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Kajian Aktivitas Antimikroba dan Proteolitik Bakteri Asam Laktat Isolat Dadih: Susu Kerbau Fermentasi Alami Dari Sumatera Barat, Indonesia

Assessment of Antimicrobial and Proteolytic Activity of Lactic Acid Bacteria Isolated from Dadih: Naturally Fermented Buffalo Milk From West Sumatra, Indonesia

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ABSTRAK

Dadih merupakan produk susu fermentasi tradisional yang berasal dari Sumatra Barat dan sejak lama merupakan bagian integral dari diet dan kebudayaan masyarakat Minangkabau. Tujuan penelitian ini adalah untuk mengevaluasi aktivitas antimikroba dan proteolitik bakteri asam laktat (BAL) yang diisolasi dari dadih. Empat sampel dadih yang digunakan berasal dari 2 lokasi yang berbeda berdasarkan metode fermentasi yang digunakan, yang kemudian diisolasi dan diseleksi berdasarkan karakteristik morfologi, genotip dan fenotip. Isolasi dilakukan menggunakan media agar *de Man Rogosa Sharpe (MRSA)* ditambah CaCO3 0.5%. Isolat dengan zona bening disekitar koloni kemudian diuji sifat morfologinya. Seluruh isolat kemudian diuji aktivitas antimikroba dan proteolitiknya menggunakan metode sumur difusi. Tahap akhir seleksi adalah identifikasi isolat berdasarkan analisis sekuens gen 16 rRNA dilanjutkan dengan profil fermentasi isolat menggunakan kit API 50 CHL. Tujuh belas isolat berhasil diisolasi dan menghasilkan aktivitas antimikroba terhadap bakteri gram positif dan negatif. Ketujuhbelas isolat juga menghasilakn aktifitas proteolitik yang tinggi. Analisis sekuen gen 16 rRNA dan profil fermentasi pada kit API 50 CHL menunjukkan bahwa 12 isolat teridentifikasi *Lactococcus lactis*, 3 isolat teridentifikasi *Lactobacillus plantarum*, dan masing-masing 1 isolat teridentifikasi sebagai *Lactobacillus pentosus* dan *Pediococcus pentosaceus.* Hasil penelitian ini menunjukkan bahwa bakteri asam laktat yang diisolasi dari dadih memiliki sifat fungsional antimikroba dengan zona hambat berkisar antara 1-6.1 mm dan aktivitas proteolitik yang berkisar antara 15-26 mm. Hal ini mendukung untuk pengembangan isolat asal dadih sebagai kultur starter susu fermentasi.

KEYWORDS:
Antimicrobial activity
Proteolytic activity
Dadih
Lactic acid bacteria

ABSTRACT

Dadih is a traditional fermented food from West Sumatra Indonesia and had become an integral diet and culture for Minangkabau tribes. The aims of this study were evaluated antimicrobial and proteolytic activity of lactic acid bacteria (LAB) isolated from dadih. Four different dadih samples was taken from 2 origins based on fermentation methods. Samples were isolated and screening for

morphological, genotype and phenotype characteristic. Isolation was conducted in de Man Rogosa Sharpe (MRS) agar containing 0.5% $CaCO_3$ followed by morphological identification. All isolates were selected based on antimicrobial and proteolytic activity with agar well diffusion method. Finally, the selected isolate were identified based on 16S rRNA partial gene sequence analysis and fermentation profile using API 50 CHL kit test. Seventeen isolates not only exhibited wide spectrum of antimicrobial activity against gram positive and negative bacteria, but also high proteolytic activity. Partial gene sequence analysis and API 50 CHL kit showed 12 isolates were identified as Lactococcus lactis, 3 isolates were identified as Lactobacillus plantarum, and 1 isolate each was identified as Lactobacillus pentosus and Pediococcus pentosaceus respectivelly. The result shows that dadih was a potential source of LAB with high antimicrobial (range between 1.0 to 6.1 mm) and proteolytic traits (range between 15 to 2.1 mm). This makes it possible to develop the functional properties of these isolates as a starter culture in milk fermentation.

1. Introduction

Dadih is traditional fermented buffalo milk in a bamboo tube from West Sumatra Indonesia. Spontaneous fermentation mainly play a role in dadih processing, and lactic acid bacteria which is naturally present in buffalo milk (Rizqiati, 2015), bamboo tubes, and the environment is the most important microorganism in this process. The presence of lactic acid bacteria (LAB) in dadih provides felicitous benefits for human health, due to the formation of bioactive components during fermentation such as lactic acid, antimicrobial compounds, and other beneficial traits as probiotics (Akuzawa et al., 2011).

Mostly in fermented milk, LAB is the main microorganism involved. Milk constituent is naturally an ideal habitat for its growth (Taye et al., 2021). Fermentation process that applied in milk preservation aims to extend the shelf life and maintain the content of milk nutrition. Recently, the purpose of milk fermentation has developed further, especially increasing its functional properties that have implications for improving human health, prevention and inhibition of pathogenic microbial toxins to get in the human body. There were many research focus in LAB bioactive components productions which is induced beneficial effects on human health (Yu et al., 2011; Sharma et al., 2013; Rashid et al., 2015; Mohammad et al., 2018).

The most important characteristic of LAB in milk fermentation was their ability to acidify milk quickly as a result of organic acids formation, such as lactic and acetic acid. LAB metabolites, including lactic and acetic acid, hydrogen peroxide, bacteriocins

and some low molecular weight compounds have antimicrobial activities (Suskovic et al., 2010; Chakoosari et al., 2014). In addition, LAB also potential to produce ethanol, bacteriocin, aroma components, exopolysaccharide and proteolytic enzymes (Ivey et al., 2013). These components play a significant role in fermented milk characteristic formation. For instance a moderate sour taste and pleasant flavour in yoghurt and cheese become either popular fermented product (Surono, 2015). Generally, *Lactobacillus*, *Lactococcus*, *Leuconostoc*, *Pediococcus* and *Streptococcus* genera were involved in milk fermentation such as yoghurt, cheese, buttermilk and kefir. These genera have good potency to increased foods safety, enhancing sensoric properties, nutrient enrichment, and other benefit that have implications for improving health (Yu et al., 2011; Sharma et al., 2013; Steele et al., 2013. Lactic acid bacteria also has an important role in protein hydrolysis to gain free amino acid during fermentation process (Lawalata and Satiman, 2015) which is used for their particular growth. The aim of this study were evaluated antimicrobial and proteolytic activity of lactic acid bacteria (LAB) isolated from Dadih.

2. **Materials and methods**

2.1. Sampling

Four dadih samples were taken from two locations based on processing methods i.e., spontaneous fermentation from Gadut at Limapuluh Kota Regency and backsloping from Kamang, Agam Regency. Forty eight hours Dadih sample were measured for acidity and aseptically taken for 10 g and put in a sterile tube (Ren and Suo, 2017).

2.2. Isolation

Five g dadih was added to 45 ml sterile saline solution (0.85%). An appropriate dilution (10^7-10^8) was made and then inoculated on to de Man Rogosa Sharpe Agar medium (MRSA) + $CaCO_3$ 0.5% (w/v) with double layer technique and incubated at 37^{o}C for 48 hours. Each plate consisted of >30 and <300 colonies ware counted. LAB viable count was expressed as log colony forming unit (CFU) per gram of sample.

2.3. Morphological Identification

Ten colonies with surrounding clear zones in each plate were randomly selected and observed the cell morphology (gram staining, cell shape), motility, and catalase test. Non motile, gram positive bacil or coccus, and negative catalase isolates were streak in fresh MRSA plates to obtained a pure culture. LAB isolates were stored in 10% (v/v) of sterile glycerol at -20 °C (Dzhakibaeva and Kebekbaeva, 2010).

2.4. Antimicrobial Activity

Antimicrobial activity was determined by the agar-well diffusion assay (Mirzaei et al., 2018). LAB culture was grown anaerobically in MRS broth at 37 °C for 24 hours to reach 10^8 CFU/ml. Three pathogenic bacteria i.e., *E. coli* ATCC 25922, *S. aureus* ATCC 25923, and *S. typhimurium* ATCC 14028 (UGM culture collection) were grown on nutrient broth medium and incubated at 30^{o}C for 24 hours. 0.1% (v/v) of the bacterial culture (10^8 CFU/ml cell density) was spread onto the nutrient agar, into which 6 mm deep wells had been dug. About 50 µL of LAB supernatant was poured into each well. After incubation at 37 °C for 24 hours, the inhibition zone around the well was measured using vernier caliper.

2.5. Proteolytic Activity

LAB proteolytic activity was evaluated based on the procedure carried out by Phyu et al., (2015). LAB isolates were incubated in MRS broth for 18 hours then poured 50 µL into 6 mm diameter wells on skim milk agar medium (w/v) composed by 0.5% casein, 0.25% yeast extract, 0.1% dextrose, 2.8% skim milk powder and 1.5% bacto agar. After 24 hours incubation, forming clear zone on plates were measured. Isolates with >6 mm clear zone (Tulini et al., 2016) considered to have high proteolytic activity.

2.6 16S rRNA Partial Gene Sequence Identification

The genome DNA of the selected LAB isolates were extracted using Presto^{TM} Mini gDNA Bacterial Kit (Geneaid). The DNA pellets were suspended in 50 µL TE buffer (Tris-EDTA) and stored at -20 °C. Lactic acid bacteria 16S rRNA gene was amplified using a universal primer 27F (5'-AGAGTTTGATCCTGGCTCAG-3') and 1492R (5'-GGTTACCTTGTTACGACTT-3') (Pang et al., 2012). A total volume PCR

of 50 ml consisted of 25 µL MyTaq HS Red Mix (Geneaid), 2 µL of each primer (10 pmol) and distilled water to achieve the final volume. Amplification was performed in Takara PCR Thermal Cycler SimpliAmp with PCR conditions as follows: pre-heating at 94 ^{o}C for 1.5 minutes, denaturation at 95 ^{o}C for 30 seconds, annealing at 50 ^{o}C for 30 seconds, and elongation at a 72 ^{o}C for 1.5 minutes, this cycle was repeated at 30 cycles, and finally performed at 72 ^{o}C for 5 minutes. PCR products were electrophoresed in 1% agarose gel. PCR products were sent to 1st BASE for sequencing 16S rDNA gene. The sequencing results were subjected into National for Biotechnology Information (NCBI) GeneBank (www.ncbi.nlm.nih.gov) and Basic Local Alignment Search Tool (BLAST) was adopted to search GeneBank database for sequence homology analysis to determine the genera of lactic acid bacteria. Species with similarity more than 97% was considered as the same.

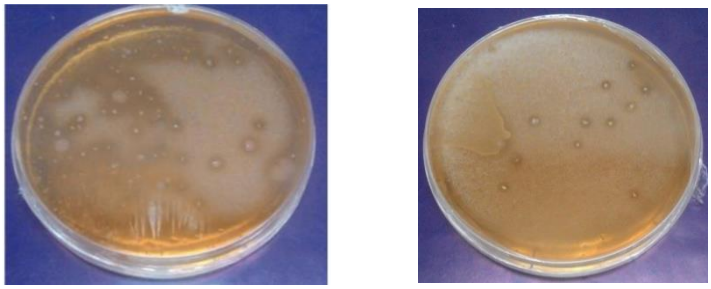
2.7 Fermentation Profile

The identification of LAB isolates was carried out by observing carbohydrate fermentation patterns using the API® 50 CHL Kit (bioMérieux, France). Isolates fermentation profile was determined using API WEB^{TM} software version 1.3.0 from bioMérieux (Jannah et al., 2016).

3. Results and discussion

3.1. Isolation and Moracidityological Identification

The number of LAB and dadih acidity ranged between 8.56 to 9.04 log CFU/g and 4.57 to 4.52 respectively. The previous study showed that the number of LAB dadih from Sianok was 7.17 log CFU/g (Jatmiko, 2010), meanwhile dadih from Pematang Panjang and Sijunjung were 10.4 log CFU/g (Syukur et al., 2014). This high bacterial population were due to many factors, including buffalo milk quality, initial bacteria population in bamboo tubes, processing methods and environmental conditions. Similar to acidity value, it varies between regions. Dadih acidity from Agam, Solok and Sijunjung were ranging from 4.47 to 4.48 (Wirawati et al., 2017). Fifty-three presumtive LAB colonies were randomly selected based on the appearance of a clear zone surrounding the colony (**Figure 1**).



(a) (b)

Figure 1. LAB colony in MRSA + CaCO3 0.5% medium. (a) dadih from Gadut; (b) dadih from Kamang

MRSA medium was a selective medium for LAB and $CaCO_3$ is used as a marker on medium. During the growth, LAB will produce lactic acid bound with $CaCO_3$ to Ca-lactate and dissolved in medium, it is formed a clear zone around the colony (Pisol et al., 2015; Phyu et al., 2015).

The morphological observation results showed that 31 isolates were gram-positive bacteria with rod or cocci shape, and negative catalase test (**Table 1**). All colonies give positive reaction in gram staining procedure. This is due to the binding of crystal violet dye to the peptidoglycan layer in gram-positive bacteria. Gram-positive bacteria have thick cell walls that resemble nets composed of 50-90% peptidoglycan, and will hold crystal violet very strongly during gram staining (Thairu et al., 2014). During gram staining, cell shape will also be seen more clearly. All isolates have been identified in rod or cocci shape and showed a negative reaction to catalase test. It's reflected by no bubble forming after cell reacted with a few drops of hydrogen peroxide (H_2O_2). Lactic acid bacteria are not able to produce catalase that converts H_2O_2 into water and oxygen. Catalase is an enzyme that catalyzes the decomposition of H_2O_2 into water and oxygen (Fugelsang and Edward 2007). It is also shown that LAB was belonged to anaerobic/facultative anaerobes group, which is no need oxygen during the growth acidityase.

Table 1. Morphological characteristic of LAB isolates

No	Isolate code	Motility	Gram staining	Catalase test	Cell shape
1	DG1	non motile	+	−	cocci
2	DG3	non motile	+	−	cocci
3	DG7	non motile	+	−	cocci
4	DG10	non motile	+	−	cocci
5	DG11	non motile	+	−	cocci
6	DG15	non motile	+	−	rod
7	DG17	non motile	+	−	rod
8	DG18	non motile	+	−	cocci
9	DG19	non motile	+	−	cocci
10	DG21	non motile	+	−	rod
11	DG23	non motile	+	−	rod
12	DG30	non motile	+	−	rod
13	DK2	non motile	+	−	rod
14	DK3	non motile	+	−	cocci
15	DK4	non motile	+	−	cocci
16	DK5	non motile	+	−	cocci
17	DK9	non motile	+	−	rod
18	DK11	non motile	+	−	rod
19	DK12	non motile	+	−	cocci
20	DK13	non motile	+	−	rod
21	DK16	non motile	+	−	rod
22	DK19	non motile	+	−	cocci
23	DK20	non motile	+	−	cocci
24	DK23	non motile	+	−	cocci
25	DK28	non motile	+	−	cocci
26	DK29	non motile	+	−	cocci
27	DK30	non motile	+	−	rod
28	DK33	non motile	+	−	rod
29	DK37	non motile	+	−	cocci
30	DK41	non motile	+	−	cocci
31	DK43	non motile	+	−	cocci

DG: dadih from Gadut; DK: dadih from Kamang

3.2 Antimicrobial Activity

Thirty one isolates which have been identified morphological properties were selected based on antimicrobial activity against food pathogenic bacteria, namely *E. coli* ATCC 25922, *S. aureus* ATCC 25923, and *S. typhimurium* ATCC 14028 using agar well diffusion method. The clear zone surrounding well shows that isolates have antimicrobial activity (**Figure 2**) and the size of the inhibition zone (mm) was presented in **Figure 3**. There were 22 isolates that had antimicrobial activity against food pathogenic bacteria.

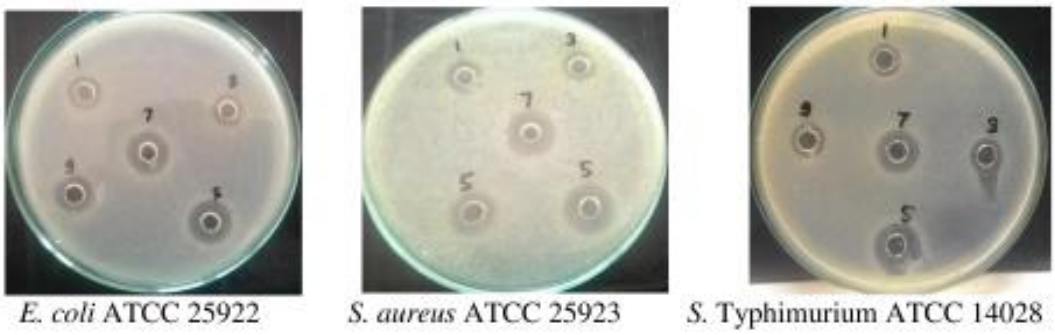


Figure 2. Clear zone surrounding the well indicated antimicrobial activities of LAB isolates

The isolates ability to inhibit pathogenic bacteria growth was create by the formation of lactic acid and other organic acids. Lactic acid is a potent outher membrane disintegrating agent, as a result of their ability to cause lipopolysaccharide release and to sensitized bacterial cell to detergents or lysozyme (Alakomi et al., 2000). These metabolites are formed during the initial growth phase (Marianelli et al., 2010).

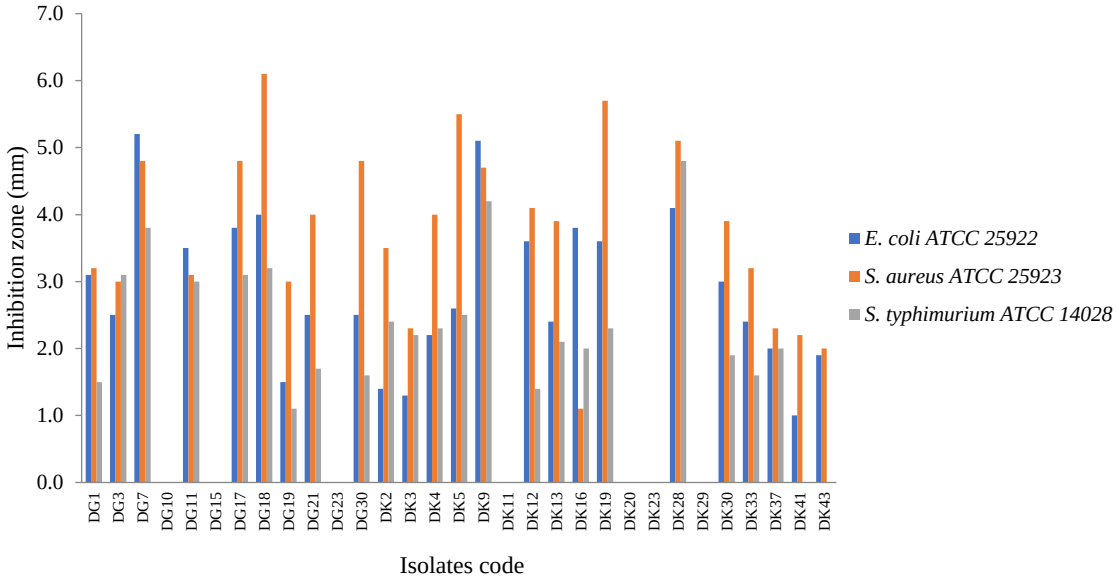


Figure 3. Size of inhibition zone (mm) antimicrobial activity of LAB isolates. DG: dadih from Gadut; DK: dadih from Kamang

Most isolates were develop a greater inhibition zone against gram positive bacteria (*S. aureus* ATCC 25923) than gram negative (*E. coli* ATCC 25922 and *S. typhimurium* ATCC 14028). Lactic acid bacteria isolated from various fermented food also had antimicrobial activity against *B. cereus*, *E. faecalis*, *E. coli*, *S. aureus* and *Listeria monocytogenes* (Fouhla et al., 2013; Chakoosari et al., 2014). Gram negative

bacteria have several resistance mechanism to organic acids. In between, the outher membrane permeability barrier which will retain antimicrobial compounds step into cell cytoplasmic was the most important factor in resistance mechanism. In addition, gram negative bacteria also shows a specific mechanism which can inactivate antimicrobial components and make them cannot penetrate the cytoplasmic membrane (Alakomi et al., 2000).

Although LAB antimicrobial activity might be due to many factors, a decrease in acidity, substrate competition, production of bactericidal and bacteriostatic compounds including bacteriocin are the main factors as antimicrobial activity. Decreasing acidity due to accumulation of lactic acid can inhibit and decay several pathogenic bacterial growth. Undissociated form of lactic acid will rapidly reduce cell internal acidity, causing a failure proton electrochemical gradient in sensitive bacteria and finally lead bactericidal and bacteriostatic effects (Castellano et al., 2017).

3.3 Proteolytic Activity

Twenty-two isolates from previous selection were tested for proteolytic activity with agar well diffusion method on Skim Milk Agar medium. The results are shown in Figure 4 .

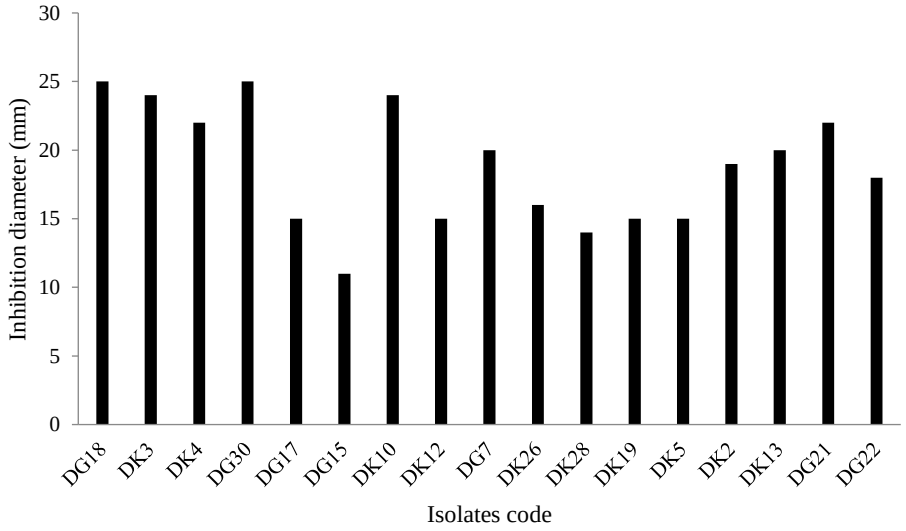


Figure 4. Size of clear zone diameter (mm) proteolytic activity of LAB isolates. DG: dadih from Gadut; DK: dadih from Kamang

Proteolytic activity in LAB was very important traits due to flavor component formation in fermented milk. The inability of LAB to generate all of the amino acids, lead proteolytic system converts proteins to peptides and then to amino acids, which is essential for bacterial growth and also contributes significantly to flavour compounds as end-products (Liu et al., 2010; Moslehishad et al., 2013). It is also stated by Moulay et al., (2006) and Savijoki et al., (2006) that during the growth acidityase, LAB proteolytic system was an important requirement through the degradation of milk casein and forming the organoleptic properties of fermented milk.

This research showed that only 17 isolates had high proteolytic activity, this was indicated by > 6 mm size of the clear zone in SMA medium. Some genera of LAB, namely *Lactococcus* and *Lactobacillus* (Addi and Guessas, 2016), *L. plantarum* and *L. mesenteroides* (Abubakr and Al-adiwish, 2017), and *Pediococcus pentosaceus* (Afriani et al., 2018) are known to have high proteolytic activity.

3.4 16S rRNA Partial Gene Sequence Identification

Identification based on 16S rRNA partial gene sequence of LAB isolates was showed in **Figure 5**. The DNA size of seventeen isolates range between 1000-1500 bp.

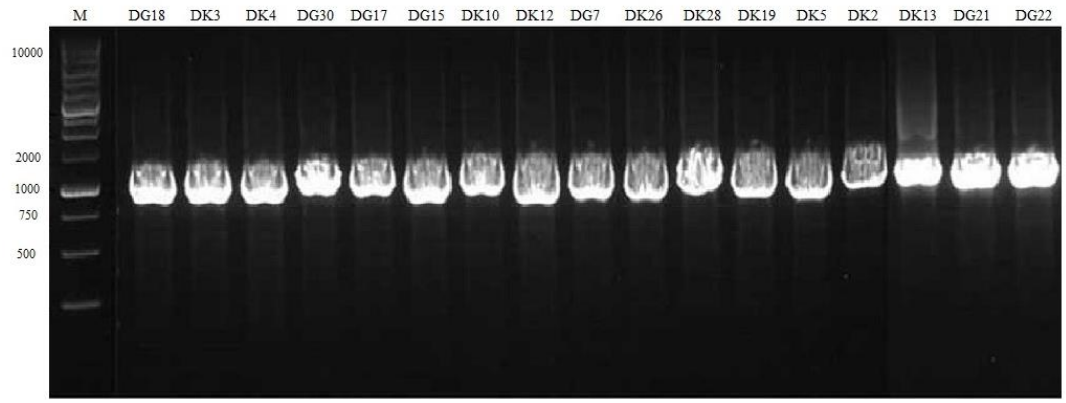


Figure 5. PCR product LAB isolates in 1% agarose gel. Line M (Ladder DNA 1 kb); Line 2-18 (LAB isolates code); DK: Dadih from Kamang, DG: Dadih from Gadut.

To confirm the species, these sequences were determined and compare with related bacteria using BLAST program at NCBI. Table 2 show the sequencing results from seventeen isolates. It is showed that 52.9% (9 isolates) were identified as *Lactococcus lactis* ssp. *lactis*, 17.65% (3 isolates) were identified as *Lactobacillus plantarum* ssp. *plantarum*, 17.65% (3 isolates) were identified as *Lactococcus lactis* ssp.

cremoris, and 5.88% (1 isolate each) were identified as *Pediococcus pentosaceus* and *Lactobacillus pentosus* (**Table 2**).

Table 2. Species identification of 17 LAB isolates partial gene sequence

Isolate code	Origin	Spesies	% ID in NCBI	Accession number
DG18	Gadut	*Lactococcus lactis* subsp. *cremoris*	98	MF098152.1
DK 3	Kamang	*Lactococcus lactis* subsp. *lactis*	97	KF879153.1
DK4	Kamang	*Lactococcus lactis* subsp. *lactis*	97	KF879153.1
DG30	Gadut	*Lactococcus lactis* subsp. *lactis*	98	KF148962.1
DG17	Gadut	*Lactobacillus plantarum* subsp. *plantarum*	98	CP031771.1
DG15	Gadut	*Lactococcus lactis* subsp. *cremoris*	99	MF098152.1
DK10	Kamang	*Pediococcus pentosaceus*	99	AB494722.1
DK12	Kamang	*Lactococcus lactis* subsp. *lactis*	98	KJ095659.1
DG7	Gadut	*Lactococcus lactis* subsp. *lactis*	98	KF148962.1
DK26	Kamang	*Lactococcus lactis* subsp. *lactis*	97	KF879153.1
DK28	Kamang	*Lactococcus lactis* subsp. *lactis*	98	KF879153.1
DK19	Kamang	*Lactococcus lactis* subsp. *lactis*	97	KF879153.1
DK5	Kamang	*Lactococcus lactis* subsp. *lactis*	98	KM485587.1
DK2	Kamang	*Lactobacillus plantarum* subsp. *plantarum*	98	KY762263.1
DK13	Kamang	*Lactobacillus pentosus*	99	CP032757.1
DG21	Gadut	*Lactobacillus plantarum* subsp. *plantarum*	99	KY762263.1
DG22	Gadut	*Lactococcus lactis* subsp. *cremoris*	99	MF098152.1

Identified species in dadih showed that *Lactococcus* and *Lactobacillus* genera were the predominant LAB in fermentation process. It was also found in another fermented dairy product from many countries, for example Hurood cheese and Jueke from Mongolia (Gao et al., 2017), traditional fermented dairy foods from Russia (Phyu et al., 2015), and fermented yak yoghurt in Sichuan China (Ren and Suo 2017).

3.5 Fermentation Profile

The fermentation profile conducted with API 50 CHL kit can be seen in Table 3. Fermentation profile of seventeen isolates was also showed that *Lactococcus lactis, Lactobacillus plantarum, Lactobacillus pentosus* and *Pediococcus pentosaceus* were the most species found in dadih fermentation. *L. lactis* ssp. *lactis* and *L. Lactis* ssp. *cremoris* are important group of LAB in milk fermentation. Both of these species have function as starter cultures, which is responsible to produce lactic acid from lactose, hydrolyze casein, and also have a significant position in citric acid fermentation and flavor formation, especially *L. lactis* ssp. *cremoris* (Samarjiza et al., 2001). Among the

genera involve in dairy fermentation, *Lactobacillus* are most heterogenous in terms of the type of fermentation that are carried out. These and other microbes, including a variety of LAB, determine the flavor, textures and other features of fermented foods (Macori and Cotter, 2018).

Table 3. Fermentation profile of LAB isolates

Type of sugar	Isolat code																
	DG18	DK3	DK4	DG30	DG17	DG15	DK10	DK12	DG7	DK26	DK28	DK19	DK5	DK2	DK13	DG21	DG22
L-arabinosa	-	-	-	-	+	-	+	-	-	-	-	-	-	+	+	+	-
D-ribosa	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
D-xylosa	+	+	+	+	-	+	+	+	+	+	+	+	+	-	+	-	+
D-glucosa	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
D-fruktosa	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
D-mannose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
L-sorbitol	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
L-rhamnosa	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Duleitol	-	-	-	-	-	-	+	-	-	-	-	-	-	-	+	-	-
D-sorbitol	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+
Methyl-AD-mannopyranosida	-	-	-	-	+	-	-	-	-	-	-	-	-	+	-	+	-
Methyl-AD-glucoopyranosida	-	-	-	-	+	-	-	-	-	-	-	-	-	+	+	+	-
N-acetylglucosamin	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-
Amygdalin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Arbutin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Esculin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Salicin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
D-cellibiosa	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
D-maltosa	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
D-laktosa	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
D-melibiosa	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+
D-sacharosa	-	-	-	-	+	-	-	-	-	-	-	-	-	+	+	+	-
D-trehalosa	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+
Inulin	+	+	+	+	-	+	+	+	+	+	+	+	+	-	-	-	+
D-melezitosa	-	-	-	-	+	-	+	-	-	-	-	-	-	+	+	+	-
D-raffinosa	-	-	-	-	+	-	-	-	-	-	-	-	-	+	-	+	-
Amidon	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-
Glycogen	+	+	+	+	-	+	-	+	+	+	+	+	+	-	-	-	+
Gentibiosa	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-
D-turanosa	+	+	+	+	-	+	+	+	+	+	+	+	+	-	+	-	+
D-Lyxosa	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
D-tegatosa	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-
D-fucosa	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-
Potassium gluconat	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+
Identified species	***L.lactis***	***L.lactis***	***L.lactis***	***L. lactis***	***L. plantarum***	***L.lactis***	***P.pentosaceus***	***L. lactis***	***L.lactis***	***L.lactis***	***L.lactis***	***L.lactis***	***L.lactis***	***L. plantarum***	***L. pentous***	***L. plantarum***	***L.lactis***

DG: dadih from Gadut; DK: dadih from Kamang

4. Conclusion

Seventeen isolates from two fermentation methods (spontaneous and backslopping) were successfully isolated and identified based on their morphological, partial gene sequence, and fermentation profiles. These isolates exhibited a wide spectrum of antimicrobial activity (ranged between 1.0 to 6.1 mm) against three

pathogenic bacteria. In addition, seventeen isolates also have high proteolytic activity (ranged between 15 to 26 mm) in medium contains milk casein. Identification based on 16S rRNA partial gene sequences and fermentation profile showed that *Lactococcus lactis, Lactobacillus plantarum, Lactobacillus pentosus* and *Pediococcus pentosaceus* were dominating LAB in dadih sample. This makes it possible to develop their functional properties as a starter culture in milk fermentation. Although further identification stage is still needed to find confirm this result, dadih as traditional Indonesian fermented milk provides information about the diversity of LAB from Indonesia.

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