isolates from goat's milk did not produce dextran. Moreover, Rizqiati et al., (2015) stated that 48 of the 52 lactic acid bacterial isolates from North Sumatera river buffalo milk did not produce dextran and only 4 isolates produced dextran. Rizqiati et al., (2016) reported that all isolate of lactic acid bacteria from Pampangan buffalo milk did not produce dextran as well. Lactic acid bacterial isolates that do not produce dextran were not included in the *Leuconostoc* group. Dextran production as a mucoid was one of the key characteristics of *Leuconostoc*. Dextran was defined as a water-soluble polysaccharide formed from α1-6 glucoside with a proportion of 0-20% (Sarwat et al., 2008).

Carbohydrate fermentation

Identification of the species based on carbohydrate fermentation pattern was done by referring to the Bergey's Manual of Determinate bacteriology. It was showed that of the 29 identified isolates of lactic acid bacteria from the Kerinci dadih, 18 isolates were *Lactobacillus plantarum*, 5 isolates were *Lactobacillus brevis*, 3 isolates were *Lactobacillus lactis*, 1 isolate was *Lactobacillus curvatus*, 1 isolate was *Leuconostoc mesentroides*, and 1 isolate was *Streptococcus faecium* (Table 5). Some researchers have utilized carbohydrate fermentation pattern to identify Lactobacillus species of fermented milk. In buffalo milk products (dadih) of Indonesia 6 species of lactic acid bacteria have been identified and obtained; *L. brevis, L. plantarum, L. casei, L. paracasei, Lactococcus lactis,* and *Leuconostoc mesenteroides*(Surono 2004b). Shi et al., (2012) also reported that 24 isolates of *Lactobacillus rhamnosus*, and 1 isolate of *Lactobacillus fermentum* were obtained from traditional fermented Mare milk, in addition to 2 isolates that were not identified. Sunaryanto and Marwoto (2013) reported that *Lactobacillus plantarum* was an isolate of lactic acid bacteria identified from the buffalo milk dadih. Nur et al., (2015) stated that *Lactobacillus plantarum* and *Lactobacillus fermentum* were two species of lactic acid bacteria identified from Dangke. Dekumpitiya et al., (2016) found that there were 5 species of lactic acid bacteria isolated from Sri Lanka buffalo milk curd; *Lactobacillus delbrueckii subsp. lactis, Lactobacillus plantarum, Lactobacillus helveticus, Lactobacillus fermentum, Lactobacillus delbrueckii* subsp. *bulgaricus,* and *Lactobacillus casei* subsp. *casei and* 2 species of *Streptococus* among genus; *Streptococcus thermophilus* and *Streptococcus lactis.*

Furthermore, it was also reported that Lactobacillus species identification was based on the carbohydrate fermentation pattern. Khedid et al., (2009) reported the highly abundant and dominant species commonly found in various dairy productswereof *Lactococcus lactis subs. lactis* (17,5%), *Lactobacillus helveticus* (10%), *S.*

Salivariussubsp.thermophilus (9.2%), *E. casseliflavus* (7.5%), *Lactobacillus casei subsp. casei* (5.8%), and *Lactobacillus plantarum* (5%). Setyawardani et al., (2011) added that there were 3 species of *Lactobacillus* identified from goat's milk; 2 isolates of *Lactobacillus rhamnosus*, 1 isolate of *Lactobacillus plantarum,* and 4 isolates of *Lactobacillus plantarum*1. Rizqiati et al., (2015) described 4 species of lactic acid bacteria found in North Sumatera river buffalo milk; 4 isolates of *Lactobacillus plantarum,* 3 isolates of *Lactobacillus brevis*, 1 isolate of *Lactobacillus pentosus,*and 2 isolates of *Lactococcus lactis*. Rizqiati et al., (2016) reported 5 species of lactic acid bacteria found in Pampangan buffalo milk of South Sumatera; 1 isolate of *Lactobacillus brevis*, 4 isolates of *Lactobacillus paracasei*ss, 1 isolate of *Lactobacillus pentosus,* 2 isolates of *Lactobacillus plantarum,* and 2 isolates of *Lactococcus lactis* ssp lactis. Also, species identification ofbacterial isolates from buffalo milk in India resulted in 6 species of lactic acid bacteria; *L. bulgaricus, L. plantarum, L. lactis, L. acidophilus, L. brevis,* and *L. rhamnosus* (Tambekar et al., 2009; Singh and Sharma, 2009; Shafakatullah and Chandra, 2014).

Identification,sequencing, and analysis of *Lactobacillus* isolates using 16S rRNA

The phylogenetic approach is the latest system of bacterial taxonomies. The relationship between bacteria is known by comparing the molecular base sequences of primarily 16S rRNA gene. Species determination of lactic acid bacterial isolates from dadih originated in Kerinci (Table 5) based on carbohydrate fermentation test showed different results once compared to molecular identification based on 16S rRNA base sequence analysis (Table 6). Identification withcarbohydrate fermentation test was less accurate and required confirmatory tests. In contrast,the molecular identification of lactic acid bacterial species was quick and accurate. This is confirmed by Conter et al., (2005), which reported that identification using carbohydrate fermentation test is less accurate and contains errors indetermination of lactic acid bacterial species.

Based on the analysis results obtained using BLAST (Table 6), and preparation of phylogenetic trees (Figure 3), from the 29 bacterial isolates original to Kerinci dadih, 5 isolates of *Lactobacillus plantarum,* 1 isolate of *Lactobacillus delbrueckii*, 13 isolates of *Enterococcus faecalis*, 1 isolate of *Enterococcus durans*, 7 isolates of *Klebsiella pneumonia*, 1 isolate of *Bacillus thuringiensies,* and 1 isolate of *Bacillus antrachis*were identified. *Lactobacillus plantarum* consists of B.2.1A, B.2.1B, B.4.2.1, B.4.3.1, B.6.2, and 1 isolate of *Lactobacillus delbrueckii* that is B.6.1.1B.

Isolate B2.1A had a close relationship with *Lactobacillus plantarum* KJ725205.1 (100% query coverage and 100% maximum identification), isolatesB2.1B, B4.3.1, and B6.2 had a close relationship with *Lactobacillus plantarum* KR816164.1 (100% query coverage and 100% maximum identification), and isolates B4.2.1 had a close relationship with *Lactobacillus plantarum* KR816164.1 (100% query coverage and 99% maximum identification). Furthermore, isolates B6.1.1B had a close relationship with *Lactobacillus delbrueckii* KJ725217.1 (100% query coverage and 100% maximum identification).

Some researchers reported the molecular identification results of lactic acid bacteria in fermented milk. Shi et al., (2012) reported that from traditional fermented mare milk 25 isolates of *Lactobacillus rhamnosus* and 2 isolates of *Lactobacillus fermentum* were identified. Sunaryanto and Marwoto (2013), reported that *Lactobacillus plantarum* is a dominant lactic acid bacterium isolated from dadih of Payakumbuh, West Sumatera. Purwati et al., (2014) reported that *Lactobacillus plantarum* is a lactic acid bacterium found in dadih that comes from Air Dingin, Solok District West Sumatera. Furthermore, Syukur et al., (2014a) reported that *Lactobacillus plantarum* is a lactic acid bacterium found in dadih that comes from Pematang Panjang, Sijunjung District, West Sumatera.

Lactobacillus plantarum

Lactobacillus plantarum is a homofermentative lactic acid bacteria that is a gram-positive facultative anaerobe. It is shaped like a straight rod with round ends, having a width of 0.9-1.2 um and a length of 3-8 um. It is non-spore forming and non-motile and is available as a single, paired or in short chains (Bringel et al., 2005).

Lactobacillus plantarum is a catalase-negative bacterium capable of melting gelatin. It can digest proteins quickly, it does not reduce nitrates, and it is tolerant of acids with optimum growth over the pH range of 4.0 - 6.8. The colony is 3 mm in size, round and smooth, compact and white color and non-translucent to light. This bacterium is capable of overhauling the complex compound into a simpler compound with the final result of lactic acid. These bacteria are able to grow at a temperature of 15ºC, generally not growing at 45ºC and its optimum growth at 30-35ºC (Bergey and Holt., 1994; Bringel et al., 2005).

Lactobacillus plantarum has a negative catalase property, capable of melting gelatin, can digest proteins quickly, not reducing nitrate and tolerant to acid with optimum growth of bacterial pH ranges from 4.0-6.8. The colony is 3 mm in size, round and smooth, compact and white color and non-translucent to light. This bacterium is capable of overhauling the complex compound into a simpler compound with the final result of lactic acid. These bacteriaare able to grow at a temperature of 15ºC, generally not growing at 45ºC and its optimum growth at 30-35ºC (Bergey and Holt., 1994; Bringel et al., 2005). *Lactobacillus plantarum* is one of the highly flexible and versatile of lactic acid bacteria species, many found in milk and its products, silage, fermented food products (sauerkraut, pickled vegetables), sausage fermentation, salted fish and in saliva, intestinal tract and feces (Bergey and Holt. 1994).

Lactobacillus delbrueckii

Lactobacillus delbrueckii is a Gram-positive bacterium, homofermentative type, anaerobic facultative, non-motile species that do not form spores but has bacilli with rounded edges (cell size 0.5-0.8 x 2.0-9.0 um), It is available as single, in pairs or in a short chain and is an important member of lactic acid bacteria in the industry (Bringel et al. 2005). This bacterium is catalase-negative, optimum growth at pH 5.5-5-8 and growth stopped at pH 3.5-3.8, therefore more present in the final process of lactic acid type fermentation. *Lactobacillus delbruekii* includes a type of thermophilic bacteria with optimum growth at 45 °C, but it can grow up to 48-52 °C but does not grow at a temperature of 15° C. Colonies are usually rough and non-pigmented. These bacteria are found in milk and processed products (yogurt and cheese). The main metabolic product of this bacterium is lactic acid, but it also produces acetal dehydes, acetone, acetoin and diacetyl in relatively low quantities. The acetaldehyde and diacetyl provide a distinctive aroma and contribute to the flavor of yogurt produced (Bergey and Holt, 1994).

V. CONCLUSION

Twenty-nine bacterial colonies originated from fermented buffalo milk (dadih) from the Kerinci district in Jambi were successfully isolated, identified and characterized using conventional methods (phenotypic characterization) and molecular analysis of PCR (genotypic characterization).

Phenotypic characterization

Morphological characteristics of the colony consist of 3 sizes (small, medium and large) and overall the colonies of white milk or cream, circular shape, edge entire and convex elevation. The morphological characteristics of the cell, overall isolates were rod-shaped, no spora, was included in Gram-positive bacteria and was distinguished over two cell sizes consisting of short, small and long, large. Physiological characteristic of the cells, overall the isolates were able to grow well at a temperature of 30ºC, 26 of isolates grew at 10ºC and 15ºC, but there were 5 of isolates that have survivability at 45ºC. The ability to grow to salt concentrations indicates that all isolates could survive at a salt concentration of 4 and 6.5%. Biochemical characteristics, all isolates were included in negative catalase bacteria, 14 of 29 isolates did not produce ammonia and only 15 of isolates produced ammonia. 22 of 29 isolates did not produce diacetyl and acetoin and only 7 of isolates produced. Overall isolates showed a positive reaction to litmus milk. It was found that 13 of 29 isolates from dadih did not produce dextran and only 16 of isolates produced dextran. Based on carbohydrate fermentation pattern obtained 18 of isolates *Lactobacillus plantarum*, 5 of isolates *Lactobacillus brevis*, 3 of isolates

Lactobacillus lactis, 1 isolate of *Lactobacillus curvatus*, 1 of *Leuconostocmesentroides* and 1 of *Streptococcus faecium.*

Genotypic characterization

Identification by using molecular analysis PCR (genotypic characterization) has detected 5 isolates of *Lactobacillus plantarum*, 1 isolate of *Lactobacillus delbrueckii*, 13 isolates of *Enterococcus faecalis*, 1 isolate of *Enterococcus durans*, 7 isolates of *Klebsiella pneumonia*, 1 isolate of *Bacillus thuringiensies* and isolate of *Bacillus antrachis*.

ACKNOWLEDGMENT

The authors thank the University of Malaya for bench work and also financial support from Agro Premier Biotech Sdn Bhd Kuala Lumpur Malaysia.

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