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ORIGINAL ARTICLE

Characterization of technological properties of lactic acid bacteria isolated from Turkish Beyaz (white) cheese

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Abstract

A total 144 lactic acid bacteria (LAB) strains were chosen and identified from 20 samples of Turkish Beyaz cheeses to be used as a starter or bioprotective culture. Genotypic characterization of these isolates revealed the presence of distinct LAB strains belonging to 15 different species including *Enterococcus faecium*, *Streptococcus lutetiensis*, *Enterococcus faecalis*, *Lactococcus lactis*, *Lacticaseibacillus paracasei*, *Lactiplantibacillus plantarum*, *Streptococcus macedonicus*, *Lactobacillus curvatus*, *Lacticaseibacillus paracasei* spp. *tolerans*, *Enterococcus durans*, *Lactobacillus delbrueckii* spp. *lactis*, *Enterococcus italicus*, *Leuconostoc pseudomesenteroides*, *Lactiplantibacillus paraplantarum*, and *Pediococcus parvulus*. The LAB strains were characterized according to their technological properties. In conclusion, many of the isolated LAB have important technological properties and in particular, *L. lactis* L52 and *S. macedonicus* L36 were found to be the most promising starter culture candidates due to their high acid-producing capacity. In addition, based on the results of antimicrobial and bacteriocinogenic activity, *L. lactis* L54 strain could be used as a bioprotective culture.

Novelty impact statement

This study is one of the most comprehensive research revealing the LAB diversity of Turkish Beyaz cheese, and it has been shown that many of the isolated LAB strains might have important technological properties to be used in white brined cheese production.

1 | INTRODUCTION

Beyaz (white) cheese is one of the most produced and consumed traditional Turkish cheese varieties. It is classified as a ripened cheese, produced from sheep's, goat's and cow's milk or a combination of these kinds of milk. The ripening period of this cheese takes 1–2 months in 14%–16% NaCl containing brine at 12–15°C. Final product has white color, hard-texture, salty acid taste, and a slight piquant flavor (Erkaya & Şengül, 2015; Hayaloglu, 2017; Hayaloglu et al., 2002; Kocak et al., 2020). The main isolates of Beyaz cheese are *Lactococcus lactis* subsp. *lactis*, *Lactococcus lactis* subsp. *cremoris*, *Lactobacillus casei* subsp. *casei*, *Enterococcus faecalis* var. *liquefaciens*, and *Weissella paramesenteroides*. However, other bacterial isolates belonging to the genus *Enterococcus*, *Leuconostoc*, *Pediococcus*, *Weisella*, and *Lactobacillus* species can also be found. It was found that during the ripening of Beyaz cheese *Lactococcus* species are abundant in the early phase of ripening, while there is an increase in *Lactobacillus* species over time (Dağdemir & Özdemir, 2008; Hayaloglu, 2017).

Especially in small-scale dairy plants, farms, or private houses, cheese is produced from raw milk without adding starter culture. It has been stated that commercial starter culture usage in traditional cheese manufacturing affects the typical aroma and characteristics. Thus, isolation and characterization of autochthonous lactic acid bacteria (LAB) from certain cheese(s) are necessary for composing unique starter culture that could be used for standardized cheese production (Dağdemir & Özdemir, 2008; Kirmaci et al., 2011).

Biopreservation can help the production of healthy and quality foods which attract the special interest of consumers. Biopreservation by LAB is mainly due to the production of a variety

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of antimicrobial compounds. Organic acids, hydrogen peroxide, CO_2, diacetyl, ethanol, reuterin, bacteriocins, and other antimicrobial metabolites of LAB can provide the protection of fermented foods against spoilage and pathogen microorganisms (Agriopoulou et al., 2020; Mani-López et al., 2018). The use of bioprotective and autochthonous LAB strains is important in the production and preservation of traditional cheeses (Topisirovic et al., 2006), and also Beyaz cheese.

In this study, the 144 LAB strains have been isolated from 20 Beyaz cheese samples manufactured in Erzurum, Turkey. Based on the technological properties, 27 of them were identified by 16S rRNA gene sequence analysis to establish them as potential starters or bioprotective cultures.

2 | MATERIAL AND METHODS

2.1 | Sampling of cheeses

Twenty samples of traditional Beyaz cheeses, prepared without adding a starter culture, were collected from different retailers, supermarkets, and dairy shops in Erzurum province Turkey. Aseptically taken samples were transferred to the laboratory at 4°C and analyzed immediately.

2.2 | Microbiological enumeration of cheeses

The samples were evaluated for their aerobic plate count (APC) (Maturin & Peeler, 1998) on Plate Count Agar (PCA) (Merck) at 30°C, yeasts and molds (ISO, 2008) on Dichloran Rose Bengal Chloramphenicol Agar (DRBC) (Merck) at 25°C, coliforms (ISO, 2010) on Violet Bile Red Agar (VRBA) (Merck) at 37°C, presumptive lactic acid bacteria (ISO, 2015) on De Man-Rogosa-Sharpe (MRS) agar (Merck) at 37°C in anaerobic conditions and enterococci (Harrigan, 1998) on Kanamycin Aesculin Azide (KAA) agar (Merck) at 35°C. Each sample was analyzed in duplicate.

2.3 | Isolation of LAB

For isolation of LAB (presumptive lactic acid bacteria from MRS agar and presumptive enterococci from KAA agar) colonies were selected according to their morphological differences (size, shape, color, etc.) and purified. Isolates were examined for Gram staining, catalase test, and microscopic shape. A total of 144 (82 from MRS, 62 from KAA) Gram-positive and catalase-negative isolates were presumptively evaluated as LAB. Stock cultures of isolates were prepared in MRS/GM17 containing 40% (v/v) glycerol and then stored at −80°C. For further analysis, LAB strains isolated from MRS were grown in MRS at 37°C, while enterococci were incubated in 0.5% glucose-containing M17 (GM17, Merck) at 35°C unless otherwise stated.

2.4 | Genotypic characterization and identification of LAB

For the genotypic characterization of potential LAB, isolates were analyzed by RAPD-PCR. DNA isolation from 18 h old culture was performed as described previously (Singh & Ramesh, 2009). RAPD-PCR analysis was conducted with primer $(GTG)_5$ (5′-GTGGTGGTGGTGGTG-3′). Following an initial denaturation at 94°C for 5 min, products were amplified by 35 cycles consisting of the following steps: denaturation at 94°C for 30 s, annealing at 47°C for 30 s, and elongation at 72°C for 150 s. The Final extension was performed at 72°C for 5 min. Finally, PCR products were separated using 1.5% (w/v) agarose gels containing ethidium bromide. Fingerprints were visualized under UV a transilluminator.

Following the RAPD-PCR selected isolates (27) were used for sequences of the 16S rRNA gene. 8F (5′-AGAGTTTGA TCCTGGCTCAG-3′) and 1541R (5′-AAGGAGGTGATCCAGCCGCA-3′) (Devereux & Willis, 1995) universal primers were used for amplification. The PCR program was: 94°C for 5 min; 35 cycles; at 94°C for 30 s, 59.5°C for 30 s, 72°C for 150 s, with a final extension of 72°C for 5 min. PCR products were sequenced by using the ABI 3130XL genetic analyzer (Applied Biosystems). Results were evaluated with the National Centre for Biotechnology Information (NCBI) database using the Blast program with a similarity criterion of 96%–100%.

2.5 | Technological characteristics

2.5.1 | Gas formation from glucose, arginine hydrolysis, proteolytic and lipolytic activity

The test for the production of CO_2 from glucose was performed in MRS broth containing Durham's tubes. Nessler's reagent was used for the arginine hydrolysis test (Drosinos et al., 2007). The proteolytic activity was determined using the MRS and GM17 agars containing 10% (w/v) skimmed milk powder. The lipolytic activity of the strains was determined using tributyrin agar. Fresh overnight 0.5 McFarland (~10^8 CFU/ml) cultures were dropped (10 µl) on modified mediums and incubated for 2 and 4 days for proteolytic and lipolytic activity respectively (Kenneally et al., 1998; Musikasang et al., 2009). The digesting ability of the tested LAB was categorized as positive when a clear zone was formed. Each experiment was done in triplicate.

2.5.2 | Growth at different temperatures, pH, and salt concentrations

Growth at different temperatures was tested by incubating strains at 4, 10, 15, and 45°C. All experiments were done in triplicate and incubated for 48 h. Sterile MRS/GM17 broths were used as the negative control, and growth at optimum temperature was used as the positive control. Growth at different pH was tested in related broth

which was adjusted to pH 2.5, 3.9, 5, 8.5, and 9 with 1 N HCl or NaOH. Sterile pH-adjusted MRS/GM17 broths were used as a negative control, and growth in MRS/GM17 broths was used as a positive control. Growth at different salt concentrations was tested in related broth supplemented with 4, 6.5, and 10% NaCl (w/v). Sterile NaCl added to MRS/GM17 broths was used as the negative control, and growth in MRS/GM17 broths was used as the positive control. Each experiment was done in triplicate. Measurements were made spectrophotometrically at 600 nm (Briges, 1953; Terzic-Vidojevic et al., 2009).

2.5.3 | Acidification activity

The acidifying activity of strains was measured in sterile reconstituted skimmed milk (10% w/v) inoculated with 1% (v/v) overnight cultures (0.5 McFarland). After incubation for 6, 12, 24, and 48 h pH (Hanna instruments) and titratable acidity values were determined according to International Dairy Federation (IDF) (IDF, 1995). Experiments were done in triplicate using non-inoculated skim milk as the negative control.

2.5.4 | Antibiotic susceptibility

Antibiotic susceptibility test of isolates was performed by disc diffusion method using nine different antibiotics including inhibitors of cell wall synthesis (ampicillin 10 µg, vancomycin 30 µg) and inhibitors of protein synthesis (gentamicin 10 µg, kanamycin 30 µg, streptomycin 10 µg, erythromycin 15 µg, clindamycin 10 µg, tetracycline 30 µg, and chloramphenicol 30 µg) recommended by European Food Safety Authority (EFSA). For this purpose, 100 µl of overnight cultures (0.5 McFarland) were spread onto related agar and antibiotic discs were placed on agar surfaces. Then, plates were incubated for 24 and 48 h for enterococci and isolates from MRS respectively (Nagpal et al., 2018). The inhibition zones were expressed as millimeters (mm) and classified as resistance or sensitive according to the zone diameter. The results were interpreted as resistant (below 15 mm), (16–20 mm), or susceptible (above 21 mm) (Sarkar et al., 2020).

2.5.5 | Detection of antimicrobial activity and bacteriocin coding genes

Antimicrobial activity of isolates against *Escherichia coli* O157:H7, *Staphylococcus aureus* ATCC 29213, *Listeria monocytogenes* ATCC 7644, *Salmonella* Typhimurium RSHMB 95091, and *Pseudomonas aeruginosa* ATCC 9027 pathogens was determined using the well diffusion method. Pathogens were grown in the Nutrient Broth (Merck) at 37°C for 24 h. LAB strains were cultured in related broth for 24 h and centrifuged at 10,000 ***g*** for 10 min. Supernatants were sterilized by filtering through a 0.45 µm pore size filter. For determination of the effect of the pH, cell-free supernatants (CFS) were neutralized by using sterile 1 N NaOH. To prevent the effect of heat-sensitive proteolytic enzymes and H_2O_2, supernatants were kept at 80°C for 10 min. In addition, supernatants were treated with proteinase K, pepsin, and trypsin at a final concentration of 0.1 mg/ml in water bath for 2 h at 37°C to eliminate the extracellular proteinaceous antimicrobials (Barbosa et al., 2016; Gutiérrez-Cortés et al., 2018). The well diffusion method was performed as described previously (Gonzalez et al., 2007; Reinheimer et al., 1990). All tests were performed in triplicate. The results were classified based on the diameter of the zones ("–" no inhibition zone, "+": >6 to <10 mm, "++": >11 to <16 mm and "+++": >16 mm) (Gupta et al., 2021).

The presence of nisin F (de Kwaadsteniet et al., 2008), enterocin A, enterocin B, enterocin L50B, enterocin P (Du Toit et al., 2000), lacticin 481 (Martinez et al., 1995), and lacticin 3147 (Martínez-Cuesta et al., 2000) bacteriocin genes was analyzed by PCR amplification. DNA isolation from 18 h old culture was performed as described previously (Singh & Ramesh, 2009). Primers and PCR conditions are described in Table S1. Bacteriocin coding genes were amplified using the following protocol: initial denaturation at 94°C for 5 min, followed by 35 cycles of denaturation at 94°C for 30 s, annealing at annealing temperature (Table S1) for 30 s, and elongation at 72°C for 30 s, with a final extension at 72°C for 5 min. PCR products were separated in 1.5% (w/v) agarose gel containing ethidium bromide and detected by UV transillumination.

3 | RESULT AND DISCUSSION

3.1 | Microbiological enumeration of cheeses

The results of the microbiological enumeration of 20 cheeses are found in Table 1 as mean, minimum, and maximum. The mean values of APC, yeasts-molds, coliform, presumptive LAB, and enterococci were 8.0, 2.86, 4.09, 7.57, and 5.53 log CFU/g, respectively. LAB and enterococci counts were similar to Yerlikaya (2018), Dağdemir and Özdemir (2008). The count of APC is similar to that of Dağdemir and Özdemir (2008), while the count of yeast-molds is similar to the results of Yerlikaya (2018), Mohammed and Çon (2021). According to the results, high counts of coliforms might be due to the usage of non-pasteurized milk and poor hygiene conditions. Therefore, it is important to use pasteurized milk and starter culture for standard and quality cheese production.

TABLE 1 Counts of main microbiological groups of Beyaz cheese[a] (log cfu/g)

	Mean	Maximum	Minimum
APC	8.00 ± 0.99	9.38 ± 0.02	6.20 ± 0.01
Yeasts-molds	2.86 ± 2.10	7.25 ± 0.02	<2
Coliform	4.09 ± 2.77	8.92 ± 0.01	<1
LAB	7.57 ± 1.03	8.98 ± 0.03	4.42 ± 0.00
Enterococci	5.53 ± 1.79	8.53 ± 0.03	2.89 ± 0.00

[a]All values are in mean ± SD, ($n = 2$).

3.2 | Isolation and characterization of LAB strains

A total of 144 lactic acid bacteria strains were isolated, 82 from MRS agar and 62 from KAA agar. According to RAPD-PCR patterns, MRS isolates are grouped into 19 (Table S2), and KAA isolates are grouped into 6 (Table S3) different clusters. According to the technological characteristics and RAPD-PCR profiles, a total of 27 isolates (at least one strain from each cluster) were identified by 16S rRNA gene sequence analysis. A total of 15 different LAB species were determined, including *Enterococcus faecium* (*E. faecium*, 30.6%), *Streptococcus lutetiensis* (*S. lutetiensis*, 13.2%), *Enterococcus faecalis* (*E. faecalis*, 12.5%), *Lactococcus lactis* (*L. lactis*, 7.6%), *Lacticaseibacillus paracasei* (*L. paracasei*, 7.6%), *Lactiplantibacillus plantarum* (*L. plantarum*, 6.3%), *Streptococcus macedonicus* (*S. macedonicus*, 4.9%), *Lactobacillus curvatus* (*L. curvatus*, 4.9%), *Lacticaseibacillus paracasei* spp. *tolerans* (*L. paracasei* spp. *tolerans*, 3.5%), *Enterococcus durans* (*E. durans*, 2.7%), *Lactobacillus delbrueckii* spp. *lactis* (*L. delbrueckii* spp. *lactis*, 2.1%), *Enterococcus italicus* (*E. italicus*, 1.4%), *Leuconostoc pseudomesenteroides* (*Leu. pseudomesenteroides*, 1.4%), *Lactiplantibacillus paraplantarum* (*L. paraplantarum*, 0.7%), *Pediococcus parvulus* (*P. parvulus*, 0.7%). The microbiota of Beyaz cheese shows that enterococci (47.2%) were the predominant group which is in agreement with the results obtained by Albayrak and Duran (2021). These findings can be explained by the salt tolerance of enterococci (Terzić-Vidojević et al., 2020). In addition, the presence of *Lactococcus*, *Lactiplantibacillus* (Formerly *Lactobacillus*), *Lactobacillus*, *Streptococcus*, *Leuconostoc*, and *Pediococcus* in white brined cheeses has also been reported by others (Frece et al., 2016; Uymaz et al., 2019). While it is known that all strains identified can be found in the cheese microbiota and can be used in cheese production, *S. lutetiensis* is associated with bovine mastitis (Chen et al., 2021). Before using this strain in fermentation, it should be subjected to safety assessments such as the presence of virulence genes and whether toxic metabolites are produced (Fugl et al., 2017).

3.3 | Technological characteristics of LAB strains

3.3.1 | Gas formation from glucose, arginine hydrolysis, proteolytic and lipolytic activities

Gas formation from glucose, arginine hydrolysis, proteolytic and lipolytic activities of isolates are found in Table 2. Among the isolates ($n = 27$), only *Leu. pseudomesenteroides* L53 (3.7%) was found to be heterofermentative. Homofermentative LAB prevalence in Beyaz cheese was also observed in other studies carried out in Turkey. In a study conducted by Albayrak and Duran (2021) all of the 40 isolates from white cheese were found homofermentative. Similarly, Mohammed and Çon (2021) found that all 12 isolates from Beyaz cheese were homofermentative. On the other hand, in the study conducted by Dağdemir and Özdemir (2008), heterofermentative lactic acid bacteria such as *Lactobacillus fermentum* and *Leuconostoc mesenteroides* were also isolated. Although heterofermentative LABs are isolated in white brined cheeses, these bacteria are not recommended to be used as starters because of their metabolic activities, which may cause adverse effects in dairy products (Haddadin, 2005). Biogenic amines are nitrogenous compounds that are mainly occurred by the decarboxylation of amino acids. Microorganisms could produce biogenic amines and consumption of foods containing large concentrations of these amines can have health risks (Linares et al., 2011; Shalaby, 1996). In the present study, it was determined that 48.14% of the LAB exhibited arginine decarboxylase activity. In addition to the decarboxylase activities of other amino acids of the strain to be used as a starter, the amounts of biogenic amines in the final product should also be determined by further studies. Proteolytic and lipolytic activities are other important properties in texture and aroma development during cheese production (Araújo-Rodrigues et al., 2021; Fox, 1989). Similar to Albayrak and Duran (2021), proteolytic activity was not detected in any of the isolated LABs. Because strains with high proteolytic activity may cause softening in Beyaz cheese, the strain(s) to be used as a starter should exhibit medium or low proteolytic activity (Dağdemir & Özdemir, 2008). Since the only strains with high/strong proteolytic activity could be detected with the used method, the activities of selected strains need to be further investigated. In our study, six of the enterococci strains showed lipolytic activity. Lipolytic activity of LAB is weak and enterococci have higher activity than other LAB genera (Schirru et al., 2012).

3.3.2 | Growth at different temperatures, pH, and salt concentrations

Table 3 presents the growth characteristics of the strains at different temperatures, pH, and salt concentrations. It was determined that 16 strains showed poor growth and the remaining 11 could not grow at 4°C. While most of the strains could grow at 10 and 15°C, *L. delbrueckii* spp. *lactis* L66, *S. lutetiensis* L55, *S. lutetiensis* E224, and *E. italicus* L57 could not grow. At 45°C, 20 strains including most of the enterococci could grow, while the remaining 7 strains could not. Most of the strains except *L. delbrueckii* spp. *lactis* L66 could grow at 4% NaCl, whereas at 6.5% NaCl *L. delbrueckii* spp. *lactis* L66, *L. lactis* L1, and *S. lutetiensis* E224 could not grow. At the 10% NaCl concentration, 13 strains showed poor growth and the remaining 14 could not grow. At low pH of 2.5 *L. paraplantarum* L34, *L. plantarum* L63, *L. paracasei* L45, *S. macedonicus* L36, and *S. macedonicus* L37 showed poor growth, and the remaining 22 strains could not grow. At pH 3.9, 8 strains developed at different rates, while the remaining 19 strains could not grow. All the strains could grow at pH 8.5 whereas at pH 5.0 *S. lutetiensis* L55, *P. parvulus* L78 and *E. italicus* L57 could not grow. At pH 9.6, all strains showed growth except *L. delbrueckii* spp. *lactis* L66 and *S. lutetiensis* L55. Beyaz cheese is matured for 30–90 days at 12–15°C, the pH of the final product is between 4.5 and 4.9, and the salt concentration is between 7% and 8% (Hayaloglu, 2017). The selection of starter cultures that can grow in the range of 12–15°C, pH 4.5–4.9, and 7%–8% NaCl concentration

TABLE 2 Arginine hydrolysis, gas formation from glucose, proteolytic and lipolytic activities of LAB

Strains	Gas formation	Arginine hydrolysis	Proteolytic activity	Lipolytic activity
L. curvatus L26	−	−	−	−
L. paraplantarum L34	−	−	−	−
L. plantarum L63	−	−	−	−
L. paracasei spp. *tolerans* L41	−	+	−	−
L. paracasei L80	−	−	−	−
L. paracasei L45	−	−	−	−
L. delbrueckii spp. *lactis* L66	−	−	−	−
Lc. pseudomesenteroides L53	+	−	−	−
L. lactis L1	−	+	−	−
L. lactis L52	−	+	−	−
L. lactis L54	−	+	−	−
S. macedonicus L36	−	−	−	−
S. macedonicus L37	−	−	−	−
S. lutetiensis L55	−	−	−	−
S. lutetiensis E224	−	−	−	−
P. parvulus L78	−	−	−	−
E. italicus L57	−	−	−	−
E. durans L43	−	+	−	+
E. faecium L14	−	+	−	−
E. faecium L56	−	+	−	−
E. faecium E208	−	+	−	+
E. faecium E229	−	+	−	−
E. faecium E260	−	+	−	+
E. faecalis L8	−	+	−	−
E. faecalis E201	−	+	−	+
E. faecalis E203	−	+	−	+
E. faecalis E237	−	+	−	+

could be advantageous. Accordingly, most of the strains (20) except *L. delbrueckii* spp. *lactis* L66, *L. lactis* L1, *S. lutetiensis* L55, *S. lutetiensis* E224, *P. parvulus* L78, *E. italicus* L57, and *Leu. pseudomesenteroides* L53 (heterofermentative) have these characteristics and could be used as starter cultures in Beyaz cheese production.

3.3.3 | Acidification activity

The changes in the pH and titration acidity of the strains at the sixth, 12th, 24th, and 48th hours are given in Figures 1 and 2, respectively. The use of strains with high acid production rate in cheese production is important for the inhibition of undesirable microorganisms and the development of taste and aroma (Aspri et al., 2017). pH and titration acidity following 6 h incubation ranged from 6.73 to 5.78 and 0.135 to 0.270 g/100 ml La, respectively. It was determined that *S. lutetiensis* E224 showed the highest acidification activity after 6 h of growth in milk. After 12 h, pH and titration acidity ranged from 6.65 to 5.12 and 0.135 to 0.441 g/100 ml La, respectively. *S. macedonicus* L36 showed the highest acidification activity at 12th hour. By the 24th hour, pH and titration acidity ranged from 6.16 to 4.45 and 0.195 to 0.813 g/100 ml La, respectively. *L. lactis* L52 showed the highest acidification activity, while *L. paraplantarum* L34 showed the lowest at this hour. The increase in acidity of milk after 24 h LABs is divided into three main groups (i): high acidifying capacity which decreases pH by more than 2 units, (ii): intermediate acidifying capacity which decreases pH by 1.5–2 units, (iii): low acidifying capacity which decrease pH less than 1 unit (Anagnostopoulos et al., 2018). When this grouping is taken into account, 5 (18.5%) of the isolates (*L. lactis* L52, *L. lactis* L1, *L. delbrueckii* subsp. *lactis* L66, *S. macedonicus* L36, and *S. lutetiensis* E224) have a high acid-producing capacity, and the remaining 22 (81.5%) isolates have low acid-producing capacity. These results are partially in agreement with the previous studies (Dağdemir & Özdemir, 2008; Domingos-Lopes et al., 2017; González et al., 2010). If evaluated after 48 h, pH and titration acidity ranged from 5.62 to 3.93 and 0.339 to 1.347 g/100 ml La, respectively. The most acidifying strain was *L. delbrueckii* spp. *lactis* L66, followed by *L. lactis* L52 (4.25 pH; 0.819 g/100 ml La) and *P. parvulus* L78 (4.41 pH; 0.753 g/100 ml La), respectively. On the other hand, *E. faecalis* L8 showed the lowest activity, followed by *E. faecalis* E203 (5.20 pH;

TABLE 3 Growth characteristics of LAB at different temperature, salt concentration, and pH

	Temperature				NaCl				pH			
Strain name	4°C	10°C	15°C	45°C	4%	6.5%	10%	2.5	3.9	5.0	8.5	9.6
L. curvatus L26	+	+++	+++	+	+++	++	−	−	−	+++	++	+
L. paraplantarum L34	+	+++	+++	+	+++	+++	+	+	+++	+++	+	++
L. plantarum L63	+	+++	+++	+	+++	+++	−	+	+++	+++	+++	+++
L. paracasei spp. *tolerans* L41	+	+++	+++	+	++	+	+	−	++	+++	+++	+++
L. paracasei L80	+	+	+++	−	+++	++	−	−	+	+++	+++	+++
L. paracasei L45	+	++	+++	+	+++	+	+	+	++	+++	+++	++
L. delbrueckii spp. *lactis* L66	−	−	−	+++	−	−	−	−	−	++	++	−
Lc. pseudomesenteroides L53	+	++	++	−	+	+	+	−	+	+++	+	+
L. lactis L1	+	+	++	−	++	−	−	−	−	++	+++	+++
L. lactis L52	+	+	++	−	++	+	+	−	−	++	+	+
L. lactis L54	+	+	++	−	++	+	−	−	−	++	+	++
S. macedonicus L36	+	+	+	+	+	+	+	+	++	+	++	+
S. macedonicus L37	+	+	+	+	+	+	+	+	+	+	+++	+
S. lutetiensis L55	−	−	−	+++	+	+	−	−	−	−	+++	−
S. lutetiensis E224	−	−	−	++	++	−	−	−	−	++	+++	+
P. parvulus L78	−	+	++	−	+	+	−	−	−	−	+	+
E. italicus L57	−	−	−	−	+	+	−	−	−	−	+++	+++
E. durans L43	+	++	+++	+++	+++	+	+	−	−	++	+	+
E. faecium L14	+	++	+++	+++	++	+	−	−	−	+	+	+
E. faecium L56	+	+	+++	+++	++	+	+	−	−	++	+	++
E. faecium E208	−	+++	+++	+++	+++	+++	+	−	−	++	+++	+++
E. faecium E229	−	+++	+++	+	+++	+++	+	−	−	++	+++	+++
E. faecium E260	−	+++	+++	+++	+++	++	−	−	−	++	+++	+++
E. faecalis L8	+	+	++	+	+	+	−	−	−	+	++	++
E. faecalis E201	−	+++	+++	++	+++	++	−	−	−	++	+++	++
E. faecalis E203	−	+++	+++	++	+++	++	+	−	−	++	+++	++
E. faecalis E237	−	+++	+++	++	+++	++	+	−	−	++	+++	++

Abbreviations: +++, absorbance close to positive control, good growth; ++, Growth; +, absorbance close to negative control, Poor growth; −, no growth.

0.468 g/100 ml La). According to our results, considering their acidification activity, strains with high acidifying capacity could be used as starter cultures.

3.3.4 | Antibiotic susceptibility

Table 4 presents the susceptibility of LAB to the different antibiotics tested. Antibiotic resistance of LAB is one of the crucial criteria for selecting these strains as starter/adjunct cultures or probiotics. Antibiotic resistance of LAB does not create a safety concern when the resistance is derived from intrinsic or chromosomal mutations. On the other hand, mobile genetic determinants of antibiotic resistance, such as plasmids and transposons, create a serious safety issue due to the potential risk of transmittance of this resistance to food or gut microbiota including pathogens (Arellano et al., 2020; Meral & Korukluoğlu, 2014; Sharma et al., 2014).

All of the LAB strains from this study were found to be susceptible to ampicillin. These results concurred with the previous studies (Meleh et al., 2020; Mohammed & Çon, 2021; Suwannaphan, 2021). *Lc. pseudomesenteroides* L53, *P. parvulus* L78, and all lactobacilli (except *L. delbrueckii* spp. *lactis* L66) were found to be resistant to vancomycin. *Leuconostoc*, *Pediococcus*, and *Lactobacillus* were reported to be intrinsically resistant to vancomycin (Ammor et al., 2007). All of the *Lactococcus* and *Streptococcus* strains from this study were sensitive to vancomycin, six strains of *Enterococcus* were found to be susceptible, and five isolates showed intermediate level sensitivity. All strains were found to be resistant to gentamicin except *Lc. pseudomesenteroides* L53. In responses of kanamycin and streptomycin, all strains were resistant except *E. italicus* L57. The widespread occurrence of gentamicin, kanamycin, and streptomycin-resistant LAB has been noted by others (Aktaş & Çetin, 2022; Aspri et al., 2017; Margalho et al., 2020; Meleh et al., 2020). It is generally known that some LAB display intrinsic resistance to gentamicin, kanamycin, and

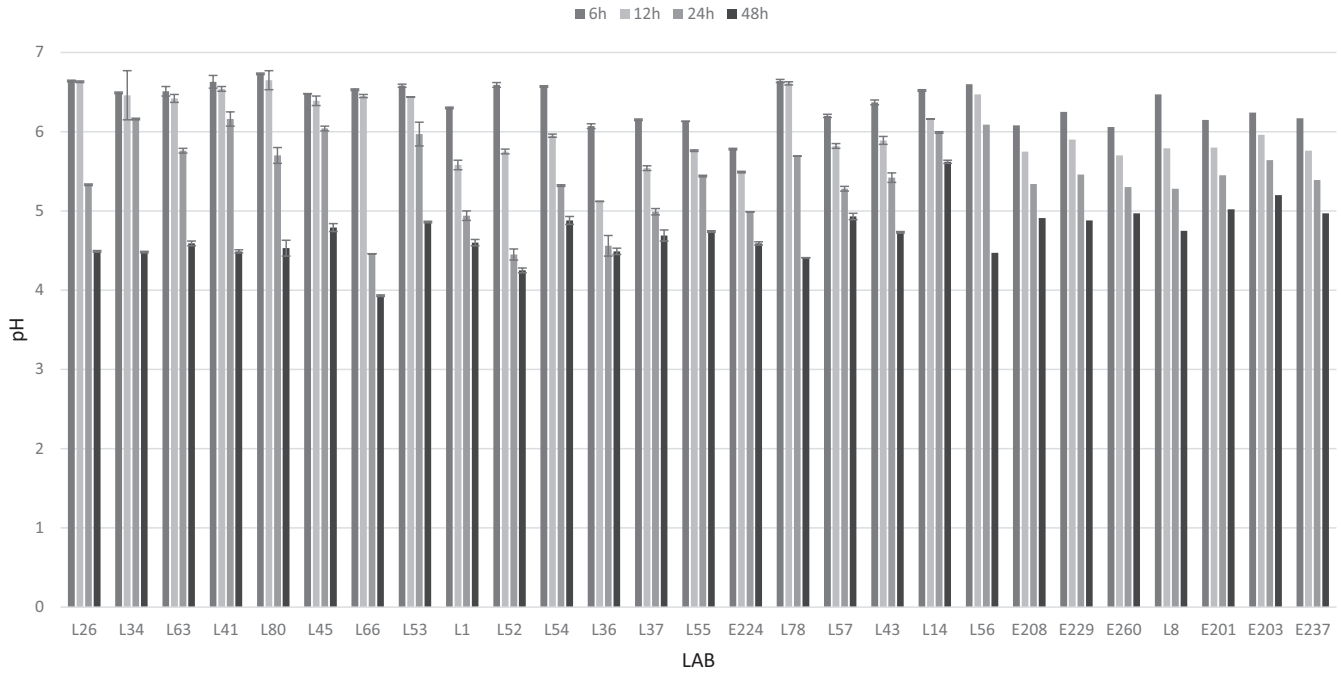


FIGURE 1 pH change in reconstituted skim milk during time. Codes represent the strain code of each strain given in Table 2. Values are represented as mean ± SD.

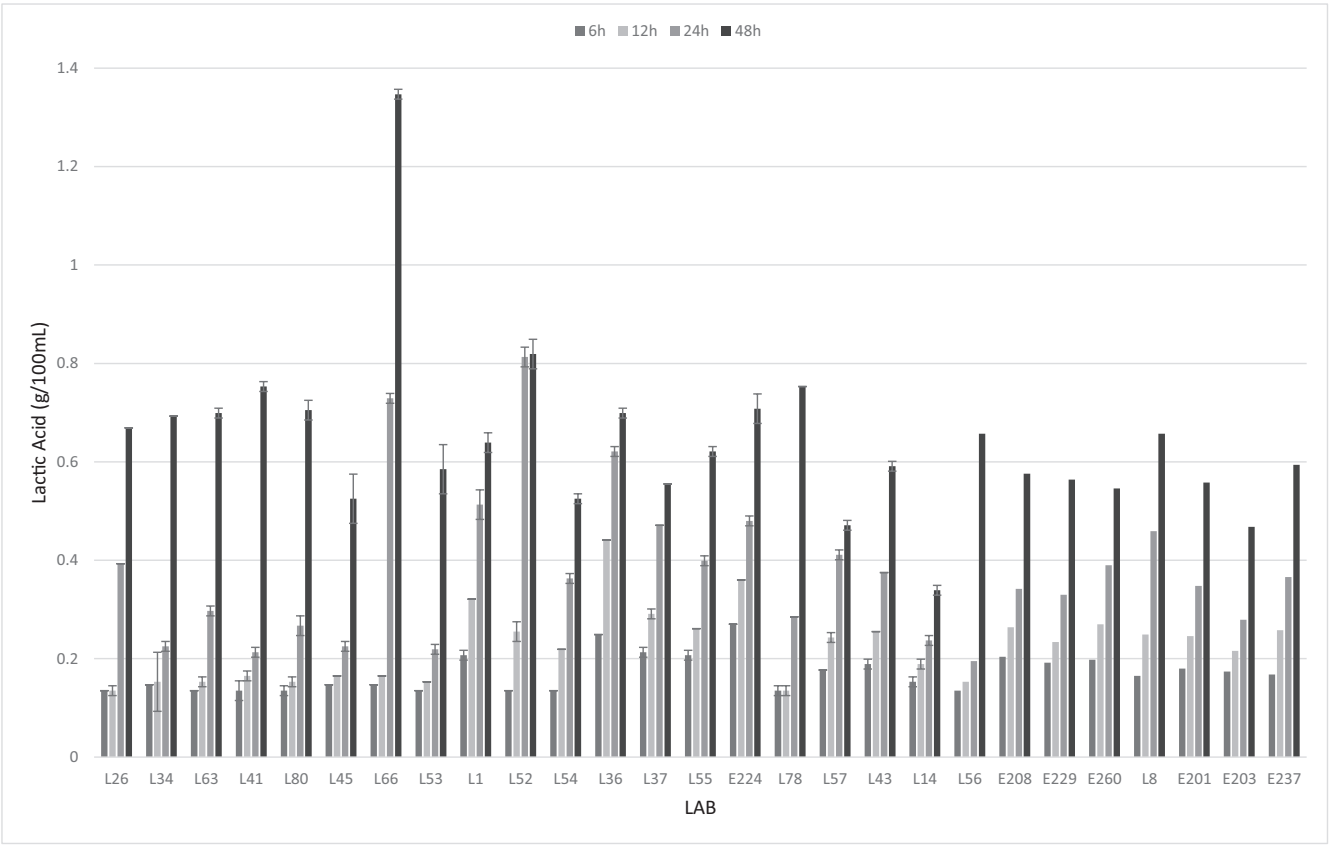


FIGURE 2 Titratable acidity change in reconstituted skim milk during time (g/100 mL^{-1} lactic acid). Codes represent the strain code of each strain given in Table 2. Values are represented as mean ± SD.

streptomycin (Ammor et al., 2007). For erythromycin, except for some enterococci, while all other 23 (85.2%) LAB strains were sensitive, *E. faecium* L56 and *E. faecium* E208 were resistant, *E. faecium* L14 and *E. faecium* E260 were intermediately sensitive. This result is in agreement with Valledor et al. (2022) who expressed that *E. faecium* isolates were resistant to erythromycin and, Holzapfel

TABLE 4 Antibiotic susceptibility of LAB

Strain code[a]	Zone of inhibition (mm)								
	AMP	VAN	GEN	KAN	STR	ERY	CLI	TET	CLA
L26	27 ± 1.4 (S)	– (R)	7 ± 1.4 (R)	8 ± 0.0 (R)	– (R)	31 ± 1.4 (S)	41 ± 1.4 (S)	34 ± 0.0 (S)	33 ± 1.4 (S)
L34	28 ± 0.0 (S)	– (R)	– (R)	– (R)	– (R)	28 ± 0.0 (S)	20 ± 0.0 (I)	21 ± 1.4 (S)	35 ± 1.4 (S)
L63	35 ± 1.4 (S)	– (R)	– (R)	– (R)	– (R)	23 ± 0.0 (S)	30 ± 1.4 (S)	23 ± 1.4 (S)	27 ± 1.4 (S)
L41	30 ± 2.8 (S)	– (R)	– (R)	– (R)	– (R)	37 ± 1.4 (S)	37 ± 1.4 (S)	38 ± 0.0 (S)	35 ± 1.4 (S)
L80	26 ± 0.0 (S)	– (R)	– (R)	– (R)	– (R)	31 ± 1.4 (S)	29 ± 1.4 (S)	37 ± 1.4 (S)	32 ± 0.0 (S)
L45	28 ± 0.0 (S)	– (R)	– (R)	– (R)	– (R)	33 ± 1.4 (S)	36 ± 0.0 (S)	36 ± 0.0 (S)	31 ± 1.4 (S)
L66	43 ± 1.4 (S)	27 ± 1.4 (S)	32 ± 0.0 (S)	– (R)	– (R)	36 ± 0.0 (S)	48 ± 0.0 (S)	45 ± 1.4 (S)	40 ± 0.0 (S)
L53	37 ± 1.4 (S)	– (R)	17 ± 1.4 (I)	12 ± 0.0 (R)	11 ± 1.4 (R)	31 ± 1.4 (S)	22 ± 0.7 (S)	33 ± 1.4 (S)	27 ± 0.0 (S)
L1	37 ± 1.4 (S)	24 ± 0.0 (S)	8 ± 0.0 (R)	9 ± 1.4 (R)	– (R)	29 ± 1.4 (S)	30 ± 0.0 (S)	40 ± 0.0 (S)	31 ± 1.4 (S)
L52	43 ± 1.4 (S)	31 ± 1.4 (S)	13 ± 1.4 (R)	14 ± 0.0 (R)	– (R)	38 ± 0.0 (S)	41 ± 1.4 (S)	47 ± 1.4 (S)	42 ± 2.8 (S)
L54	41 ± 1.4 (S)	28 ± 0.0 (S)	9 ± 1.4 (R)	9 ± 1.4 (R)	– (R)	33 ± 1.4 (S)	34 ± 2.8 (S)	43 ± 1.4 (S)	37 ± 1.4 (S)
L36	29 ± 1.4 (S)	29 ± 1.4 (S)	13 ± 1.4 (R)	9 ± 1.4 (R)	– (R)	31 ± 1.4 (S)	41 ± 1.4 (S)	39 ± 1.4 (S)	38 ± 0.0 (S)
L37	37 ± 1.4 (S)	27 ± 1.4 (S)	12 ± 0.0 (R)	– (R)	– (R)	31 ± 4.2 (S)	43 ± 1.4 (S)	36 ± 0.0 (S)	37 ± 1.4 (S)
L55	30 ± 2.8 (S)	22 ± 0.0 (S)	– (R)	– (R)	– (R)	24 ± 2.8 (S)	32 ± 0.0 (S)	32 ± 0.0 (S)	26 ± 2.8 (S)
E224	35 ± 1.4 (S)	20 ± 0.0 (I)	– (R)	– (R)	– (R)	28 ± 0.0 (S)	32 ± 0.0 (S)	31 ± 1.4 (S)	26 ± 0.0 (S)
L78	38 ± 0.0 (S)	– (R)	– (R)	– (R)	– (R)	30 ± 0.0 (S)	37 ± 1.4 (S)	24 ± 0.0 (S)	29 ± 1.4 (S)
L57	61 ± 1.4 (S)	37 ± 1.4 (S)	15 ± 1.4 (R)	22 ± 0.0 (S)	27 ± 1.4 (S)	42 ± 0.0 (S)	31 ± 1.4 (S)	13 ± 1.4 (R)	49 ± 1.4 (S)
L43	40 ± 0.0 (S)	24 ± 0.0 (S)	8 ± 0.0 (R)	8 ± 0.0 (R)	– (R)	31 ± 1.4 (S)	41 ± 1.4 (S)	41 ± 1.4 (S)	32 ± 0.0 (S)
L14	40 ± 0.0 (S)	37 ± 1.4 (S)	6 ± 0.0 (R)	– (R)	– (R)	16 ± 0.0 (I)	30 ± 0.0 (S)	40 ± 0.0 (S)	29 ± 1.4 (S)
L56	42 ± 0.0 (S)	24 ± 0.0 (S)	– (R)	– (R)	– (R)	11 ± 1.4 (R)	49 ± 1.4 (S)	13 ± 1.4 (R)	37 ± 1.4 (S)
E208	30 ± 0.0 (S)	20 ± 0.0 (I)	12 ± 0.0 (R)	8 ± 0.0 (R)	– (R)	13 ± 0.0 (R)	32 ± 0.0 (S)	31 ± 1.4 (S)	30 ± 0.0 (S)
E229	32 ± 0.0 (S)	20 ± 0.0 (I)	8 ± 0.0 (R)	7 ± 0.0 (R)	– (R)	30 ± 0.0 (S)	10 ± 0.0 (R)	31 ± 1.4 (S)	27 ± 1.4 (S)
E260	25 ± 0.0 (S)	20 ± 0.0 (I)	10 ± 0.0 (R)	– (R)	8 ± 0.0 (R)	20 ± 0.0 (I)	33 ± 1.4 (S)	29 ± 1.4 (S)	29 ± 1.4 (S)
L8	40 ± 0.0 (S)	24 ± 0.0 (S)	– (R)	– (R)	– (R)	30 ± 0.0 (S)	22 ± 0.0 (S)	36 ± 0.0 (S)	28 ± 0.0 (S)
E201	30 ± 0.0 (S)	17 ± 1.4 (I)	7.5 ± 0.7 (R)	9 ± 1.4 (R)	– (R)	23 ± 1.4 (S)	12 ± 0.0 (R)	29 ± 1.4 (S)	25 ± 1.4 (S)
E203	27 ± 1.4 (S)	17 ± 1.4 (I)	8.5 ± 2.1 (R)	14 ± 0.0 (R)	– (R)	21 ± 1.4 (S)	10 ± 0.0 (R)	32 ± 2.8 (S)	27 ± 1.4 (S)
E237	28 ± 0.0 (S)	16 ± 0.0 (I)	– (R)	– (R)	– (R)	24 ± 0.7 (S)	11 ± 1.4 (R)	33 ± 1.4 (S)	25 ± 1.4 (S)

Abbreviations: AMP, ampicillin (10 μg); VAN, vancomycin (30 μg); GEN, gentamycin (10 μg); KAN, kanamycin (30 μg); STR, streptomycin (10 μg); ERY, erythromycin (15 μg); CLI, clindamycin (10 μg); TET, tetracycline (30 μg); CLA, chloramphenicol (30 μg); –, no inhibition zone; R, (resistant) ≤15 mm; I, (intermediate) = 16–20 mm; S, (susceptible) ≥21 mm.

[a]Codes represent the strain code of each strain given in Table 2. Values are represented as mean ± standard deviation.

et al. (2018) stated that erythromycin resistance is an intrinsic property of this species. While 22 (81.5%) of strains were susceptible to clindamycin, *L. paraplantarum* L34 (3.7%) was intermediately sensitive, 4 (16.7%) of them were resistant including *E. faecium* E229 and most of the *E. faecalis* strains (E201, E203, E237). Similar to our results Jahan et al. (2013) revealed that 10 of the 13 *E. faecium* and all 15 *E. faecalis* strains, isolated from fermented meat products, were resistant to clindamycin. For tetracycline, *E. italicus* L57 and *E. faecium* L56 strains (7.40%) were resistant and 25 (92.6%) of the strains were sensitive. All of the isolated strains were sensitive to chloramphenicol. Consequently, several patterns were determined for the antibiotic resistance of LAB isolated in this study. Although the resistance detected seems mostly instinctive, further research must be done to prove the absence of potentially transferable resistance genes.

3.3.5 | Detection of antimicrobial activity and bacteriocin coding genes

The use of starter cultures with antimicrobial activity generally originating from bacteriocin production can help the control of spoilage and pathogenic microorganisms present in the fermentation (Gao et al., 2014; Lozo et al., 2021). All the strains were tested for

TABLE 5 Antimicrobial activity of LAB

Strains	*Escherichia coli* O157:H7			*Staphylococcus aureus* ATCC 29213			*Listeria monocytogenes* ATCC 7644			*Salmonella* typhimurium RSHMB 95091			*Pseudomonas aeruginosa* ATCC 9027		
	pH	E	H	pH	E	H	pH	E	H	pH	E	H	pH	E	H
L. curvatus L26	–	+	+	–	–	++	–	++	++	–	–	+	–	++	++
L. paraplantarum L34	–	++	–	–	+++	+++	–	–	–	–	+	–	–	++	++
L. plantarum L63	–	–	+	–	+++	+++	–	–	–	–	+	–	–	+++	+++
L. paracasei spp. *tolerans* L41	–	–	+	–	++	++	+	–	–	–	–	–	–	++	–
L. paracasei L80	–	++	–	–	++	++	–	–	++	–	+	+	–	+++	++
L. paracasei L45	–	–	–	–	++	++	–	–	–	–	++	–	–	–	++
L. delbrueckii spp. *lactis* L66	–	–	–	–	–	–	–	–	–	–	–	+	–	–	–
Lc. pseudomesenteroides L53	–	+	–	++	++	–	–	++	++	–	++	+	–	–	–
L. lactis L1	–	++	++	–	++	++	–	++	++	–	++	+	–	++	++
L. lactis L52	–	+	+	–	++	++	–	–	++	–	++	++	–	–	–
L. lactis L54	–	–	+	–	++	++	++	+++	+++	–	++	++	–	–	–
S. macedonicus L36	–	+	–	–	++	++	–	–	–	–	–	–	–	+	–
S. macedonicus L37	–	–	–	–	++	–	–	–	–	–	–	–	–	++	–
S. lutetiensis L55	–	–	–	–	–	–	–	–	+	+	+	–	–	++	–
S. lutetiensis E224	–	–	–	–	++	++	–	–	–	–	–	–	–	–	–
P. parvulus L78	–	–	–	–	–	–	–	–	+	–	–	–	–	+	–
E. italicus L57	–	–	–	–	–	–	–	–	–	–	–	–	–	++	–
E. durans L43	–	++	–	–	+++	+++	–	–	–	–	++	–	–	–	++
E. faecium L14	–	–	–	–	++	+++	–	–	–	–	–	–	–	+++	++
E. faecium L56	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
E. faecium E208	–	–	–	–	–	–	++	–	+	–	–	–	–	–	–
E. faecium E229	–	–	–	–	–	+	–	–	–	–	–	–	–	–	–
E. faecium E260	–	–	–	++	–	–	++	–	+	–	–	–	–	–	–
E. faecalis L8	–	–	–	–	++	–	–	–	–	–	–	–	–	++	+
E. faecalis E201	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
E. faecalis E203	–	–	–	–	–	–	++	–	–	–	–	–	–	–	–
E. faecalis E237	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–

Abbreviations: –, no inhibition zone; +, >6 to <10 mm; ++, >11 to <16 mm; +++, >16 mm; H, heat treatment; E, enzyme treatment.

antimicrobial activity against *E. coli*, *S. aureus*, *L. monocytogenes*, *S.* Typhimurium and *P. aeruginosa*, and the results are shown in Table 5. The antibacterial activity of CFS was mainly due to organic acids produced by LAB. These findings are concurred with the other studies (Petkova et al., 2021; Silva et al., 2020; Suwannaphan, 2021). On the other hand, neutralized CFS of *L. paracasei* spp. *tolerans* L41, *L. lactis* L54, *E. faecium* E208, *E. faecium* E260, and *E. faecalis* E203 showed activity against *L. monocytogenes*; *Lc. pseudomesenteroides* L53 and *E. faecium* E260 showed activity against *S. aureus*; *S. lutetiensis* L55 showed activity against *S.* Typhimurium. Considering these results, antimicrobial activity of both heat and enzyme-treated CFS of *L. paracasei* spp. *tolerans* L41 and *E. faecalis* E203 might be due to a heat-sensitive proteinaceous substance production. Similarly, antimicrobial activity of both pH neutralized and enzyme-treated CFS of *Lc. pseudomesenteroides* L53 and *S. lutetiensis* L55 might be due to the production of a non-protein substance like H_2O_2. Furthermore, antimicrobial activity of both pH neutralized and heat-treated CFS against *L. monocytogenes* may be an indication of bacteriocin production. The addition of proteinase K, pepsin, and trypsin to the CFS of *L. lactis* L54, *E. faecium* E208, and *E. faecium* E260 resulted in loss the of antimicrobial activity against *L. monocytogenes*. Nisin F, lacticin 481, and lacticin 3147 genes were investigated for *L. lactis* L54 and enterocin A, enterocin B, enterocin L50B, and enterocin P genes for both *E. faecium* E208, and *E. faecium* strains in order to reveal the source of their antimicrobial activity. The PCR amplification revealed that *L. lactis* L54 was positive for the presence of nisin F bacteriocin (Figure 3) while both *E. faecium* E208, and *E. faecium* were found negative for the presence of any enterocin genes. Considering other technologic characteristics, *L. lactis* L54 could be used as a bioprotective culture in the production of Beyaz cheese.

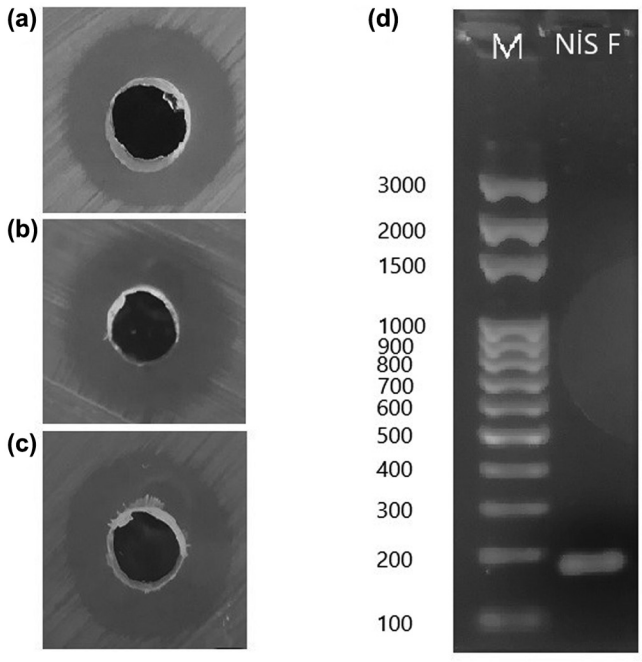


FIGURE 3 Antibacterial activity of *L. lactis* L54 against *L. monocytogenes* (a: pH adjustment, b: enzyme treatment, c: heat treatment). Agarose gel electrophoresis of the nisin F PCR product obtained from *L. lactis* L54. M, marker; Nis F, Nisin F gene (180 bp) (d).

4 | CONCLUSION

In conclusion, this study is one of the most comprehensive research revealing the LAB diversity of Turkish Beyaz cheese, and it has been shown that many of the isolated LAB strains might have important technological properties to be used in white brined cheese production. Considering the conditions in which the Beyaz cheese was produced, it was found that 20 of the 27 strains were able to adapt to these conditions. In particular, *L. lactis* L52 and *S. macedonicus* L36 were found to be promising candidates due to their high acid-producing capacity in addition to their other technological properties. As well as the other technological features, when the antimicrobial and bacteriocinogenic activities of the strains were evaluated, it was found that *L. lactis* L54 strain could be used as bioprotective culture. Despite these favorable properties, the genetic basis of antibiotic resistance of the strains and characterization of produced bacteriocin need to be conducted in further studies. In addition, the effects of technological properties on cheese should be examined by using the strains singly or in combination in the production of model Beyaz cheese.

AUTHOR CONTRIBUTIONS

Hacer Meral Aktaş contributed to conceptualization, methodology, validation, investigation, writing – original draft, writing – review & editing, and visualization. Ahmet Erdoğan contributed to conceptualization, project administration, supervision, and funding acquisition.

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CONFLICT OF INTEREST

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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