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Harnessing Indigenous Fermentation: Probiotic Lactic Acid Bacteria from West Sumatera's Dadih

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Abstract

The potential of lactic acid bacteria (LAB) isolated from dadih, a traditional fermented buffalo milk from West Sumatra, was explored as functional starter cultures for yogurt production. The use of local LAB is considered essential due to Indonesia's dependence on imported cultures, which often exhibit inconsistent performance and suboptimal quality. This study selected bacterial isolates based on their ability to grow in highly acidic media and their tolerance to bile salt treatment. Molecular identification was conducted by amplifying the 16S rRNA gene using the PCR method. Specific LAB isolates with high probiotic potential were further characterized based on their tolerance to acid and bile salts. The results showed that several LAB strains from dadih, such as Lactococcus lactis, Weissella paramesenteroides, and Weissella sp., exhibited good probiotic properties. These isolates could effectively ferment milk and produce yogurt with organoleptic qualities comparable to commercial cultures. In addition, Serratia marcescens, known as an opportunistic pathogen, was also detected during the isolation process; some of its strains are known to participate in fermentation processes. These findings support the utilization of local LAB as an alternative to imported cultures, promoting local food production, improving the nutritional value of fermented dairy products, and preserving traditional culinary heritage.

Keywords: *Dadih, potential yogurt strater, Lactococcus lactis, Weissella paramesenteroides, Weissella sp.*

Introduction

Indonesia remains dependent on imported starter cultures for yogurt production, primarily due to persistent challenges in achieving the desired quality, stability, and consistency in locally produced cultures. Locally sourced starters often exhibit variability in microbial composition, which may result in inconsistent sensory attributes such as flavor, texture, and shelf life—key parameters that influence consumer acceptability and market competitiveness [1]–[3]. In addition, many locally produced starters fail to meet the stringent safety and quality standards required for commercial-scale yogurt production, thereby limiting their broader application [4]. In contrast, imported starter cultures are preferred for their superior consistency and reliability, driving both producers and consumers to favor foreign products despite the potential for developing competitive local alternatives [5].

One promising indigenous source of probiotic cultures is *dadih*, a traditional fermented buffalo milk product native to West Sumatra, Indonesia. *Dadih* is naturally fermented in bamboo tubes, a process that supports the spontaneous growth of diverse Lactic Acid Bacteria (LAB). Previous studies have identified several LAB species from *dadih*, including *Lactobacillus plantarum*, *Lactobacillus fermentum*, and *Lactobacillus casei*, which are known for their functional probiotic properties [6],[7]. These bacteria not only play a key role in the fermentation process but also

confer health benefits, including improved gut health and enhanced immune function.

Recent research has increasingly focused on the isolation and characterization of LAB strains from *dadih* to evaluate their potential as functional starter cultures for yogurt production. For instance, Amelia et. al, reported that specific LAB strains isolated from *dadih* exhibited desirable probiotic traits, including acid and bile tolerance, as well as antimicrobial activity—properties essential for their survivability and efficacy in the gastrointestinal tract and in fermented dairy applications. Similarly, [8] demonstrated that strains such as *Lactobacillus casei* and *Lactobacillus rhamnosus* not only facilitated milk fermentation but also enhanced the nutritional and functional attributes of yogurt. In addition, [9] found that selected LAB strains from *dadih* could produce yogurt with organoleptic characteristics comparable to those obtained using commercial imported starters. Furthermore, [7] investigated the technological potential of these strains, particularly their ability to synthesize exopolysaccharides, which contribute to improved viscosity, mouthfeel, and overall yogurt texture.

Given these promising findings, LAB strains derived from *dadih* may serve as viable candidates for the development of high-quality, locally sourced starter cultures for yogurt production. The present study aims to isolate, screen, and characterize LAB strains from *dadih* collected from various regions in West Sumatra, with the objective of identifying potential probiotic cultures suitable for use as competitive yogurt starters in the Indonesian dairy industry.

Although several studies have reported the isolation and probiotic characterization of LAB from *dadih* [7],[9], most have focused on limited parameters, such as acid and bile salt tolerance or antimicrobial activity, without integrating these traits into a comprehensive selection pipeline for potential starter cultures. Furthermore, few studies have combined phenotypic screening with molecular identification and functional assessment in yogurt production, which is crucial to ensure both technological performance and probiotic efficacy. This gap in the literature underscores the need for systematic research that not only identifies robust indigenous LAB strains but also evaluates their ability to produce yogurt with sensory qualities comparable to commercial starters. Addressing this gap is essential for reducing dependency on imported cultures, supporting local dairy industries, and preserving Indonesia's unique fermentation heritage. Therefore, the present study was designed to isolate, screen, and molecularly identify LAB strains from *dadih* collected across various regions in West Sumatra, and to assess their probiotic potential for application as competitive yogurt starter cultures.

Experimental

Equipment

The equipment used in this study included Petri dishes (Anumbra) , inoculating loop, test tubes (Iwaki), hot plate and magnetic stirrer (Corning), pH meter, Erlenmeyer flasks (Pyrex), micropipettes(iPipette), L-shaped spreader, incubator (Gallenkamp), centrifuge, a colony counter(Stuart Scientific), UV-Vis spectrophotometer (Shimadzu), and a laminar airflow cabinet.

Material

Fermented buffalo milk (*dadih*) was obtained from a traditional producer located in Nagari Air Dingin Solok, Sijunjung, Bukittinggi, and Lintau, under aseptic conditions and transported to the laboratory under refrigerated conditions for further analysis. The materials used in this study included, NaCl 0,9%, MRS agar medium, MRS broth medium, calcium carbonate ($CaCO_3$), glycerol, alcohol, Mueller-Hinton Agar (MHA) medium, sulfuric acid (H_2SO_4), bile salts, and sodium hydroxide (NaOH).

Methods

Isolation of Lactic Acid Bacteria (LAB)

The isolation of lactic acid bacteria was performed following [10] method. One milliliter of *dadih* was transferred into 9 mL of sterile 0.9%

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physiological saline and homogenized. Serial dilutions were carried out up to a concentration of 10^{-9}. Each dilution was subsequently inoculated onto MRS agar media and incubated at 37°C for 48 hours. Colonies with distinct morphological characteristics were sub-cultured and purified using the streak plate method on sterile MRS agar. Purified single colonies were then inoculated onto MRS agar supplemented with 0.2% $CaCO_3$. Colonies that formed clear zones around their growth area were identified as acid-producing bacteria and further isolated on sterile MRS agar [10].

Bile Salt Tolerance Test

The bile salt tolerance of LAB isolates was assessed following [11] step. MRS broth medium was supplemented with 0.3% (w/v) bile salts. LAB isolates were pre-cultured in MRS broth and incubated at 37°C for 18 hours. The culture was then inoculated into 5 mL of bile-supplemented MRS broth at a 1% inoculum (50 µL). The bacterial growth at 0 hours was used as the control. Incubation was carried out at 37°C for 4 hours. Growth was monitored by measuring turbidity using a spectrophotometer at 600 nm. LAB isolates with survival rates above 20% were considered bile salt tolerant. The survival percentage was calculated using the following formula:

$$\text{Survival percentage (\%)} = \frac{\text{OD treatment-OD Control}}{\text{OD Control}} \times 100\ \%$$

where:
OD treatment = Optical density at bile salt treatment
OD control = Optical density control (at 0 hours)

Acid Tolerance Test

The acid tolerance of LAB isolates was evaluated by a modified method of [12]. MRS broth medium adjusted to pH 2 and pH 3 with 0.1 N HCl. LAB isolates were initially grown in MRS broth and incubated at 37°C for 18 hours. The culture was then inoculated into acidified MRS broth (pH 2 and 3) at a bacterial concentration of 1% (v/v). The bacterial growth at 0 hours served as the control. Incubation was carried out at 37°C for 48 hours. Bacterial growth was determined based on turbidity measurements using a spectrophotometer at 600 nm. LAB isolates showing survival percentages greater than 20% were considered acid-tolerant. The percentage of survival was calculated using the following formula:

$$\text{Survival percentage (\%)} = \frac{\text{OD treatment-OD Control}}{\text{OD Control}} \times 100\ \%$$

where:
OD treatment = Optical density at low pH treatment
OD control = Optical density control (at 0 hours)

Gram Staining

The morphological characterization of LAB isolates was conducted through Gram staining. The procedure began with the preparation of microscope slides by cleaning them with alcohol and allowing them to dry. A bacterial smear was prepared by suspending a bacterial colony in sterile distilled water and transferring it onto the slide, followed by heat fixation to attach the cells. The smear was stained with crystal violet for 1 minute, rinsed with running water, and then treated with iodine solution for another minute. After rinsing, the slide was decolorized with ethanol and counterstained with safranin for 30 seconds. Once dry, the stained bacteria were observed under a light microscope at 100× magnification using oil immersion[13].

Molecular Identification

Genomic DNA of bacterial isolates was extracted using the guanidium thiocyanate-EDTA-sodium lauryl sarcosinate (GES) method (Pitcher et al., 1989), followed by purification with the GeneJET Genomic DNA Purification Kit. DNA quality and concentration were measured using a NanoDrop spectrophotometer. The 16S rRNA gene was amplified by PCR using universal primers 27F (5′-AGA GTT TGA TCC TGG CTC AG-3′) and 1492R (3′-GGT TAC CTT GTT ACG AAC TT-5′).

The 25 µL PCR reaction mixture contained 4 µL of DNA template, 1 µL of each primer, 12.5 µL of GoTaq Green Master Mix (Promega), and 6.5 µL of nuclease-free water. PCR was performed with an initial denaturation at 95°C for 3 min, followed by 35 cycles of denaturation at 95°C for 30 s, annealing at 50°C for 30 s, and extension at 72°C for 60 s, with a final extension at 72°C for 7 min. PCR products were visualized via electrophoresis on a 1% agarose gel stained with GelRed, using a 1 kb DNA ladder for size estimation. Amplified products were sequenced (1st Base, Malaysia) using both forward and reverse primers[14].

Raw sequence data were aligned using Geneious software, and chromatograms were examined for quality. Clean, high-resolution peaks were saved in FASTA format. Homologous sequences were identified using BLASTn against the NCBI GenBank database. Multiple sequence alignment was performed with Clustal X (v2.1), and non-aligned regions were edited using BioEdit. Phylogenetic analysis was conducted using MEGA (v11) with the neighbor-joining (NJ) method, employing the Kimura 2-parameter model and 1000 bootstrap replications. The best substitution model was determined using the "Find Best DNA/Protein Models" feature in MEGA.

Results and Disscusion

Lactic Acid Bacteria Isolates

The isolation of lactic acid bacteria (LAB) present in *dadih* was conducted to obtain single bacterial colonies with potential probiotic properties. A screening process was carried out using several test variables to ensure that only potential isolates were selected. The initial LAB isolation results 16 isolates from Sijunjung's *dadih,* 14 isolates from Lintau's *dadih*, 12 isolates form Bukittinggi's *dadih,* and 11 isolates from Solok's *dadih,* which perform ability in producing acid by neutralizing $CaCO_3$. These results are shown in Table 1 and Figure 1.

The specific medium used for LAB isolation in this study was de Man, Rogosa, and Sharpe (MRS) agar. Although MRS is classified as a selective medium, its application still requires modification to achieve optimal conditions suited to particular bacterial types[15].

In the initial isolation phase of this study, non-LAB strains were able to grow on the surface of MRS agar. Therefore, subsequent isolations were performed by supplementing the medium with 0.2% $CaCO_3$. Calcium carbonate ($CaCO_3$) serves as an indicator for lactic acid-producing bacteria. $CaCO_3$ forms a suspension in the agar medium, as it is insoluble in water but can dissolve under acidic conditions, resulting in the release of CO_2 gas. The presence of LAB can be identified by the appearance of a clear halo surrounding bacterial colonies, indicating the dissolution of $CaCO_3$ by lactic acid produced by the bacteria [16].

Table 1. Description of each lactic acid bacteria isolated from different dadih

Sample code	Origin	Fermentation time (days)	Number of isolate
SJJ	Muaro Sijunjung, Kabupaten Sijunjung	4	16
LTU	Nagari Tanjung Bonai, Lintau Buo, Tanah Datar	2	14
BKT	Bukittinggi, Agam	2	12
SLK	Nagari Aie Dingin, Kabupaten Solok	7	11

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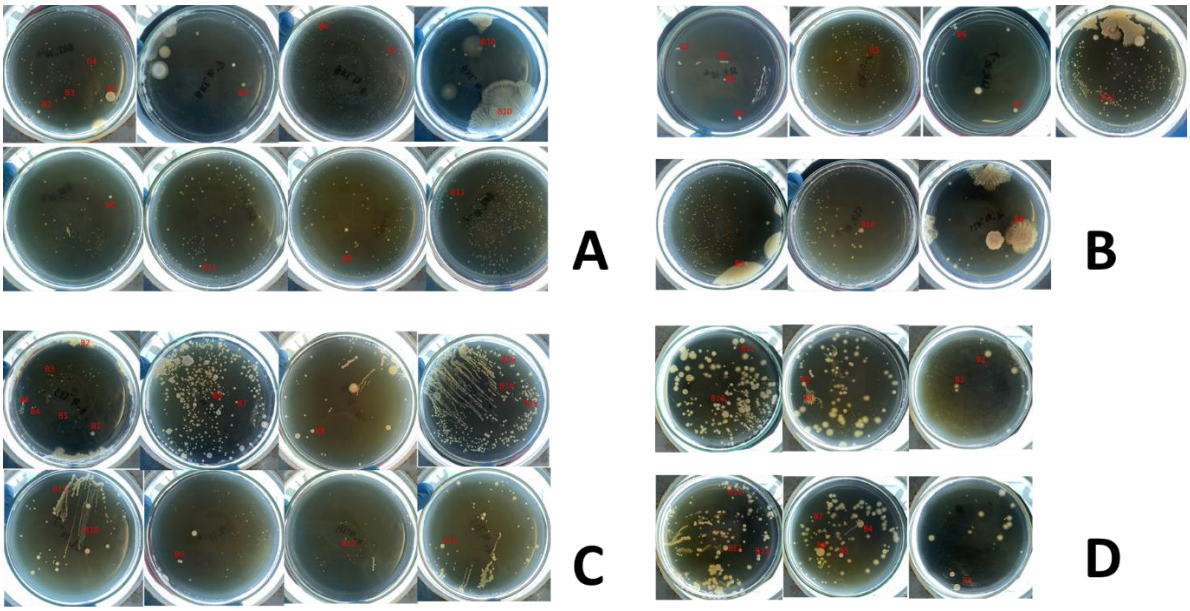

Figure 1. Lactic acid bacteria isolated from dadih samples collected in (A) Nagari Balingka, Bukittinggi; (B) Nagari Aie Dingin, Solok; (C) Sijunjung; and (D) Nagari Tanjung Bonai, Lintau.

Table 2. Screened LAB isolated from Dadih

Dadih	Halo zone	B1	B2	B3	B4	B5	B6	B7	B8	B9	B10	B11	B12	B13	B14	B15	B16
SJJ		+	-	+	+	-	-	+	+	+	+	-	-	+	+	+	+
LTU		+	-	+	+	+	-	-	+	-	-	+	+	+	+		
SLK		-	+	+	-	-	+	+	+	+	+	-					
BKT		-	-	-	+	+	+	-	+	-	-	+	-				

This subsequent isolation step eliminated several non-acid-producing isolates and retained only those capable of producing lactic acid. The results of this selection are shown in Table 2.

Probiotic Potential of Lactic Acid Bacteria (LAB)

Further screening was conducted to select bacterial isolates with probiotic characteristics, based on their ability to survive under acidic conditions, specifically at low pH, and in the presence of bile salts. According to Diza, a generally accepted characteristic for probiotics is the ability of microorganisms to remain viable throughout the digestive process. Their resistance to extreme conditions such as gastric acidity and the presence of bile salts in the intestine is particularly important. This enables beneficial bacteria to colonize the digestive tract and support the body's metabolic processes, ultimately contributing to immune function [17]. The screening results for potential probiotic candidates based on their tolerance to bile salts are presented in **Figure 2.**

The bile salt tolerance test was performed to assess the ability of each isolate to survive in the presence of bile salts, simulating conditions in the small intestine. Isolates that could maintain viability under these conditions were considered more likely to survive gastrointestinal transit and thus demonstrate potential probiotic functionality.

The acid tolerance test was conducted to evaluate the capacity of each isolate to withstand low pH conditions similar to those found in the human stomach. This step is crucial for determining whether the bacteria can survive gastric passage before reaching the intestine.

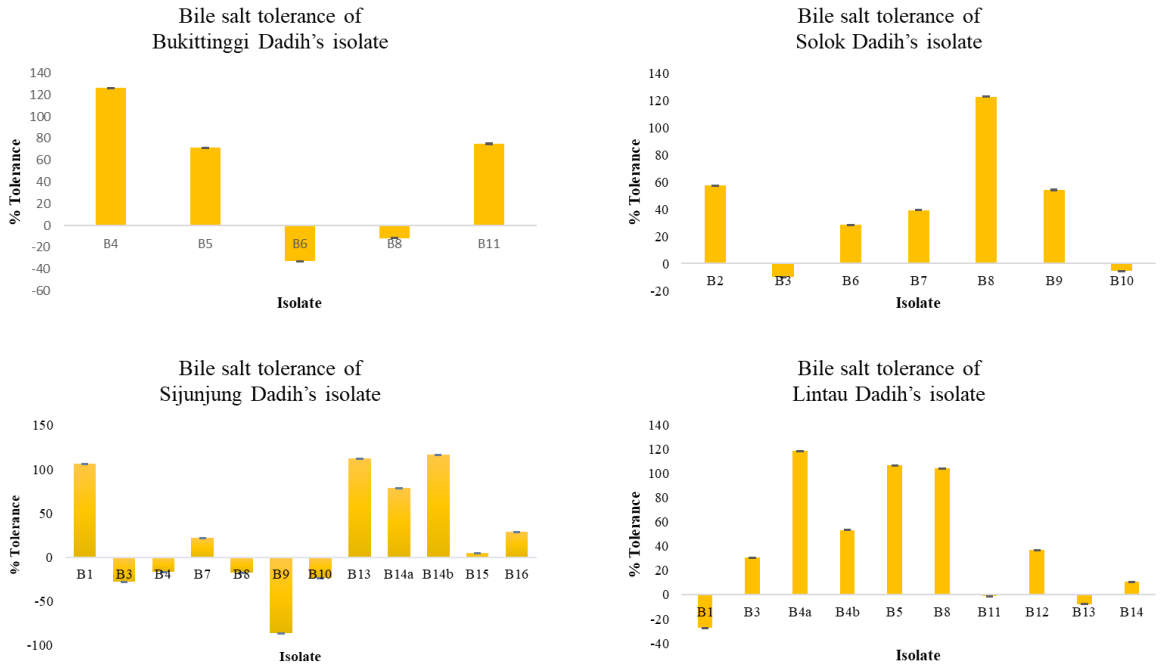

Figure 2. Bile salt tolerance percentage graph of each bacterial isolate

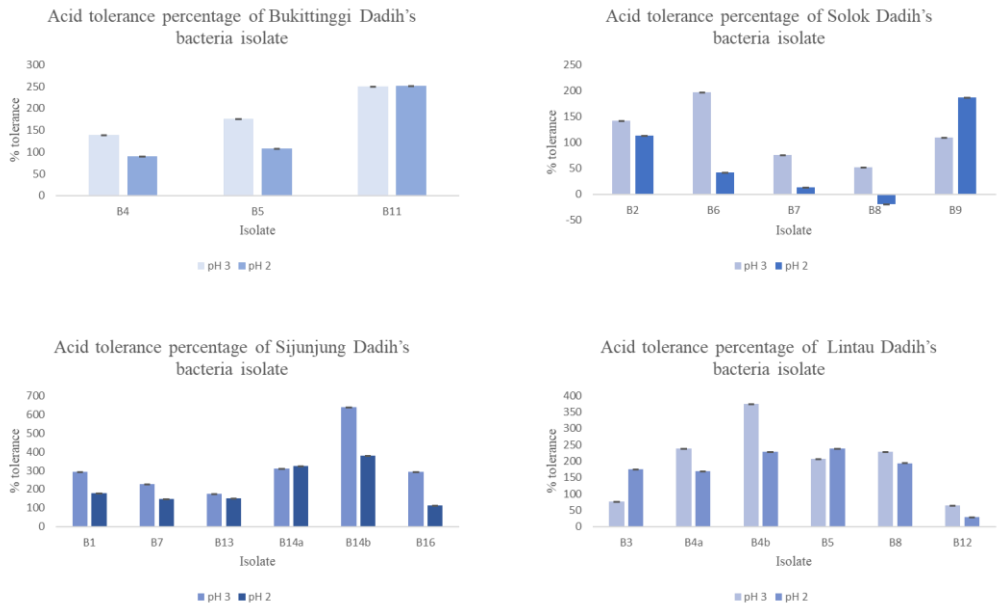

Figure 3. Tolerance of acid (%) in different isolates

Bacterial strains with resistance percentages within the minimum range of 20–40% have good survivability. In contrast, strains with values below this threshold are regarded as having poor survival capacity[18]. Therefore, isolates with resistance below 20% were eliminated, while those exceeding this level underwent further screening, specifically acid tolerance tests under low pH conditions. The low pH tolerance test results are presented as a bar graph in **Figure 3** below.

Bacterial isolates with growth percentages exceeding 5% demonstrated tolerance to low pH conditions. Therefore, isolates with acid resistance above this threshold were subjected to Gram staining for morphological identification.

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Gram Staining

The results of the Gram staining test are presented in **Table 3**. Based on the study's results, 18 candidate isolates from various *dadih* samples were identified as performing characteristic criteria for lactic acid bacteria (LAB). These included isolates from Sijunjung (SJJ): B1, B7, B13, B14a, B14b, and B16; from Lintau (LTU): B3, B4a, B4b, B5, B8, and B12; from Solok (SLK): B2, B6, and B9; and from Bukittinggi (BKT): B1, B4, and B5. Four isolates which perform the best characteristics as LAB and probiotics based on the screening test (isolates LTU B4a, LTU B12, SJJ B7, and SJJ B13), Gram staining shown in **Figure 4**.

Table 3. Gram staining identification

Dadih	Halo zone	B1	B2	B3	B4a	B4b	B5	B6	B7	B8	B9	B11	B12	B13	B14	B14b	B16
SJJ		bc	-	-	-	-	-	-	bc	-	-	-	-	bc	bc	bc	bc
LTU		-	-	cs	cs	bc	bc	-	-	cs	-	-	bc	-	-	-	-
SLK		-	cs	-	-	-	-	bc	-	-	cs	-	-	-	-	-	-
BKT		-	-	-	Bc	-	bc	-	-	-	-	bc	-	-	-	-	-

notes: bc: bacillus, cs:coccus

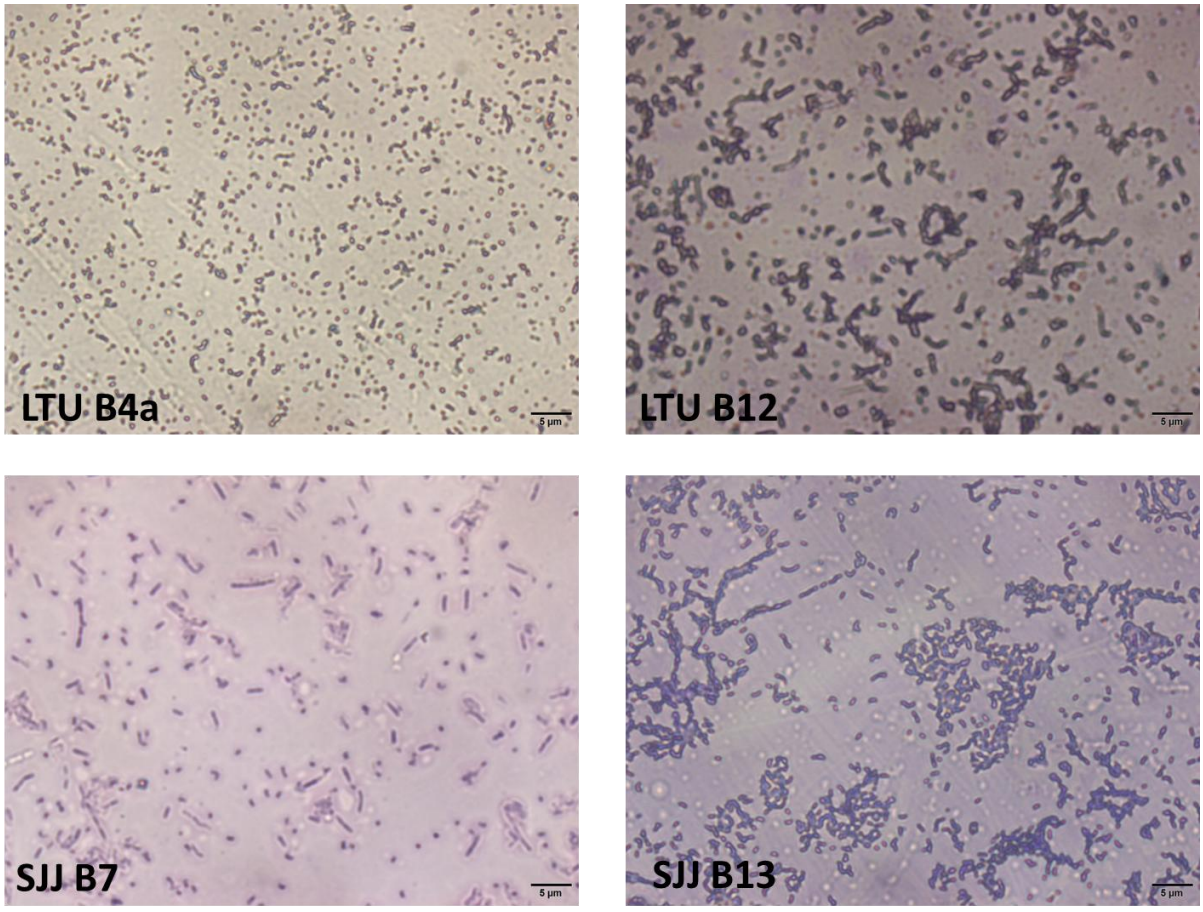

Figure 4. Gram staining of isolates LTU B4a, LTU B12, SJJ B7, and SJJ B13

The progressive screening strategy allowed us to track the performance of each isolate from initial acid production screening, through bile salt and acid tolerance tests, to Gram staining and final molecular identification. Four isolates—LTU B4a, LTU B12, SJJ B7, and SJJ B13—emerged as the top candidates based on their consistent performance across all probiotic-related assays and were therefore selected for 16S rRNA sequencing.

Molecular Identification and phylogenetic Analysis

This study successfully isolated several bacterial strains from *dadih*, a traditional fermented dairy product native to West Sumatra. Four isolates with the highest probiotic potential were selected for molecular identification using BLAST analysis based on their DNA sequences.

The identification results revealed that the isolates belonged to the genera *Lactococcus*, *Serratia*, and *Weissella*, with varying levels of sequence similarity (see **Table 4**).

The phylogenetic tree analysis (**Figure 5**) studies the relationships of each isolate, where the LTU B4a isolate is related to *Lactococcus lactis* subsp. hordniae strain XYR, the LTU B12 isolate is related to *Serratia marcescens* strain ABRL068, the SJJ B7 isolate is related to *Weissella confusa* strain GC, and the SJJ B13 isolate is related to *Weissella* sp. strain NBRC 107219.

Isolate B4a was identified as *Lactococcus lactis* strain 0S2R7, with 88.18% similarity. *Lactococcus lactis* is a well-known LAB commonly used in dairy fermentations due to its ability to convert lactose into lactic acid. This bacterium indicates that LAB plays a significant role in the fermentation process of *dadih*, particularly in the development of its characteristic texture and flavor. *Lactococcus lactis* plays a pivotal role in the fermentation of dairy products, serving as a key microorganism in both industrial and artisanal settings. This bacterium is renowned for converting lactose into lactic acid, which is crucial for the acidification process in dairy fermentation. This process not only preserves the product but also contributes to flavor and texture development in various dairy products such as cheese, butter, and fermented milk. Additionally, *L. lactis* is recognized for its probiotic properties, which offer health benefits beyond basic nutrition[19],[20].

L. lactis metabolizes lactose into lactic acid, which lowers the pH of the dairy product, inhibiting the growth of spoilage organisms and pathogens [19],[20], the bacterium also breaks down milk proteins into peptides and amino acids, contributing to the flavor profile of fermented products [21],[22]. Different subspecies, such as *L. lactis* subsp. lactis and *L. lactis* subsp. cremoris, are used as starter cultures to produce specific flavors and textures in cheeses like Cheddar, Brie, and Camembert[23],[24].

L. lactis produces bacteriocins, such as nisin, which have antimicrobial properties and can act as natural preservatives in food products [23]. The bacterium also synthesizes exopolysaccharides (EPSs), which improve the texture and mouthfeel of fermented dairy productsOther bioactive compounds produced include gamma-aminobutyric acid (GABA) and short-chain fatty acids, which have potential health benefits[19],[21].

Table 4. BLAST profile of each isolate

No	Sample	Species	Strain	*Query coverage* (%)
1	Isolat LTU B4a	*Lactococcus lactis*	0S2R7	88,18
2	Isolat LTU B12	*Serratia marcescens*	ABRL068	94,85
3	Isolat SJJ B7	*Weissella confusa*	G C	77,06
4	Isolat SJJ B13	*Weissella sp.*	NBRC 107219	86,27

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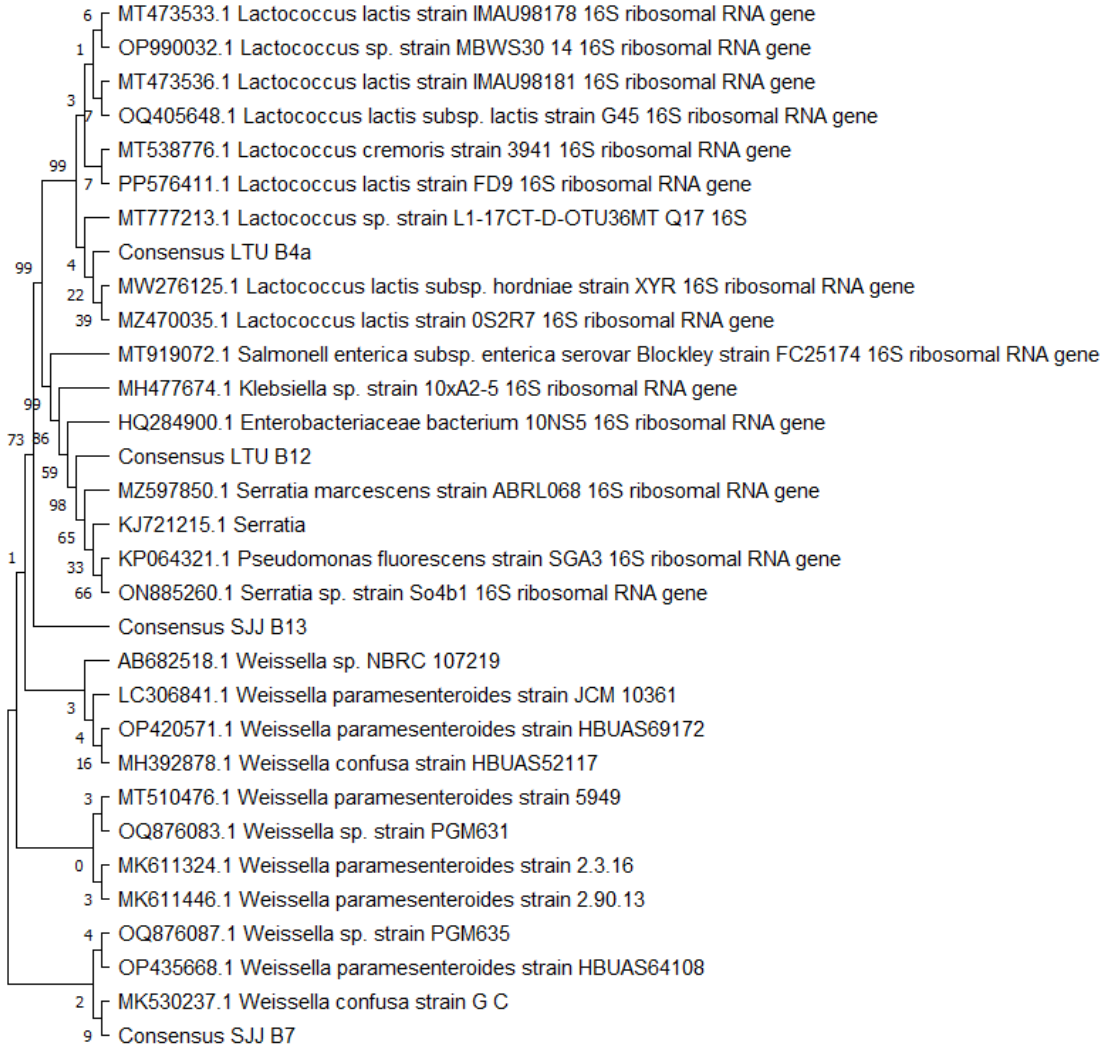

Figure 5. Phylogenetic tree analysis

L. lactis is considered a probiotic, contributing to gut health by maintaining microbial balance in the gastrointestinal tract[25]. It has been associated with immune system enhancement and the prevention of gastrointestinal infections[26]. The bacterium's ability to survive in the gastrointestinal tract and its production of health-promoting metabolites make it a valuable component of functional foods[23],[25].

Isolate B12 showed the highest similarity (94.85%) with *Serratia marcescens* strain ABRL068. Although *S. marcescens* is not classified as a genuine LAB, its presence could be attributed to environmental or equipment contamination during fermentation. This bacterium is commonly found in moist environments and can grow in dairy products. However, its presence should be monitored, as some strains are opportunistic pathogens. The ability of bacteria to survive under acidic gastric conditions and in the presence of bile salts is a common criterion used to select strains with probiotic potential. However, this characteristic excludes lactic acid bacteria (LAB). *Serratia* spp., although not classified as LAB, possess several cellular defense mechanisms, such as proton pumps and robust cell membranes, which enable them to withstand extreme environments, including low pH and bile salts. Moreover, certain *Serratia* strains are known for their high osmotic and pH tolerance, allowing them to pass through initial screening stages. Additionally, some *Serratia* species can produce small amounts of organic acids, sufficient to dissolve $CaCO_3$ and form clear zones, thus

yielding positive results during acid-producing bacterial screening.

Isolate SJJ B7 was identified as *Weissella confusa*, with 77.06% similarity. *Weissella confusa* is a heterofermentative LAB frequently found in traditional fermented foods. In addition to producing lactic acid, this bacterium can synthesize exopolysaccharides, contributing to the viscosity and texture of the final product. The relatively low sequence similarity suggests the possibility of a local strain or a previously unreported species. Isolate SJJ B13 was identified as *Weissella* sp. strain NBRC 107219, with 86.27% similarity. This result indicates that the isolate belongs to the genus *Weissella*, although its species could not be definitively identified. This finding suggests the presence of a unique microbial diversity in *dadih* from West Sumatra, highlighting opportunities for further exploration of its functional potential, including probiotic properties and antimicrobial compound production.

In comparison with previous studies, the *Lactococcus lactis* isolate identified in this study demonstrated acid and bile tolerance within the range reported for probiotic dairy strains used in yogurt production [19],[23]. Similar to reports by Amelia et al, our *L. lactis* isolate exhibited robust growth in acidic conditions, suggesting good survivability in the gastrointestinal tract. However, unlike certain commercial L. lactis strains that produce high levels of bacteriocins (nisin), our isolate requires further characterization to determine its antimicrobial spectrum against foodborne pathogens.

The Weissella isolates (*W. confusa* and *Weissella* sp.) obtained in this study showed comparable acid and bile tolerance to those described by Harnentis et al., from West Sumatran *dadih*. These strains are notable for their potential exopolysaccharide (EPS) production, which can improve yogurt texture and mouthfeel[9]. However, the relatively lower sequence similarity for *W. confusa* (77.06%) suggests a possible novel or local variant, which warrants deeper genetic and functional studies to determine its unique traits[27].

The detection of *Serratia marcescens* in *dadih* aligns with occasional reports of non-LAB species in traditional fermented foods[28]. While certain *S. marcescens* strains have been found in fermentation environments and can contribute to biochemical changes, this bacterium is widely recognized as an opportunistic pathogen capable of causing human infections. For this reason, its inclusion in probiotic applications is not recommended without rigorous safety assessments, including virulence gene screening and antimicrobial resistance profiling. In this study, the presence of *S. marcescens* is reported solely as part of the microbial profile of *dadih* and is excluded from consideration as a starter culture candidate for yogurt production. Its occurrence in *dadih* may be due to environmental contamination or the complexity of natural fermentation microbiota. Any potential utilization of *S. marcescens* in fermentation processes would require rigorous safety evaluations, including virulence gene detection and antimicrobial resistance profiling, before any consideration for food-related applications[28],[29].

Overall, these findings reinforce the probiotic potential of indigenous *L. lactis* and *Weissella* strains from *dadih* while highlighting the need for careful microbiological safety screening of all isolates, particularly those belonging to genera with known pathogenic members.

Conclusions

The research on lactic acid bacteria (LAB) isolated from West Sumatra's *dadih* has identified several key species, specifically L*actococcus lactis, Weissella paramesenteroides, Weissella spp., and Serratia marcescens*. *Lactococcus lactis* is crucial in dairy fermentation, particularly in cheese and yogurt production, contributing to the development of flavor and texture while possessing probiotic properties that enhance gut health and digestion. *Weissella paramesenteroides* is known for its ability to ferment a wide range of carbohydrates, improving the nutritional profile of fermented foods and promoting a healthy gut microbiota. *Weissella spp*. plays a significant role in

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fermentation, contributing to the flavor and preservation of various fermented foods, and can produce antimicrobial compounds that inhibit spoilage organisms. Although *Serratia marcescens* is primarily recognized as an opportunistic pathogen, certain strains can be involved in fermentation processes, potentially contributing to specific fermentation characteristics under certain conditions. The detection of *S. marcescens* highlights the importance of thorough safety screening in natural fermentation studies to ensure that only non-pathogenic and beneficial microorganisms are selected for food production. Further research can explore their specific health benefits and applications in enhancing the quality and safety of fermented dairy products, promoting both health and cultural heritage.

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