Research Article

Selection of Potential Lactic Acid Bacteria as a Candidate Probiotic on Cheese Making by Product Whey

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Abstract | Whey is a by-product of the cheese-making process which may contain lactic acid bacteria (LAB). The objectives of this study were to isolate, identify, and characterize probiotic lactic acid bacteria from whey, a by-product of cheese production. This study consisted of three stages: (1) isolation, identification of LAB obtain from whey, a by-product of cheese production, morphology, physiology, and biochemistry; (2) *in vitro* probiotic characteristics testing, including survival at pH 3, resistance to 0.3% bile salt media, and antimicrobial activity against *Escherichia coli*: O157, *Pseudomonas*, *Listeria inokua*, and *Klebsiella pneumonia*; and (3) Molecular identification of LAB by analysis of the base sequence of the 16S RNA gene. The whey for this study was collected in Lasi Farm in Agam Regency, West Sumatra, Indonesia. The data was subsequently subjected to descriptive analysis. The bacteria obtained were rod-shaped that were catalase negative and homofermentative. Lactic acid bacteria were found to have antibacterial activity against pathogenic bacteria such as *E. coli, Pseudomonas, Listeria inokua* and *Klebsiella pneumonia*. Base on the phylogenetic analysis, the bacteria isolated were closely related to *Limosilactobacillus fermentum*, a probiotic bacteria candidate.

Keywords | Lactic acid bacteria, *Limosilactobacillus fermentum*, Antimicrobial, Probiotic

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INTRODUCTION

Whey is more than a by product of the cheese-making process. Whey is a yellow liquid with a sour and slightly salty taste that is separated from the curd during the cheese-making process or a liquid that has been drained of fat and casein and contains 80% protein (de Wit, 2001). Even as a waste, the nutritional content of whey can still be utilized and processed (Laleye et al., 2008) (Philippopoulos and Papadakis, 2001) and (Salvatore et al., 2014). According to (Lievore et al., 2015), There are two whey-based milk coagulation methods: sweet whey and sour whey. On the other hand, sour whey is the by-product of acidifying milk to cause it to coagulate. In contrast, sweet whey is obtained through chymosin enzymatically, has an acidity of 6-7, and is also known as cheese whey. According to (Sinha et al., 2007), whey protein contains essential nutrients and is widely accepted as a functional food ingredient a Generally Recognized as Safe (GRAS) substance is commercial whey protein.

Whey on the market can be in the form of whey protein concentrate (WPC), sweet whey powder (SWP), whey protein isolate (WPI), and special WPC, which can be used as fermented drinks and yogurt (Hugunin et al., 2009). Based on biological value, whey protein is superior to the protein produced from other products such as soy, casein, and eggs (Pescuma et al., 2010; Shiby and Mishra,

2013). According to (Walsh et al., 2010; Melia et al., 2017), Probiotics have many benefits, like stopping the growth of pathogenic organisms, preventing intestinal, vaginal, and diarrheal infections, and boosting the immune system.

The Food and Agriculture Organization and the World Health Organization define probiotics as "live microorganisms when consumed in sufficient quantities to have a health effect on the host. Probiotics must meet several criteria, including being taxonomically clear, surviving in human intestinal conditions, surviving in sufficient numbers with effective doses throughout the shelf life, being supported by at least one positive human clinic trial, and being safe to use. The FDA considers most probiotics to be generally recognized as safe (GRAS) microorganisms (Dicks and Botes, 2010).

Consumer interest in functional foods or beverages containing probiotics and prebiotics continues to increase, creating a vast market to develop (Rathore et al., 2012; Walsh et al., 2010). LAB is isolated from various types of milk, such as goat milk (Melia et al., 2017) and buffalo milk (Rizqiati et al., 2015). Furthermore, the lactic acid bacteria isolate can be applied to the processing of fermented milk products which are beneficial for health, such as fermented goat milk (Melia et al., 2020, 2022; Kurnia et al., 2021), traditional cheese (Terzic-Vidojevic et al., 2014; Montel et al., 2014).

Because the potential for probiotics in whey from Lasi Farm's cheese production has never been explored traditionally or molecularly, this research is vital to be done.

MATERIALS AND METHODS

Sampling

Samples were obtained from Lasi Farm, Agam Regency, West Sumatra, Indonesia. Whey A and whey B samples were obtained twice and stored in a cold box until they were examined.

Isolation of lactic acid bacteria

de Mann, Rogosa, and Sharp (MRS) broth and MRS agar (Merck, Germany) were used to isolate the LAB. In order to isolate LAB, 1 mL of whey was added to 9 mL of sterile distilled water. Subsequently, the isolates were serially diluted up to the seventh dilution. A total of 100 mL of sample was spread plate plated on MRS agar for 48 hours at 37°C. One single colony was chosen for further testing. The selected colonies were further examined for morphological characteristics such as form and color, as well as biochemical characteristics such as Gram stain test, catalase, and fermentation type (Kopermsub and Yunchalard, 2010).

Acid resistance test

The acid resistance test was carried out using the modified method of Pato (2003) and Kocabay and Cetinkaya (2020). MRS broth (9 mL) was added with 5N HCL to justify pH 3 and used as a control. 1 mL of LAB culture was added to the MRS broth and incubated for 90 minutes at 37°C using the spread plate method, and this was used as stock culture. One mL was then spread plate grown on MRS agar media and incubated for 48 hours at 37°C. After counting the colonies, the CFU/mL concentration was calculated. The following formula was used to calculate the survival rate:

Survival rate % = log CFU N1/log CFU N0 × 100

N1= Total number of the cells that survived after each pH treatment, N0= Total number of alive cells before the treatment.

Bile salt resistance test

The bile salt resistance test was carried out using a modified method of (Pato, 2003; Kocabay and Cetinkaya, 2020). Bacteria from lactic acid were put into a test tube with 9mL of MRS broth with ox gall 0.3% and 9 mL of MRS broth without ox gall (without ox gall). Then, it was kept at 37°C for five hours. They were put on agar media with a dilution of 10-6 and kept at 37°C for 48 hours. The colonies were counted, and the CFU/mL was calculated and plotted. The following formula was used to calculate the survival rate:

Survival rate % = log CFU N1/log CFU N0 × 100

N1= Total number of the cells that survived after each pH treatment, N0= Total number of alive cells before the treatment.

Antimicrobial activity test

Antimicrobial activity testing was carried out using the modified well diffusion agar method (Yang et al., 2012; Rossi et al., 2021; Pato et al., 2020, 2022). LAB isolates were inoculated into MRS broth and then incubated for 24h at 37°C. The LAB supernatant was collected by centrifugation at 14.000 rpm for 15 min at 37°C. Furthermore, the cell-free LAB supernatant was an antimicrobial substrate to be tested for its antimicrobial activity with the well diffusion agar method using Nutrient Agar (Merck). The tested bacteria were *Escherichia coli*, *Pseudomonas*, *Listeria monocytogene*, and *Klebsiella pneumonia*. The clear zone formed was measured after incubation for 24 hours.

Genomic DNA isolation of lactic acid bacteria and 16S rRNA

The 16S rRNA sequence was used to identify the bacteria. The DNA from the bacteria was taken out with the PrestoTM Mini gDNA bacteria kit (GBB100 Geneaid).

24F: S′ AGA GTT TGA TGG CT 3′ and 1541R: S′ AAG GAG GTG ATC CCG CA 3′ were used to make DNA. In total, 50 liters of water, bacteria DNA, and Dream Taq DNA polymerase ('Thermo Scientific) were used in the PCR process. To start with, we did 3min of pre-PCR. We did 35 cycles of 95°C for 30sec, 50–50°C for 30 seconds, and 72–72°C for 1 minute 30 seconds. Then, we did 10 minutes at 72°C for post-PCR. The PCR products were put on a 1% agarose gel with ethidium bromide at a 5 g/ml concentration. It took 45 µl of electrophoresis in a concentration of 1X TBE (Tris Borate EDTA) buffer to separate the two groups. Following that, a UV transilluminator (Vilber Lourmat) and a UV-filtered digital camera were used to see and record the bands (Olympus SP 500-UZ) (Feliatra et al., 2019).

Phylogenetic analysis

The alignment analysis of sequences was carried out (Basic Local Alignment Search Tool) by comparing the obtained sequences (query) with those in the Gene Bank database at NCBI (http://www.ncbi.nlm.gov). MEGA v7.0 was used for phylogenetic analysis to create a phylogenetic tree.

Statistical analysis

The data obtained were calculated as the mean and then analyzed using descriptive methods.

RESULTS AND DISCUSSION

Total lactic acid bacteria and morphological and biochemical properties

As shown in Table 1, the total LAB found in whey A was 8.0 x 10^9 CFU/ml, while the total lactic acid bacteria found in whey B was 1.2 x 10^{10} CFU/ml. Furthermore, the morphological characteristics of LAB from whey, which was round (cocci), rod-shaped, and cream-colored, have been observed for the benefit of probiotics.

Table 1: Total lactic acid bacteria.

Sample	Total LAB (CFU/mL)
Whey A	80 x 10^8
Whey B	124 x 10^8

Acid resistance

There were 38 isolates of lactic acid bacteria. However, two of them were acid resistant (pH 3), namely W5 isolate from whey A and W7 from whey B. Acid resistance of Lactic acid bacteria can be seen in Table 2. Following an acid resistance test, a viability test was performed. The viability of isolate W5 was 82.35%, while isolate W7 was 93.97%.

Bile salt resistance

LAB viability in the small intestine was essential because it relates to its potential as probiotics. LAB resistance during incubation on MRS with 0.3% bile salts. The resistance of isolates W5 and W7 to bile salts in vitro using oxgall was shown in Table 3. In the presence of bile salts, isolate W5 had 44.23% viability, while isolate W7 had 49.31% viability.

Table 2: Acid resistance of isolates W5 and W7.

LAB isolate	Total bacteria (x 10^7 CFU/mL)		Viability (%)
	Control	pH 3	
W5	68	56	82.35
W7	83	78	93.97

Table 3: Bile salt resistance of isolates W5 and W7.

LAB isolate	Total bacteria (x 10^7 CFU/mL)		Viability (%)
	Control	*Ox gall* 0.3%	
W5	156	69	44.23
W7	146	72	49.31

Antimicrobial activity

Table 4 showed the inhibitory activity of LAB against pathogenic bacteria (Gram-positive and Gram-negative). The two LAB isolates inhibited the growth of pathogenic bacteria differently. The highest inhibition activity of pathogenic bacteria was shown by W7 isolate against the pathogenic bacterium *E. coli* with a zone of inhibition diameter of 7.5 mm. The lowest bacterial inhibitory activity was shown by W5 isolate against *Pseudomonas* bacteria with a zone of inhibition diameter of 2.5 mm. The zone of inhibition diameter from W5 and W7 was between 2.5-7 mm. Thus, both isolates had an antimicrobial activity with low inhibitory activity.

Table 4: Antimicrobial activity of isolates W5 and W7.

Sample	The diameter of the clear zone (mm)			
	Escherichia coli O157	*Pseudomonas*	*Listeria inokua*	*Klebsiella pneumoniae*
W5 isolate	5.5 ± 0.5	2.5 ± 0.5	4.0 ±1.0	3.0 ± 0.5
W7 isolate	7.5 ± 0.5	4.0 ± 1.0	7.0 ± 0.3	0
Ampisilin	5.0 ± 0.2	5.0 ± 0.5	6.0 ± 0.1	0
Kanamicin	6.0 ±0.1	6.0 ± 0.3	3.0 ± 0.5	5.0 ± 0.1

Sequentially, the inhibitory activity of W5 isolates was: *E.coli O157>Listeria inokua>Klebsiella pneumonia>Pseudomonas*. Meanwhile, for W7 isolate were *E. coli O157>Listeria inokua>Pseudomonas*. In general, LAB isolates showed inhibition activity against gram-negative bacteria (higher *E.coli*) than other pathogenic bacteria.

PCR amplification of the 16S rRNA gene

Figure 1 showed the PCR amplification of the 16S rRNA gene in W5 and W7 isolates. Furthermore, Figures 2 and 3 show the entire nucleotide sequences of W5 and W7

with 1476pb and 1468pb, respectively. Figure 4 presented phylogenetic trees based on 16S rRNA gene sequence analysis. The sequencing results of W5 and W7 isolates were compared to Gene Bank data using the BLAST program on the NCBI website (http://www.ncbi.nlm.nih.gov) and revealed a 99.80% (W5), 99.59% (W7) similarity rate with *Limosilactobacillus fermentum*HFD1 for W5 isolate and *Limosilactobacillus fermentum SK152* for W7 isolate (Tables 5 and 6).

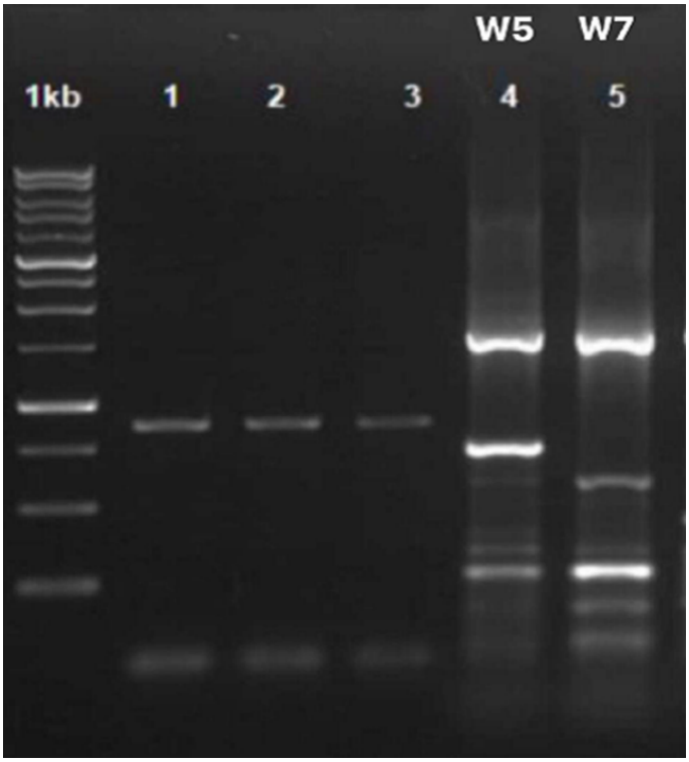

Figure 1: The PCR amplification of ribosomal RNA gene using l1492R and 27F. W5 and W7 are the isolated whey lactic acid bacteria (M = 1 kB DNA Ladder).

ATGCAAGTCGAACGCGTTGGCCCAATTGATTGATGGTGCTTGCACCTGATTGATTTTGGTCGCCAA
CGAGTGGCGGACGGGTGAGTAACACGTAGGTAACCTGCCCAGAAGCGGGGGACAACATTTGGAA
ACAGATGCTAATACCGCATAACAGCGTTGTTCGCATGAACAACGCTTAAAAGATGGCTTCTCGCT
ATCACTTCTGGATGGACCTGCGGTGCATTAGCTTGTTGGTGGGGTAACGGCCTACCAAGGCGATG
ATGCATAGCCGAGTTGAGAGACTGATCGGCCACAATGGGACTGAGACACGGCCCATACTCCTAC
GGGAGGCAGCAGTAGGGAATCTTCCACAATGGGCGCAAGCCTGATGGAGCAACACCGCGTGAGT
GAAGAAGGGTTTCGGCTCGTAAAGCTCTGTTGTTAAAGAAGAACACGTATGAGAGTAACTGTTCA
TACGTTGACGGTATTTAACCAGAAAGTCACGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTA
GGTGGCAAGCGTTATCCGGATTTATTGGGCGTAAAGAGAGTGCAGGCGGTTTTCTAAGTCTGATG
TGAAAGCCTTCGGCTTAACCGGAGAAGTGCATCGGAAACTGGATAACTTGAGTGCAGAAGAGGG
TAGTGGCACTCCATGTGTAGCGGTGGAATGCGTCGATATATGGAAGCACACCAGTGGCGAAGGC
GGCTACCTGGTCTGCAACTGACGCTGAGACTCGAAAGCATGGGTAGCGAACAGGATTAGATACC
CTGGTAGTCCATGCCGTAAACGATGAGTGCTAGGTGTTGGAGGGTTTCCGCCCTTCAGTGCCGGA
GCTAACGCATTAAGCACTCCGCCTGGGGAGTACGACCGCAAGGTTGAAACTCAAAGGAATTGAC
GGGGGCCCGCACAAGCGGTGGAGCATGTGGTTTAATTCGAAGCTACGCGAAGAACCTTACCAGG
TCTTGACATCTTGCGCCAACCCTAGAGATAGGGCGTTTCCTTCGGGAACGCAATGACAGGTGGTG
CATGGTCGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTTGT
TACTAGTTGCCAGCATTAAGTTGGGCACTCTAGTGAGACTGCCGGTGACAAACCGGAGGAAGGTG
GGGACGACGTCAGATCATCATGCCCCTTATGACCTGGGCTACACACGTGCTACAATGGACGGTAC
AACGAGTCGCGAACTCGCGAGGGCAAGCAAATCTCTTAAAACCGTTCTCAGTTCGGACTGCAGGC
TGCAACTCGCCTGCACGAAGTCGGAATCGCTAGTAATCGCGGATCAGCATGCCGCGGTGAATACG
TTCCCGGGCCTTGTACACACCGCCCGTCACACCATGAGAGTTTGTAACACCCAAAGTCGGTGGGG
TAACCTTTTAGGAGCCAGCCGCCTAAGGTGGGACAGATGATTAGGGTGAAGTC□

Figure 2: Nucleotide sequence of W5 isolate (1476pb).

TGCAAGTCGAACGCGTTGGCCCGATTGATTGATGGTGCTTGCACCTGATTGATTTTGGTCGCC
AACGAGTGGCGGACGGGTGAGTAACACGTAGGTAACCTGCCCAGAAGCGGGGGACAACATT
TGGAAACAGATGCTAATACCGCATAACAGCGTTGTTCGCATGAACAACGCTTAAAAGATGG
CTTCTCGCTATCACTTCTGGATGGACCTGCGGTGCATTAGCTTGTTGGTGGGGTAATGGCCTA
CCAAGGCGATGATGCATAGCCGAGTTGAGAGACTGATCGGCCACAATGGGACTGAGACACG
GCCCATACTCCTACGGGAGGCAGCAGTAGGGAATCTTCCACAATGGGCGCAAGCCTGATGG
AGCAACACCGCGTGAGTGAAGAAGGGTTTCGGCTCGTAAAGCTCTGTTGTTAAAGAAGAAC
ACGTATGAGAGTAACTGTTCATACGTTGACGGTATTTAACCAGAAAGTCACGGCTAACTACG
TGCCAGCAGCCGCGGTAATACGTAGGTGGCAAGCGTTATCCGGATTTATTGGGCGTAAAGA
GAGTGCGGGCGGGTTTCTAAGTCTGATGTGAAAGCCTTCGGCTTAACCGGAGAAGTGCATCG
GAAACTGTATAACTTGAGTGCAGAAGAGGGTAGCGGCACTCCATGTGTAGCGGTGGAATGC
GTAGATATATGGAAGAACACCAGTGGCGAAGGCGGCTACCTGGTCTGCAACTGACGCTGAG
ACTCGAAAGCATGGGTAGCGAACAGGATTAGATACCCTGGTAGTCCATGCCGTAAACGATG
AGTGCTAGGTGTTGGAGGGTTTCCGCCCTTCAGTGCCGGAGCTAACGCATTAAGCACTCCGC
CTGGGGAGTACGACCGCAAGGTTGAAACTCAAAGGAATTGACGGGGGCCCGCACAAGCGGT
GGAGCATGTGGTTTAATTCGAAGCTACGCGAAGAACCTTACCAGGTCTTGACATCTTGCGCC
AACCCTAGAGATAGGGCGTTTCCTTCGGGAACGCAATGACAGGTGGTGCATGGTCGTCGTCA
GCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTTGTTACTAGTTGCC
AGCATTAAGTTGGGCACTCTAGTGAGACTGCCGGTGACAAACCGGAGGAAGGTGGGGACGA
CGTCAGATCATCATGCCCCTTATGACCTGGGCTACACACGTGCTACAATGGACGGTACAACG
AGTCGCGAACTCGCGAGGGCAAGCAAATCTCTTAAAACCGTTCTCAGTTCGGACTGCAGGCT
GCAACTCGCCTGCACGAAGTCGGAATCGCTAGTAATCGCGGATCAGCATGCCGCGGTGAAT
ACGTTCCCGGGCCTTGTACACACCGCCCGTCACACCATGAGAGTTTGTAACACCCAA
AGTCGGTGGGGTAACCTTTTAGGAGCCAGCCGCCTAAGGTGGGACAGATGATTAGGG

Figure 3: Nucleotide sequence of W7 isolate (1468pb).

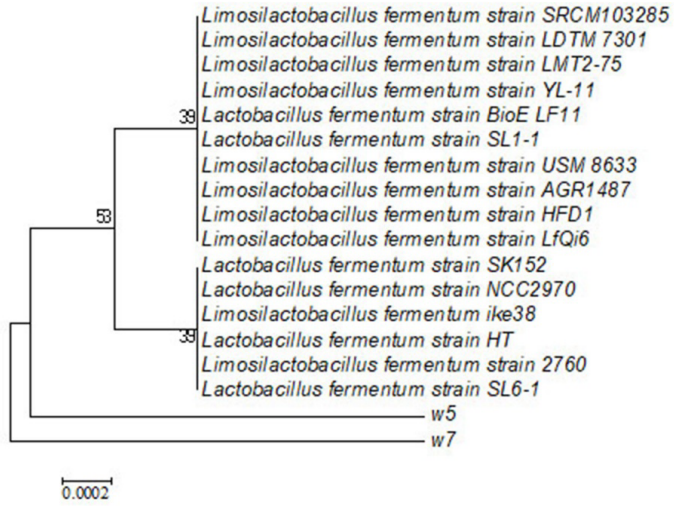

Figure 4: Phylogenetic tree of isolate W5 dan W7.

Total whey lactic acid bacteria and morphological and biochemical properties of lactic acid bacteria

The presence of LAB in whey was caused by the nutritional content of whey, such as lactose that supports LAB growth. According to de Wit (2001), whey contains protein, lactose, vitamins, and minerals. The nutritional composition of whey depended on the animal breed, feed, and lactation period. Lactose content in gouda cheese whey was 47g/L. Lactic acid bacteria naturally occur in various raw materials due to nutrients that promote their growth, as with research by (Melia et al., 2019; Rizqiati et al., 2015). The results were in line with a study by (Ogier et al., 2002) stating that the genus *Lactobacilli* was the main genus isolated from milk and its dairy products.

Table 5: BLAST Analysis of W5 isolate.

Description	Max Score	Total Score	Query Cover	E value	Per. Ident	Accession
Limosilactobacillus fermentum strain HFD1	2649	13216	100%	0.0	99.80%	CP050919.1
Limosilactobacillus fermentum strain AGR1487	2649	13229	100%	0.0	99.80%	CP047585.1
Limosilactobacillus fermentum strain USM 8633	2649	13202	100%	0.0	99.80%	CP045034.1
Lactobacillus fermentum strain SL1-1	2649	2649	100%	0.0	99.80%	MN435796.1
Lactobacillus fermentum strain BioE LF11	2649	2649	100%	0.0	99.80%	MK779053.1
Limosilactobacillus fermentum strain YL-11	2649	13198	100%	0.0	99.80%	CP034193.1
Limosilactobacillus fermentum strain LMT2-75	2649	13193	100%	0.0	99.80%	CP034099.1
Limosilactobacillus fermentum strain SRCM103285	2649	13189	100%	0.0	99.80%	CP035054.1
Limosilactobacillus fermentum strain LDTM 7301	2649	13193	100%	0.0	99.80%	CP031195.1
Limosilactobacillus fermentum strain LfQi6	2649	13180	100%	0.0	99.80%	CP025592.1

Table 6: BLAST Analysis of W7 isolate.

Description	Max Score	Total Score	Query Cover	E value	Per. Ident	Accession
Lactobacillus fermentum strain SK152	2621	13058	100%	0.0	99.59%	CP016803.1
Lactobacillus fermentum strain NCC2970	2621	13085	100%	0.0	99.59%	CP017151.1
Limosilactobacillus fermentum ike38	2621	13047	100%	0.0	99.59%	AP024320.1
Limosilactobacillus fermentum strain HFD1	2617	13062	100%	0.0	99.52%	CP050919.1
Limosilactobacillus fermentum strain AGR1487	2617	13067	100%	0.0	99.52%	CP047585.1
Limosilactobacillus fermentum strain USM 8633	2617	13058	100%	0.0	99.52%	CP045034.1
Lactobacillus fermentum strain HT	2617	2617	100%	0.0	99.52%	MN589592.1
Limosilactobacillus fermentum strain 2760	2617	13053	100%	0.0	99.52%	CP044354.1
Lactobacillus fermentum strain SL6-1	2617	2617	100%	0.0	99.52%	MN435805.1
Lactobacillus fermentum strain SL1-1	2617	2617	100%	0.0	99.52%	MN435796.1

Before molecularly identifying LAB using 16S rRNA, it is necessary to examine their morphology. The morphological characteristics of lactic acid bacteria isolated from whey were also involved, including round (cocci), rod-shaped, and cream-colored bacteria. Furthermore, Salminen et al. (2004) explained that LAB belonged to Gram-positive, non-sporing, spherical, or rod-shaped bacteria, catalase-negative, non-motile, and facultative aerobic.

Acid resistance

Of the 38 LAB isolates, two isolates had acid resistance (pH 3), namely W5 isolate from whey A and W7 from whey B. One of the characteristics of bacteria having potential as probiotics was their resistance to gastric conditions to survive at low pH conditions. The result was in line with studies by (Pato, 2003; Shi et al., 2012; Ren et al., 2014), stating that LAB, including probiotics, must be tolerant of gastric and small intestine conditions so that it must be tolerant of low pH on lysozyme enzymes, gastric acid, and bile salts. From Table 2, LAB isolates from whey had a high viability rate of more than 50%. The same results were also obtained in Lin et al. (2006) and Ramadhanti et al. (2021), which found LAB had viability above 50% at low pH conditions. This high viability value indicates that LAB has a high digestive tract survival ability. This result was in line with a study stating that LAB with a viability rate above 50% at low pH conditions indicates LAB has high viability in the digestive tract.

Bile salt resistance

LAB viability in the small intestine is essential because it relates to its potential as probiotics. This study used 0.3% bile salt. The bile salt concentration in the small intestine ranges from 0.15 to 0.6%, depending on the food consumed (Fernández et al., 2003). Therefore, LAB probiotics must survive in bile salt conditions with these concentrations. According to Rizqiati et al. (2015), in normal humans, the time required for food to transit in the small intestine was about 4–6 hours, and in the large intestine was about 24–48 hours.

The isolate was able to withstand bile salt quite well, where the LAB viability for the W7 isolate was 49.31%, and the W5 isolate was 44.23% (Table 3). Bezkorovainy (2001) stated that *Bifidobacteria* and *Lactobacillus* are beneficial bacteria for human health known as probiotics. Because isolates W5 and W7 have at least 20-40% resistance to gastric acid and bile salt, they can be considered prospective probiotic candidates.

LAB survived in bile salt conditions because LAB synthesized the enzyme bile salt hydrolase (BSH) to deconjugate bile salts. This study had also been demonstrated by Moser and Savage (2001), who used Lactobacillus strains isolated from the human intestine and dairy products. According to Canchaya et al. (2006), the production of this BSH enzyme is regulated by the BSH gene in bacteria. Furthermore, according to Pennacchia et al. (2004), bile salt secreted in the small intestine can damage the cell membrane of bacterial probiotics by hydrolyzing lipids and fatty acids.

Antimicrobial activity

Antimicrobial activity was a criterion possessed by the LAB to be categorized as probiotics. Antimicrobial activity was essential because it was related to LAB's inhibiting pathogenic bacteria. In this research, the inhibitory activity of W5 isolates was sequenced as follows: *E. coli O157* > *Listeria inokua* > *Klebsiella pneumonia* > *Pseudomonas*. Meanwhile, the *E. coli* O157 isolate was followed by *Listeria inokua* and *Pseudomonas*. LAB isolates inhibited gram-negative bacteria (particularly *E. coli*) more than other pathogenic bacteria. The same result was also obtained by Bao et al. (2010), cell-free supernatant *L. fermentum* inhibited the growth of Gram-positive (*L. monocytogenes* and *S. aureus*) and Gram-negative (*E. coli, S. flexneri,* and *S. typhimurium*) bacteria. Lactic acid bacteria isolated from dadih were also able to inhibit the growth of S. aureus and P. carotovorum subsp. carotovorum (Pato et al., 2021, 2022)

LAB probiotics could promote the growth of beneficial microorganisms, decrease the number of pathogenic microbes, and help to avoid food intolerance and allergies (Liévin-Le Moal and Servin, 2014; Sidira et al., 2014; Kia et al., 2016). There were four categories of the zone of inhibition, namely very strong zone with a zone of inhibition diameter of 20 mm, strong with the zone of inhibition diameter of 15 – 20 mm, moderate with a zone of inhibition diameter of 10 – 14 mm, and low with the zone of inhibition diameter of 5 – 9 mm (Nandi et al., 2017).

The antimicrobial activity of *L. fermentum was* found by Kang et al. (2017); García et al. (2012); Lehri et al. (2017), respectively, against *Staphylococcus aureus, Helicobacter pylori,* and *Campylobacter jejuni*. The ability of L. *fermentum* to generate organic acids, primarily lactic and acetic acids, as well as antimicrobial peptides, was credited with its bacteriostatic action.

Lactic acid bacteria exerted antibacterial activity in the host's intestine by producing organic acids (lactic, acetic, formic, propionic, and butyric acids), bacteriocins (nisin and pediocin), and other antibacterial peptides (da Silva Sabo et al., 2015).

PCR amplification of the 16S rRNA gene

This study discovered that isolates W5 and W7 had good LAB viability to pH 3 and 0.3 percent ox gall and antimicrobial activity against pathogenic bacteria. According to molecular identification with 16S rRNA, lactic acid bacteria had similarities to *Limosilactobacillus fermentum* HFD1 for W5 isolates and *Limosilactobacillus fermentum* SK152 for W7 isolates. The genome and taxonomy of the *Lactobacillaceae* have just recently been studied and evaluated. Its previous scientific name, *Lactobacillus fermentum*, has been changed to *Limosilactobacillus fermentum* as; a result, Zheng et al. (2020) and Melia et al. (2017) also found *L. fermentum* L23, a probiotic strain isolated from buffalo milk. Rodríguez-Sojo et al. (2021) explained that *Limosilactobacillus fermentum* CECT5716 was a good potential probiotic with anti-inflammatory and immunomodulatory properties. For medical uses and food preservation, a study on *L. fermentum* has been developed in preclinical and clinical studies (Naghmouchi et al., 2019).

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NOVELTY STATEMENT

The novelty of this research is to find lactic acid bacteria that are close to *Limosilactobacillus fermentum* which have the potential as probiotics from Cheese Making By-Product Whey.

AUTHORS CONTRIBUTION

This manuscript's materials preparation, data analysis, and text writing were all done by all of the authors.

Conflict of interest

The authors have declared no conflict of interest.

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