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Exploring Probiotic Potential: Molecular Characterization of Lactic Acid Bacteria Isolated from the Dadih of West Sumatra

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ABSTRACT

Dadih, a traditional fermented milk product from West Sumatra, Indonesia, is recognized for its rich community of beneficial probiotic bacteria, cultivated through natural fermentation in bamboo tubes. This study aimed to isolate and identify lactic acid bacteria (LAB) from Dadih using molecular techniques, specifically 16S rRNA gene sequencing, to evaluate their probiotic potential. Samples were collected from Lima Puluh Kota Regency. Probiotic candidates were selected based on their ability to withstand gastrointestinal stress conditions, including exposure to pH 2.0 and 0.3% bile salts. Safety and efficacy were assessed through antibiotic resistance profiling, while antibacterial activity was tested against pathogenic strains, including *Salmonella, Propionibacterium acnes, Escherichia coli O157, Staphylococcus aureus, Acinetobacter baumannii,* and *Listeria monocytogenes*. Among the isolates, strain MD1 exhibited strong probiotic properties, demonstrating 92.58% survival under bile salt conditions and 75% survival at low pH. It showed resistance to ampicillin, erythromycin, and penicillin, and intermediate resistance to tetracycline. PCR amplification of the 16S rRNA gene using primers 27F and l1492R yielded a 1,485bp product. Molecular analysis revealed a 97% sequence similarity between isolate MD1 and *Levilactobacillus brevis*. These findings suggest that *Levilactobacillus brevis* from Dadih holds significant probiotic potential. Its resilience under gastrointestinal conditions and antimicrobial activity against foodborne and clinical pathogens indicate promising applications in both human nutrition and animal product processing industries. As a natural microbial resource, it offers a sustainable alternative for enhancing food safety and supporting gut health through functional fermentation. This study highlights the importance of preserving traditional fermented foods as untapped reservoirs of novel probiotic strains for biotechnological innovation.

Key words: Bile Salts, Dadih, LAB, Probiotics, 16S rRNA.

INTRODUCTION

Probiotics are live microorganisms that, when consumed in adequate amounts, confer health benefits on the host (FAO 2013). These beneficial effects are primarily linked to modulation of the gut microbiota, which plays a crucial role in digestion, immunity, and overall well-being (Meng et al. 2018). To be effective, probiotics must survive the harsh conditions of the gastrointestinal tract, including low pH and bile acids (Suharti et al. 2020). Therefore, the FAO and WHO recommend a minimum of 10^6 CFU/g of live cells in functional foods to ensure therapeutic efficacy (Neffe-Skocińska et al. 2018). Maintaining viability remains a challenge due to environmental stressors, including oxygen exposure, pH fluctuations, and interactions with other microbes or harmful metabolites (Terpou et al. 2019). Strategies like strain selection, encapsulation, and synbiotic formulations are used to enhance stability. Lactic acid bacteria (LAB) are among the most commonly used probiotic genera—Gram-positive, acid-tolerant, and often classified as GRAS. They serve as starter cultures in fermentation and exhibit antimicrobial activity against pathogens, supporting food safety. Their dual role in food production and health promotion makes LAB a cornerstone in functional food development (Dowarah et al. 2018).

Incorporating probiotic bacteria into dairy products represents an optimal approach to restoring the equilibrium of the intestinal microbiota. Dairy food

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products must satisfy specific criteria to be considered a valuable medium for delivering probiotic bacteria. The requirements outlined ensure the effective delivery of probiotic cultures as dietary supplements, thereby enhancing their beneficial impact. In Indonesia, there is considerable diversity in local food sources that contain probiotics, with Dadih from West Sumatra being a notable example (Hatti-Kaul et al. 2018; Abesinghe et al. 2020).

Dadih is a traditional fermented dairy product originating from the Minangkabau people of West Sumatra, Indonesia, made by spontaneously fermenting buffalo milk in bamboo tubes for 24 to 48 hours. This natural fermentation fosters a prosperous and resilient community of indigenous lactic acid bacteria (LAB), including *Lactobacillus, Pediococcus, Enterococcus, Weissella,* and *Leuconostoc*. These microbes exhibit strong probiotic potential due to their tolerance to acidic and basic conditions, antimicrobial properties, and ability to colonize the human intestinal tract (Anggraini et al. 2019; Amelia et al. 2020; Mandalia 2023). LABs play a central role in the fermentation process, contributing functional benefits such as antimicrobial, antioxidant, antidiabetic, immunomodulatory, hypocholesterolemic, and antimutagenic activities (Hertati et al. 2023; Herlina & Setiarto 2024; Pramana et al. 2025). Nutritionally, Dadih contains 6.83%–7.90% protein, 5.11%–5.92% fat, and 74.18%–75.71% water, with a pH of 5.0–5.3, reflecting its stable and nutritious composition (Alzahra et al. 2024). Metagenomic studies across regions such as Bukittinggi, Limapuluh Kota, Agam, and Solok confirm the dominance of LAB, though strain-level identification remains incomplete (Collado et al. 2007; Venema & Surono 2019; Fatdillah et al. 2021). Molecular techniques such as 16S rRNA gene sequencing have identified specific probiotic strains, including *Pediococcus acidilactici* producing GABA, *Lactobacillus plantarum*, and *Enterococcus faecalis* isolates (Anggraini et al. 2019; Harnentis et al. 2019; Yuliana & Azhar 2022; Aritonang et al. 2022). These findings underscored Dadih's value as a natural reservoir of functional probiotics with promising applications in gut health and food safety.

Although many studies have confirmed the probiotic properties of lactic acid bacteria (LAB), research to identify LAB in Dadih continues due to the diversity of its microbiota. This aligns with the findings of Fatdillah et al. (2021) and Rossi et al. (2025), who noted that different Dadih-producing regions harbor diverse microbial communities. Therefore, this study aims to molecularly identify LAB from Dadih in Lima Puluh Kota Regency, West Sumatra, using 16S rRNA sequencing, and to evaluate their probiotic potential. This research hypothesizes that the LAB isolated from Dadih in Lima Puluh Kota possesses significant probiotic properties that can be broadly developed for functional food products to support consumer health.

MATERIALS AND METHODS

Sample collection

A total of 5 Dadih samples were collected from Lima Puluh Kota Regency, West Sumatra, Indonesia, and tested 3 times. Bacterial cultures were selected for their probiotic properties after being grown in de Man, Rogosa, and Sharpe (MRS) medium (Merck, Germany) for 24 hours at 37°C and molecularly identified.

Isolation of LAB from Dadih

Lactic acid bacteria isolates were grown in liquid De Man, Rogosa, and Sharpe (MRS) medium (Merck, Germany) by the serial dilution technique, then spread onto MRS agar (Merck, Germany). These were incubated at 37°C under anaerobic conditions for 48 hours. The isolates underwent initial analysis based on Gram staining, morphology, and catalase activity (Barrow et. al., 1993; Davoodabadi et al. 2015).

Acid Tolerance Test of LAB.

To assess acid tolerance, MRS medium was modified to a pH of 3.0 using 5N hydrochloric acid (HCl). A 1mL culture sample was introduced into 9mL of this adjusted MRS medium and incubated anaerobically at 37°C for 1.5 hours. Following incubation, the viable bacteria count was determined by plating serial dilutions on MRS agar. Acid tolerance was evaluated by comparing the viable culture counts on MRS agar after 2 hours of incubation. The plate count method was used to determine survival rates (%) on MRS agar after 0 and 1.5 hours of incubation. The measurement was repeated three times.

Bile Salt Tolerance Test of LAB.

The bile concentration in the human intestine is estimated to be 0.3%. Therefore, the experiment was carried out using this concentration, with slight modifications, as described by Hossain et al. (2018). 9mL of sterilized MRS medium containing 0.3% bile was inoculated with 1mL of the overnight culture. The tubes were then incubated at 37°C for five hours. Tolerance to bile salts was assessed by comparing the counts of viable cultures on MRS agar after 5 hours of incubation. The plate count method was used to determine survival percentages on MRS agar at the start and after 5 hours of incubation. The measurement was repeated three times.

Antibiotic resistance

Lactic acid bacteria isolates were tested for resistance to antibiotics recommended by the Clinical and Laboratory Standards Institute (2009). Following Madhava and Reddy (2013), the method for testing antibiotic sensitivity and resistance of lactic acid bacteria was evaluated using different antibiotics on MRS agar inoculated with probiotic bacteria. Antibiotic discs were placed on the agar surface at 37°C for 24 hours. Four types of antibiotics were tested: ampicillin (10µg), erythromycin (15µg), penicillin (10µg), and tetracycline (30µg).

Antimicrobial Activity Test against Pathogenic Bacteria

The Antimicrobial Activity Test against Pathogenic Bacteria was conducted using the well-diffusion assay developed by Mirzaei et al. (2018) with slight modifications. The test bacteria used to assess the antibacterial activity were *Escherichia coli* O157:H7, *Staphylococcus aureus* ATCC 25923, *Acinetobacter baumannii*, *Salmonella*, *Propionibacterium acnes*, and *Listeria monocytogenes*. The cell-free supernatant produced by lactic acid bacteria cultured in MRS medium for 24 h at 37°C under anaerobic conditions was centrifuged at 10,000rpm for 5 min at 4°C. Fifty microliters of supernatant were placed in wells containing Mueller-Hinton Agar (Merck) medium, where the pathogenic bacteria were grown. The size of the inhibition zone, an indicator of the isolate's antibacterial activity, was measured after 24 h. The measurement was repeated three times.

Molecular Identification of Isolated LAB Strains Using 16S rRNA Gene Sequencing

Lactic acid bacteria isolates were cultured in MRS broth at 37°C for 24 h. Genomic DNA was extracted using the Extrap Soil DNA Kit Plus Ver. 2. Amplification of the 16S rRNA of the isolates was performed by polymerase chain reaction (PCR) using a 16S rRNA gene fragment of approximately 1.5 KB, with universal primers 27F (5'-AGAGTTTGATCCTGGCTCAG-3') and 1492R (5'-GGTTACCTTGTTACGACTT-3'. The procedure began with an initial denaturation at 95°C for 5 min, followed by 25 cycles of denaturation at 94°C for 1 min, annealing at 56°C for 1 min, extension at 72°C for 1.5 min, and a final extension at 72°C for 7 min. The resulting DNA was isolated by electrophoresis at 100 V for 21 min, using 1% agarose in ×1 TAE buffer. A gel documentation system was used to capture images of bands within the gel. Purification was conducted using a rapid gene gel/PCR extraction kit (Nippon Genetics, Germany), and the resulting sequences were analyzed using the BLAST program available at the NCBI gene bank database (http://blast.ncbi.nlm.nih.gov/Blast.cgi). Sequence alignments were performed using BioEdit, and a phylogenetic tree was constructed using MEGA version 6.

Statistical Analysis

All experiment was conducted in triplicate, and the results were calculated in a Microsoft Excel spreadsheet and presented as Mean±SD.

RESULTS AND DISCUSSION

Morphological and biochemical characteristics of LAB isolates from Dadih.

Table 1 shows the characteristics of lactic acid bacteria isolates from Dadih. The isolated LABs are generally rod-shaped, gram-positive, with a colony size of 1-3mm, cream-colored, and catalase-negative. According to other studies (Setyawardani et al. 2019; Jafari et al. 2021; Jeyagowri et al. 2023), lactic acid bacteria isolated from different sources exhibit characteristics such as rod shape, single or paired colonies, and negative catalase activity. LAB can be identified at the genus level using its morphological characteristics. Hoque et al. (2010) state that the genus *Lactobacillus* can be identified by Gram staining and specific biochemical tests. *Lactobacillus* species are stationary, gram-positive, catalase-negative, non-spore-forming rods that vary in length from coccobacilli to long, thin bacilli.

The acid resistance of LAB isolates

Human gastric pH is estimated to range from 1.5 to 3.5 (Table 2). Therefore, resistance to pH three is often used in in vitro tests to assess gastric acid tolerance. Of the 20 Dadih LAB isolates tested morphologically and biochemically, only 6 exhibited acid resistance (pH 3) above 50%. Isolates LAB demonstrated acid resistance ranging from 53.97% to 75.00%. These results are higher than those reported by Abdullah et al. (2022), where *Lactobacillus plantarum* isolated from Dadih from Halaban, Indonesia, had an acid resistance (pH 3) of 65.98%, but lower than that of *Lactiplantibacillus pentosus* at 96.24% (Susmiati et al. 2024) and *Lactobacillus* at pH 2.5, 84.91% (Hernentis et al. 2019). Susanti et al. (2007) showed that most lactic acid bacteria exhibit reduced growth rates at low pH, resulting in decreased cell viability, a condition that varies among strains. Exposure to highly acidic conditions can cause membrane damage and depletion of internal components, such as magnesium, potassium, and lipids, potentially leading to the death of acid-sensitive bacteria.

Table 1: Characterization of LAB isolate from Dadih

Isolate of LAB	Characterization of LAB				
	Shape	Gram Staining	Size (mm)	Color	Catalase
MD1	Rods	Positive	1	Cream	Negative
MD2	Rods	Positive	1	Cream	Negative
MD3	Cocci	Positive	2	Cream	Negative
MD4	Rods	Positive	3	Cream	Negative
MD5	Cocci	Positive	1	Cream	Negative
MD6	Rods	Positive	1	Cream	Negative
MD7	Cocci	Positive	3	Cream	Negative
MD8	Cocci	Positive	2	Cream	Negative
MD9	Rods	Positive	2	Cream	Negative
MD10	Rods	Positive	3	Cream	Negative
MD11	Cocci	Positive	3	Cream	Negative
MD12	Rods	Positive	1	Cream	Negative
MD13	Cocci	Positive	1	Cream	Negative
MD14	Cocci	Positive	1	Cream	Negative
MD15	Rods	Positive	1	Cream	Negative
MD16	Rods	Positive	1	Cream	Negative
MD17	Rods	Positive	3	Cream	Negative
MD18	Rods	Positive	3	Cream	Negative
MD19	Cocci	Positive	1	Cream	Negative
MD20	Cocci	Positive	1	Cream	Negative

Table 2: Result of acid resistance test (pH 3)

Isolate	Viability (%)
MD1	75.00±0.27
MD7	68.89±0.12
MD10	65.82±1.24
MD11	53.97±5.21
MD15	74.07±1.11
MD20	54.54±0.99

Values are presented as mean±SD.

Resistance of the LAB isolates to bile salts

Isolates that resisted low pH conditions were assessed for their tolerance to bile salts. One of the most important characteristics of probiotics is their resistance to bile salts, which break down membrane lipids, leading to cell leakage and death (Choi et al. 2015). According to Byakika et al. (2019), tolerance to bile salts ranges from 0.1% to 0.7%. The test results in Table 3 show two selected LAB isolates with relatively high bile salt resistance: isolate MD1 at 92.58% and isolate MD15 at 86.09%. Harnentis et al. (2019) reported that *Lactobacillus spp.* resisted bile salt at 50.07% and Poddar et al. (2023) stated that *Pedicococcus acidilactici* was resistant up to 67.3%. Furthermore, Harun et al. (2020) and Abdullah et al. (2022) reported bile salt resistance of 6% and 54.90%, respectively, for *L. plantarum* isolated from Dadih.

Table 3: Result of the bile salt tolerance test

Isolate	Viability (%)
MD1	92.58±1.98
MD15	86.09±0.73

Values are presented as mean±SD.

Yuliana et al. (2023) also reported that LAB isolates from Dadih were resistant to 0.3% bile salts. The ability of lactic acid bacteria to endure bile salts is a significant attribute, as it determines their effectiveness in the digestive system, particularly in the upper small intestine, where bile salts are introduced. Acting as surfactants, bile salts activate lipolytic enzymes from the pancreas. These enzymes interact with fatty acids in the bacterial cytoplasmic membrane, altering its structure and permeability and thereby affecting the bacteria's bile salt resistance. The resistance of lactic acid bacteria to bile salts is associated with the enzyme bile salt hydrolase (BSH), which hydrolyzes conjugated bile salts, thereby reducing their toxic effects on cells (Susanti et al. 2007).

Lactic Acid Bacteria Resistance to Antibiotics

The sensitivity of lactic acid bacteria isolates to antibiotics was shown in Table 4. All lactic acid bacteria isolates were resistant to ampicillin and penicillin, except for MD15, which showed intermediate susceptibility to erythromycin, and MD1, which showed intermediate susceptibility to tetracycline. This was consistent with the findings of Sadrani, Dave, and Vyas (2014), who reported that lactic acid bacteria were resistant to erythromycin and penicillin.

Table 4: Results of Lactic Acid Bacteria Resistance to Antibiotics

Isolate of LAB	Type of Antibiotic			
	Amphycilin	Erytomycin	Penicillin	Tetracyclin
MD1	R	R	R	I
MD15	R	I	R	S

R (Resistance), I (Intermediate), S (Sensitive).

Antimicrobial activity of LAB isolates

Next, the probiotic candidate test from Dadih evaluated antibacterial activity against the pathogenic isolate MD1, which showed the highest bile salt resistance. As shown in Table 5, isolate MD1 exhibited antimicrobial activity against pathogenic bacteria, such as *Escherichia coli* O157, *Staphylococcus aureus, Acinetobacter baumannii, Salmonella, Propionibacterium acnes*, and *Listeria monocytogenes.* The antimicrobial activity of isolate MD1 was slightly higher than that of ampicillin and penicillin (positive controls).

Harun et al. (2020) and Pato et al. (2019) reported that *L. plantarum* and *Lactobacillus casei* subsp. *casei* R-68 (LCR-68) isolated from Dadih also inhibited *Escherichia coli*, as did *Lactobacillus spp.* from Dadih in the study by Harnentis et al. (2019), which also inhibited *E. coli, S. aureus,* and *S. Enteritidis*. Similarly, Wirawati and Widodo (2021) reported that isolates of *L. plantarum, L. lactis*, and *Pediococcus pentosaceus* exhibit antimicrobial activity. Abdullah et al. (2022) reported that *L. plantarum* from Dadih exhibited a relatively high inhibitory effect against *Escherichia coli* O157 (21.16mm), *Staphylococcus aureus* (23.17mm), *Listeria innocua* (19.24mm), and *Listeria monocytogenes* (18.23mm). Likewise, Yuliana et al. (2023) found that lactic acid bacteria isolates from Dadih could inhibit *E. coli* and *Salmonella*. Dadrasnia et al. (2021) stated that *L. plantarum* and *Lactobacillus delbrueckii* from Dadih of Kerinci origin exhibited antimicrobial activity against *Salmonella enteritidis* strain ATCC BAA-711, *Salmonella enteritidis* ATCC BAA-71, *Escherichia coli* strain ATCC 35401, and *Staphylococcus aureus* strain ATCC 2592.

Although the production of organic acids is commonly credited for the significant antimicrobial effects of LAB, it is crucial to acknowledge that antimicrobial peptides and other metabolites produced by these strains might also contribute to these effects (Somashekaraiah et al. 2019). According to Li and Gu (2018), probiotic bacteria produce antimicrobial substances or secondary metabolites that can inhibit the growth of harmful microbes during the fermentation of various food products. *Lactobacillus pentosus* produces bacteriocins, which are natural antimicrobial peptides. These bacteriocins have potential applications as natural preservatives in the food industry, exhibiting antibacterial activity against pathogens such as *Salmonella* and *Staphylococcus aureus*, thereby enhancing food safety and shelf life (Pramana et al. 2025). As in the studies by Heidari et al. (2021) and Pang et al. (2025), lactic acid bacteria produce bacteriocins active against pathogens that cause foodborne illnesses, *including Listeria monocytogenes, Bacillus cereus, Staphylococcus aureus,* and *Escherichia coli*.

Molecular Characterization of Isolated LAB Strains via 16S rRNA Gene Sequencing

The results of probiotic selection from six LAB isolates obtained from Dadih, following acid and bile salt tolerance tests and assessment of the ability to inhibit pathogenic bacteria, led to the selection of isolate MD1 as a probiotic candidate for molecular identification. The nucleotide sequence determines the length of the 16S rDNA region amplified. The lengths of 16S rDNA

Table 5: Results of antimicrobial activity against pathogenic bacteria

Sample	Pathogen Bacteria (mm)					
	EC	SA	AB	S	PA	L
MD1	9±0.23	10±0.08	10±1.20	11±0.77	9±0.20	11±1.11
Ampicillin	7±0.44	9±0.31	8±0.89	10±1.56	7±0.55	8±0.87
Penicillin	8±0.61	9±0.04	8±0.22	10±3.01	9±1.80	8±0.99

EC: *Escherichia coli O157,* SA: *Staphylococcus aureus*, AB: *Acinetobacter baumannii,* S: *Salmonella*, PA: *Propionibacterium acnes*, L: *Listeria monocytogenes.* Values are presented as mean±SD.

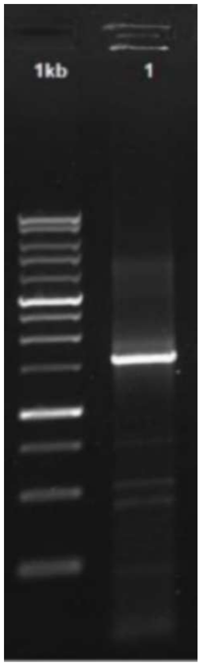

Fig 1: The amplification of the ribosomal RNA gene by PCR with the primers l1492R and 27F (M = 1 kB DNA Ladder, MD1 = 1485pb)

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TGCCTATACTGCAAGTCGAACGAGCTCCCGTTGAAGTGACGTGCTTGCACTGATTTCAACAATGAAGCGAGTG
GCGAACTGGTGAGTAACACGTGGGAAATCTGCCCAGAAGCAGGGGATAACACTCTGGGAAACAGGTGCTAA
TACCGTATAACAACAAAATCCGCATGGATTTTGTTTGAAAGGAGGCTTCGGCTATCACTTCTGGATGATCCCGC
GGCGTATTAGTTAGTTGGTGAGGTAAAGGCCCACCAATACGATGATACGTAGCCGACCTGAGAGGGTAATCG
GCCACATTGGGACTGAGACACGGCCCAAACTCCTACGGGAGGCAGCAGTAGGGAATCTTCCACAATGGACGA
AAGTCTGATGGAGCAATGCCGCGTGAGTGAAGAAGGGTTTCGGCTCGTAAAACTCTGTTGTTAAAGAAGAAC
ACCTTTGAGAGTAACTGTTCAAGGGTTGACGGTATTTAACCAGAAAGCCACGGCTAACTACGTGCCAGCAGCC
GCGGTAATACGTAGGTGGCAAGCGTTGTCCGGATTTATTGGGCGTAAAGCGAGCGCAGGCGGTTTTTTAAGT
CTGATGTGAAAGCCTTCGGCTTAACCGGAGAAGTGCATCGGAAACTGGGAGACTTGAGTGCAGAAGAGGAC
AGTGGAACTCCATGTGTAGCGGTGGAATGCGTAGATATATGGAAGAACACCAGTGGCGAAGGCGGCTGTCT
AGTCTGTAACTGACGCTGAGGCTCGAAAGCATGGGTAGCGAACAGGATTAGATACCCTGGTAGTCCATGCCG
TAAACGATGAGTGCTAAGTGTTGGAGGGTTCCGCCCTTCAGTGCTGAGCTATGCATAAGAATACCGCCTGGG
GAGTACGACCGCAAGGTTGAAACTCAAAGGAATTGACGGGGGCCCGCACAAGCGGTGGAGCATGTGGTTTA
ATTGGAAGCTACGCGAAGACCCTTACCAGTTCTTGACATCTTATGCCAATCTTAGAGATAAGACGTTCCCTTCG
GGGACAGAATGACAGGTGGTGCATGGTTGTCGTCAGTTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACG
AGCGCAACCCTTATTATCAGTTGCCAGCATTCAGTTGGGCATTTTGGTGAGACTGCCGGTGCCAAACCGGAGG
AAGGTGGGGATGACGTCAAATCATCATGCCCCTTATGCCCTGGGCTACACACGTGTTCCAATGGACGGTACAA
CGAGTTGGGAATTCGGGAGGTTAAGCTAATTTCTTAAACCCGTTTTCAGTTCGGATTGTAGGCTGCAATTCGC
CTACATGAAGTTGGAATCGTTAGTAATCGCGGATCAGCATGCCGCGGTGAATACGTTCCCCGGCCCTTGTACA
CACCGCCCGTCACACCATGAGAGTTTGTAACACCCAAAGCCGGTGAGATACCCTTCAGGAGTCAGCCGTCTAA
GGTGGGACAGATGATTAGGGTGAAGTCGTAACAAGGTA
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Fig 2: Nucleotide sequence of isolate MD1 of Dadih.

fragments derived from various lactobacilli vary. 16S rDNA segments from several lactobacilli species were typically about 1500bp in length (Mirzaei H and Barzgari A 2012). The electrophoresis results are shown in Fig. 1. Amplification of the ribosomal RNA gene by PCR with the primers l1492R and 27F was 1485bp, and the nucleotide sequence of isolate MD1 is displayed in Fig. 2.

A phylogenetic tree based on 16S rRNA gene sequences is shown in Fig. 3. Sequence results for the Dadih isolate, compared with GenBank data using the BLAST program on the NCBI website (http://www.ncbi.nlm.nih.gov), showed 97% similarity to *Levilactobacillus brevis. Levilactobacillus brevis* is a species of rod-shaped, gram-positive lactic acid bacteria that is heterofermentative, producing CO, lactic acid, acetic acid, and ethanol during fermentation. *L. brevis* is the type species of the genus *Levilactobacillus* (Zheng et al. 2020).

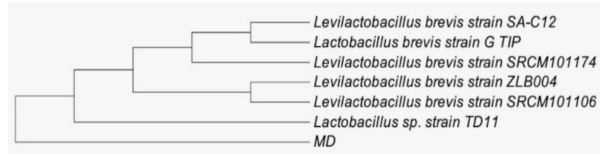

Fig 3: Phylogenetic isolate of MD1 from Dadih.

Conclusion

Following the probiotic selection process for lactic acid bacteria isolates derived from Dadih, which assessed their resistance to acidic conditions and bile salts, antibiotic resistance, and their capacity to inhibit pathogenic microorganisms, one promising probiotic isolate was identified: isolate MD1. Furthermore, molecular identification using 16S rRNA gene sequencing showed that isolate MD1 had 97% similarity to *Levilactobacillus brevis.* This isolate has potential for further development in the livestock product-processing industry as a source of probiotics beneficial to health.

DECLARATIONS

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Data availability statement: All data supporting the findings of this study are available within the manuscript.

Ethical Statement: Ethical approval was not required because no experimental animals were used in this study.

Author's Contributions: The experiment was conceived and designed by SM and IJ. The experiments were carried out by SM and ZS. The laboratory analyses were performed by IA and ZS. The data analysis was done by ZS. The primary researchers and authors of this article were SM, IJ, RDS, and ZS. SM, RDS, and IA wrote and edited the piece.

Generative AI Statement: The authors declare that no Gen AI/DeepSeek was used in the writing/creation of this manuscript.

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Abbreviations: LAB, lactic acid bacteria; MRS, de man, rogosa, and sharpe medium; FAO, food and agriculture Organization; WHO, world health organization; GRAS, generally recognized as safe; GABA, gamma-aminobutyric acid; PCR, polymerase chain reaction; DNA, deoxyribonucleic acid; RNA, ribonucleic acid; rRNA, ribosomal ribonucleic acid; BSH, bile salt hydrolase; ATCC, American type culture collection; CFU, colony forming unit; BLAST, basic local alignment search tool; NCBI, national center for biotechnology information; MEGA, molecular evolutionary genetics analysis; EC, *Escherichia coli O157*; SA, *Staphylococcus aureus*; AB, *Acinetobacter baumannii*; S, *Salmonella*; PA, *Propionibacterium acnes*; L, *Listeria monocytogenes.*

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