Journal of Food Science and Technology (Iran)

Homepage: www.fsct.modares.ir

Scientific Research

Investigation of probiotic potential of lactic acid bacteria isolated from Lighvan cheese

Mehdi Hoseinzadeh[1], Samad Vajdi Hokmabad [2] and Jalil Khandaghi * [3,4]

1. MSc Graduate of Microbiology, Sarab Branch, Islamic Azad University, Sarab, Iran.
2. Assistant professor of Animal Sciences, Sarab Branch, Islamic Azad University, Sarab, Iran.
3. Assistant professor of Food Science and Technology, Sarab Branch, Islamic Azad University, Sarab, Iran.
4. *Corresponding author: Department of food Biotechnology, Biotechnology Research Center, Tabriz Branch, Islamic Azad University, Tabriz, Iran.

ARTICLE INFO

Article History:
Received:2024/5/23
Accepted:2024/11/24

Keywords:

Probiotic,

Lighvan cheese,

Lactobacillus,

Enterococcus

DOI: **10.22034/FSCT.22.158.270.**

*Corresponding Author E-gh2002_haghayegh@yahoo.com

khandaghi@iausa.ac.ir

ABSTRACT

The main reason for the presence of specific microbiota, particularly lactic acid bacteria in dairy products made from raw milk, such as Lighvan cheese, is the traditional methods of preparing these products. A large number of these bacteria are probiotic microorganisms, and for example, many species of Lactobacillus, Enterococcus and Pediococcus bacteria presented in foods have shown probiotic properties. In the current study, genus-specific PCR detection for the Lactobacillus, Enterococcus, and Pediococcus genera was used to confirm the isolates of lactic acid bacteria that had been phenotypically isolated from 25 samples of traditional Lighvan cheese. Subsequently, the probiotic properties of the isolates, including resistance in simulated gastrointestinal tract conditions, antagonistic properties, auto-aggregation and co-aggregation attributes, antibiotic resistance and hemolytic activities of the selected strains were evaluated. Based on biochemical and molecular detection tests, among the 84 selected strains, 58 isolates were identified as Lactobacillus (29 isolates), Enterococcus (18 isolates) and Pediococcus (11 isolates). Screening of these isolates for survival in human GI tract conditions showed that 26.1% of the isolates (14 isolates) had resistance levels above 50%. Also, nine isolates of the selected strains showed potent antibacterial activity against *Listeria monocytogenes* and *Staphylococcus aureus*, as well as *Aspergillus flavus* and *Penicillium citrinum* molds. Moreover, based on the ability of auto-aggregation and co-aggregation features, four isolates LS106, LS71, LS33 and LS6 (belonging to Lactobacillus and Enterococcus genera) were selected. Finally, the investigation of antibiotic resistance revealed the sensitivity of these isolates to majority of the used antibiotics, so that all isolates were susceptible to tetracycline and ampicillin. Also, not one of these four isolates exhibited hemolytic properties. As a result, these isolates are recommended as suitable candidates for usage in fermented food products, taking advantage of their beneficial properties.

[Downloaded from fsct.modares.ac.ir on 2025-04-14]

[DOI: 10.22034/FSCT.22.158.270]

1- Introduction

Consumption of fermented dairy products has a major impact on consumer health because of the presence of beneficial microbial flora and their metabolites. Traditional techniques of preparing dairy products manufactured from raw milk from various animals including sheep, goats, and cows are responsible for the presence of certain microbiota in these products, which are typically belonging to the lactic acid bacteria [1]. Research has demonstrated that these beneficial bacteria can assist the food industry in producing health-conscious goods by producing metabolites such as organic acids, hydrogen peroxide, and bioactive peptides such as bacteriocins and bacteriocin-like substances [2].
Cheese is a high-protein, calcium, and phosphorus-containing dairy product. The diverse methods used to produce cheese, such as curdling, pressing, and ripening, are what have caused various types of cheese to arise [3]. One of the Iran's traditional fermented dairy products is Lighvan cheese, which is a type of starter-free, semi-hard cheese, manufactured from a blend of sheep and goat milk in the Lighvan village in Tabriz, Iran. The annual cheese production in the Lighvan region exceeds 3,150 tons, and because of distinct flavor, it is the most popular traditional Iranian cheese. The most essential trait that distinguishes Lighvan cheese from other similar products is the use of raw milk without adding starter culture in its formulation [4].
Lactic acid bacteria (LAB) are a diverse group of gram-positive, non-spore-forming cocci/bacilli categorized according to their morphological, metabolic, and physiological traits. Their primary metabolite is lactic acid, which is created through carbohydrate fermentation [5]. These bacteria have numerous applications in the food sector, as lactic fermentation not only extends food shelf life but also improves its organoleptic properties [6]. Lactic acid bacteria are also the most common probiotic microorganisms, with numerous Lactobacillus, Enterococcus, Leuconostoc, and Pediococcus bacteria found in food exhibiting probiotic properties [7].
Due to the great importance of these bacteria, the isolation and identification of novel species of these bacteria from various food sources is of great importance. Generally, identification of lactic acid bacteria is performed based on various phenotypic characteristics (cell morphology, growth conditions, and carbohydrate fermentation pattern) [8], but these methods do not provide clear classification results, particularly when it comes to differentiating LABs strains. Therefore, molecular methods, such as sequencing of 16/23 S rRNA genes or genus- and species-specific PCR tests, are used as an effective tool to identify this group of bacteria in fermented foods [9]. Accordingly, several studies have been conducted on the application of molecular techniques to identify LAB isolates from various foods [1, 10].
The beneficial features of probiotics include increasing the bioavailability of nutrients, modifying and modulating the microbial flora of the digestive tract, antagonistic effects against pathogenic bacteria, reducing serum cholesterol levels, and preventing cancer [11]. To be effective, probiotics must be resistant to the low pH and bile salts in order to survive in the upper digestive tract and then colonize by adhering to intestinal epithelial cells [12]. Other key probiotic requirements of these microorganisms include assuring consumer safety, the most notable of which is the absence of resistance to common therapeutic antibiotics [13].
Even though probiotic isolates from traditional Iranian fermented foods have been the subject of several studies in recent years [14,15], the search for additional effective strains is still ongoing because the microflora of these products can vary by region due to seasonal conditions and other environmental factors [16]. This supports the search for wild isolates with probiotic potential in each geographical region's fermented products. Given the foregoing, the primary objective of the current study is to isolate and identify the lactic acid bacteria population in traditional Lighvan cheese, as well as to establish the probiotic properties and safety aspects of these isolates.

2- Materials and methods

2-1- Sample collection

This study was conducted in the winter of 1402 to isolate and identify lactic acid bacteria from traditional Lighvan cheese. For this, 25 samples of traditional Lighvan cheese were randomly collected from manufacturing sites in Lighvan village and delivered on ice to the laboratory in Tabriz.

2-2- Isolation of lactic acid bacteria

For the initial isolation of lactic acid bacteria, their phenotypic characteristics were used [17, 18]. This was accomplished by adding one gram of cheese sample to nine milliliters of sterile saline solution, and then cultivating it on the surface of MRS agar medium after preparation of ten-fold serial dilutions. The plates were incubated for 24-72 hours at 30 and 42 °C and under anaerobic conditions to provide the best growth conditions for mesophilic and thermophilic bacteria. Five colonies with different morphology were selected from each plate and after confirming that the isolates were gram-positive and catalase-negative, the phenotypic groups were divided as follows.

[Downloaded from fsct.modares.ac.ir on 2025-04-14]
[DOI: 10.22034/FSCT.22.158.270]

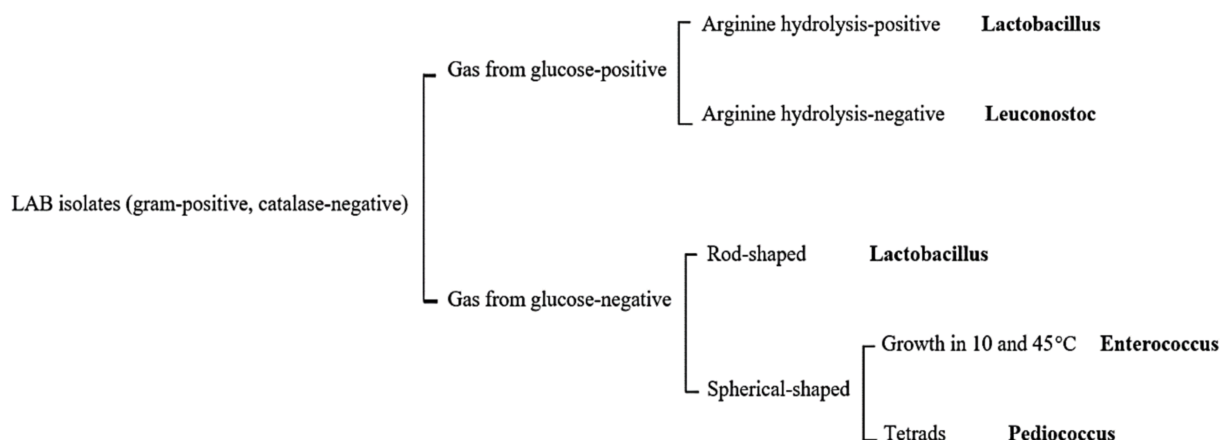

Figure1. Differentiation scheme for lactic acid bacteria isolated from Lighvan cheese

2-3- Confirmation by molecular identification

Molecular identification of isolates was performed following DNA extraction using an extraction kit (Pouya Gene Azma-PD115-50, Iran). For this purpose, a PCR reaction with a final volume of 25 μL containing: 10 ng/μL template DNA, 0.4 μM of each genus-specific primer, 0.2 μM dNTP, 2 μM MgCl2, 2.5 μL PCR buffer, and one unit of Taq DNA Polymerase was performed in a thermal cycler (PTC 200 Waltham, USA). The thermal cycles used for all primers included an initial denaturation of 5 min at 95°C and final cycle of the reaction mixtures for 7 min at 72°C. 30 cycles of template denaturation at 95°C for 45 s, primer annealing at 61°C for 50 s, and elongation at 72°C for 1 min were carried out for the Pediococcus-specific primer. 30 cycles were performed using the touchdown method for the primers specific to Lactobacillus and Enterococcus. These cycles included denaturation at 95°C for 45 s, primer annealing for 50 s from 59 to 56°C for the Enterococcus-specific primer, and primer annealing for 50 s from 59 to 52°C for the Lactobacillus-specific primer, followed by primer extension at 72°C for 1 min. The sequences of the primers used in this study are given in Table 1. The PCR product was electrophoresed in a 1.5% gel agarose containing ethidium bromide (Bio-Rad, USA).

Table1. Genus-specific primers used for the detection of LAB isolates from Lighvan cheese

Primers	Primers Sequence (5'-3')	Amplicon size (bp)	Reference
Lactobacillus genus-specific primers			
LbLMA1-rev	F: CTCAAAACTAAACAAAGTTTC	209	[۱۹]
R16-1	R: CTTGTACACACCGCCCGTCA		
Enterococcus genus-specific primers			
Ent1	F: TACTGACAAACCATTCATGATG	112	[۲۰]
Ent2	R: AACTTCGTCACCAACGCGAAC		
Pediococcus genus-specific primers			
Pedio_F	F: GAACTCGTGTACGTTGAAAAGTGCTGA	701	[۲۱]
Pedio_R	R: AGTGGAACTCCATGTGTAG		

2-4- Investigation of probiotic properties

2-4-1- Resistance to simulated human GI tract condition

To assess the viability of LAB isolates in simulated human gastric juice, the simultaneous tolerance of the strains to acid and pepsin (the pH of the microbial suspension was adjusted to 2.5 and 3 mg/ml of pepsin was added to it) was investigated. After one and three hours of incubation at 37°C (considering the normal duration of food retention in the human stomach), the samples were surface-cultured on MRS agar medium, and the number of surviving bacteria was determined [22]. Additionally, by inoculating fresh cell pellets of LAB isolates in MRS broth containing 0.3% bile salt (*w/v*) and 1 mg/ml pancreatin (pH = 8), the isolates' resistance to conditions similar to those of the small intestine was ascertained. After incubation for two and four hours at 37°C (given the typical duration of food retention in human gut), the surviving bacteria were counted [22] and finally, the selected bacteria that demonstrated greater resistance to bile and acid were then employed for further work.

[Downloaded from fsct.modares.ac.ir on 2025-04-14]
[DOI: 10.22034/FSCT.22.158.270]

2-4-2- Antagonistic effects

The well diffusion method was used to determine the antibacterial activity of selected LAB isolates against the gram-positive pathogenic bacteria (*Listeria monocytogenes* and *Staphylococcus aureus*) and two gram-negative indicator bacteria *Escherichia coli* and *Salmonella enterica* [10]. First, pathogenic bacteria were surface cultured on the MRS agar and after 24 hours incubation, wells with 5 mm diameter were created in plates and 50 µl of filtered (through a 0.2 µm filter) fresh selected LAB suspensions was added to each well. To do this, the neutralized, catalase-treated filtrates (pH = 7.2) were utilized. After overnight incubation of the plates at 37°C, clear inhibition zones with a diameter greater than 10 mm were considered as strong antimicrobial activity.

To investigate the antifungal activity, *Aspergillus flavus* and *Penicillium citrinum* were used. For this, PDA medium with concentrations of 10–100% (*v/v*) of filtered suspensions of LAB isolates was prepared and a disc of the test fungi (by cutting a piece from the outer edge of the fungal colony) with a diameter of approximately 5 mm was seeded in its center. The plates were incubated for one week at 25 °C and the minimum inhibitory concentration (MIC) for each mold was obtained. Then, the mycelial growth of fungi (average of two perpendicular diameters) was measured in the treated (T) and control (C) plates and growth inhibition percentage (I) at this concentration was calculated as follows [23].

$$I (\%) = [(C-T)/C] \times 100$$

2-4-3- Auto-aggregation and Co-aggregation properties

These properties were determined by spectrophotometric method [24]. After incubation of LAB isolates in MRS broth (24 hours, 37°C) and preparation of half McFarland standard bacterial suspension, the cell pellet was harvested by twice centrifugation (5 minutes, 4°C at 3500 rpm) in Behsan, MC10 centrifuge and after washing, resuspended in the same volume of phosphate buffer. Then the resulting mixture was vortexed and incubated at 37 °C for 4 h without agitation. The aggregation percentage was obtained using the following equation.

$$\text{Auto-aggregation } \% = (A_F/A_0) \times 100$$

A_F and A_0 are respectively the final and initial absorbance of the LAB isolate suspension, measured at 620 nm using a spectrophotometer (Cecil-Aquarius, UK).

To investigate the co-aggregation feature, after preparing the bacterial suspensions using the mentioned approach, equal volumes of LAB suspension were mixed with each of *Escherichia coli* and *Salmonella enterica*, vortexed and were left to stand for 4 hours at 37°C. Finally, the co-aggregation was calculated according to the following equation.

$$\text{Co-aggregation } \% = [(A_P + A_L)/2 - (A_{mix})/(A_P + A_L)/2] \times 100$$

A_P, A_L and A_{mix} are the absorbance of the pathogenic bacteria suspension, LAB isolates and their mixture, after 4 hours of incubation at 620 nm, respectively.

2-4-4- Antibiotic resistance

The sensitivity of selected lactic acid bacteria to common therapeutic antibiotics was determined by the disk diffusion method [25]. For this purpose, after preparing a lawn culture from a fresh suspension of LAB isolates (equal to 0.5 McFarland turbidity standard) on MRS agar medium, antibiotic disks including vancomycin (30 µg), cefixime (5 µg), penicillin (10 units), clindamycin (2 µg), tetracycline (30 µg), chloramphenicol (30 µg), ampicillin (10 µg), erythromycin (5 µg), azithromycin (15 µg), gentamicin (10 µg), and ceftriaxone (30 µg) prepared from PadtanTeb, Iran were seeded on to the agar at proper distances apart. The inhibition zone diameters (average of two perpendicular diameters) of formed after 24 hours of incubation at 37°C was recorded.

2-4-5- Hemolytic activity

LAB isolates were cultured in blood agar medium containing 5% sheep blood and incubated (48 hours at 37°C) to assess the hemolytic potency. Beta and alpha hemolysis were defined by the development of a clear hemolysis zone or formation of a green halo surrounding the colonies, respectively [26].

3- Results and Discussion

3-1- Isolation of lactic acid bacteria

Following phenotypic tests, 84 (67.2%) of 125 white or gray colonies isolated and purified from various MRS agar plates were identified as Lactobacillus (39 isolates), Enterococcus (25 isolates), and Pediococcus (20 isolates) and assessed by PCR test using particular primers for the bacterial genera mentioned above. Finally, 58 isolates were identified as Lactobacillus (29), Enterococcus (18), and Pediococcus (11). Figure 1 depicts one of the gel images of the molecular confirmation of LAB isolates from Lighvan cheese samples.

[Downloaded from fsct.modares.ac.ir on 2025-04-14]

[DOI: 10.22034/FSCT.22.158.270]

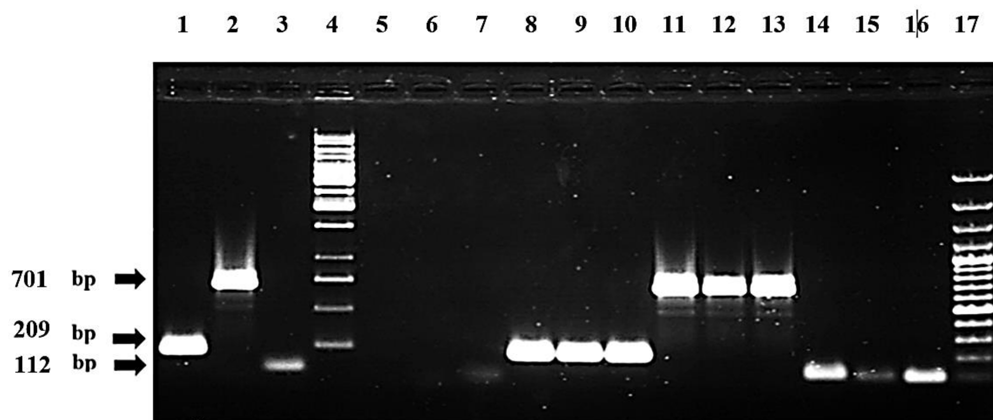

Figure2. Image of the 1.5% gel agarose (Invitrogen, G501802) of PCR products of Lighvan cheese isolates using genus-specific primers for Lactobacillus, Enterococcus and Pediococcus genera described in Table1. Lane 4: 1Kbp marker (Sinaclon SL 7051, Iran); Lane 17: 100bp marker (Sinaclon SL 7031, Iran); Lanes 1-3: positive control for *Lactobacillus plantarum* (PTCC 1058), *Pediococcus pentosaceus* (ATCC 25744) and *Enterococcus faecalis* (PTCC 1237); Lane 5-7: negative control (without template DNA); Lanes 8–16: DNA samples isolated from Lighvan cheese.

Since lactic acid bacteria play a major role in the ripening and preparation of various fermented foods, it is of particular importance to incorporate these bacteria, which also have probiotic features, in food products to enhance consumer health [27]. However, because of their higher environmental compatibility, probiotic bacteria found in regionally specific fermented foods are of greater interest [28], and accordingly, numerous studies have been conducted to identify and introduce the LAB bacterial population of various traditional Iranian foods and evaluate their probiotic properties [5, 29]. In this context, various species of the genus Lactobacillus [30], Enterococcus [10], and Pediococcus [31] with probiotic properties have been previously isolated from Iranian fermented foods.

3-2- Probiotic properties of isolates

3-2-1- Resistance to simulated human digestive tract condition

Table 2 lists the LAB isolates that survived more than 50% in simulated human stomach and intestine conditions and were selected for further study. Probiotic bacteria must be able to survive for 2-3 hours in human gastric juice (with a pH of about 3 and pepsin enzyme) and then safely pass through the gut (with the presence of bile salts and digestive enzymes such as pancreatin) within 4 hours to exert their beneficial effects [32, 33]. Thus, the most crucial and initial feature of a probiotics is thought to be resistance to bile salts and acid. In the current study, 26.1% of the LAB isolates that belonged to the genera Lactobacillus, Enterococcus, and Pediococcus demonstrated good resistance (above 50%) under simulated GI tract conditions. This finding contrasts with other studies found that lactic acid bacteria isolated from fermented dairy products had a high survival rate (71–76%) [34, 35]. Also, other researchers' reports of LAB isolates' lower resistance to simulated human gastrointestinal conditions are in line with the findings of the present study [10, 36].

Table2. Survival of LAB isolates under artificial human GI tract conditions

Isolates	Survival Rate (%) after 3 h in Simulated Gastric juice [a]	Survival Rate (%) after 4 h in Simulated gut juice [b]	LAB genus
LS6	68.68±0.92	51.65±1.13	*Enterococcus*
LS9	74.14±0.38	77.95±0.78	*Lactobacillus*
LS13	79.92±0.51	54.59±0.89	ND
LS17	66.98±1.14	53.34±0.34	*Enterococcus*
LS18	53.33±0.97	54.93±0.72	*Lactobacillus*
LS33	61.82±0.48	56.39±0.31	*Lactobacillus*
LS37	72.23±0.41	75.37±0.43	*Lactobacillus*
LS50	51.20±0.43	47.42±0.76	ND
LS58	73.64±0.52	78.39±0.42	*Enterococcus*
LS59	86.90±0.69	70.62±0.17	*Enterococcus*

[Downloaded from fsct.modares.ac.ir on 2025-04-14]

[DOI: 10.22034/FSCT.22.158.270]

LS64	74.19±0.51	78.01±0.27	*Pediococcus*
LS71	56.49±1.13	54.63±0.55	*Lactobacillus*
LS83	52.20±0.01	58.04±0.48	ND
LS85	48.68±0.92	51.65±1.13	*Pediococcus*
LS99	74.14±0.38	77.95±0.78	*Pediococcus*
LS106	79.92±0.51	54.59±0.89	*Enterococcus*
LS118	66.98±1.14	53.34±0.34	*Lactobacillus*

Data are mean ± standard deviation of three independent experiments.

[a], phosphate-buffered saline solution (adjusted to pH 3) containing 3 mg mL^{-1} pepsin.

[b], PBS solution containing 0.3% bile salts and 1 mg mL^{-1} pancreatin.

3-2-2- Antagonistic effect

Nine and six of the 14 isolates selected in the previous test, respectively, had substantial inhibitory effects (with an inhibition zone greater than 10 mm) against *Staphylococcus aureus* and *Listeria monocytogenes* (Table 3). The isolates' antimicrobial effects on gram-negative pathogens were lower, with seven and five isolates, respectively, displaying antimicrobial properties on *Escherichia coli* and *Salmonella enterica*, and only three and one LAB isolates providing an inhibition zone larger than 10 mm (Table 3). This study supports the findings of other researchers by showing that lactic acid bacteria had greater antibacterial effects on gram-positive bacteria than on gram-negative pathogenic bacteria [34, 35]. The antimicrobial effects of LABs are attributed to various metabolites, such as acid, hydrogen peroxide, or their bacteriocin [37]. Since filtered LAB suspensions were examined in this work after neutralization and catalase enzyme treatment, the effects indicated can be attributed to bacteriocin metabolites. Other studies have also pointed out the role of these compounds in the LABs' antimicrobial properties [2, 38].

Table3. Antibacterial effect of selected LAB isolates against indicator pathogens

Isolates	Zone diameter (mm) around			
	Salmonella enterica (PTCC 1709)	*Escherichia coli* (PTCC 1276)	*Staphylococcus aureus* (PTCC 1764)	*Listeria monocytogenes* (PTCC 1163)
LS6	0	5.48±0.16	7.21±0.26	12.01±0.33
LS9	6.26±0.06	17.47±0.23	12.64±0.28	16.50±0.21
LS17	0	0	0	0
LS18	0	0	11.26±0.05	10.58±0.19
LS33	2.30±0.17	13.59±0.19	21.58±0.20	20.49±0.14
LS37	0	8.54±0.09	0	13.43±0.18
LS58	0	0	0	2.64±0.21
LS59	12.31±0.15	11.43±0.18	18.41±0.20	22.24±0.23
LS64	0	0	0	7.26±0.05
LS71	4.43±0.10	8.54±0.25	13.71±0.19	18.35±0.10
LS85	0	0	8.65±0.24	11.30±0.17
LS99	0	0	0	4.53±0.24
LS106	0	0	16.38±0.15	15.54±0.09
LS118	5.73±0.10	2.46±0.10	0	0

Data are mean ± standard deviation of three independent experiments.

Also, analysis of the impact of various concentrations of the filtered suspension of 14 selected LAB isolates on the suppression of *Aspergillus flavus* and *Penicillium citrinum* colonies' radial growth revealed that all isolates, except for three (LS18, LS64, and LS99), had possessed antifungal effects. Table 4 depicts the minimum inhibitory concentration (MIC) of each mold and the inhibition percentages of the selected isolates at this concentration. To date, the antifungal activities of different bacteria including LABs have been established against a wide spectrum of molds [39, 40, 41]. Even the application of *Lactobacillus plantarum* antifungal substances as fungicides against plant pathogenic molds has been stablished [23].

Table4. Antifungal effect of selected LAB isolates against indicator molds

Isolates	MIC (%)	Growth inhibition (%) of *Aspergillus flavus* (PTCC 5004)	MIC (%)	Growth inhibition (%) of *Penicillium citrinum* (PTCC 5304)
LS6	30	14±0.10	30	24±0.04
LS9	40	10±0.09	50	9±0.55

[Downloaded from fsct.modares.ac.ir on 2025-04-14]

[DOI: 10.22034/FSCT.22.158.270]

LS17	70	16±0.22	70	13±0.36
LS18	-	-	-	-
LS33	20	21±0.25	20	29±0.30
LS37	100	15±0.30	70	24±0.34
LS58	60	25±0.17	40	10±0.20
LS59	30	21±0.48	30	22±0.11
LS64	-	-	-	-
LS71	20	27±0.07	20	25±0.06
LS85	40	23±0.53	30	18±0.65
LS99	-	-	-	-
LS106	20	20±0.11	30	31±0.23
LS118	100	9±0.40	100	11±0.30

Data for growth inhibition (%) are mean ± standard deviation of three independent experiments.

3-2-3- Auto-aggregation and Co-aggregation properties

The results of the ability of selected LAB isolates to auto-aggregation and co-aggregation with *Escherichia coli* and *Salmonella enterica*, are shown in Table 5. Accordingly, auto-aggregation, aggregation with *Salmonella enterica*, and aggregation with *Escherichia coli* ranged from 21.22 to 60.78, 31.49 to 33.88, and 30.14 to 07.80, respectively. The isolates selected for further experiments were those whose results were recorded above 50%. Bacterial aggregation, which contributes to their adhesion and colonization in gut epithelial cells, is regarded as one of the most significant and vital capabilities of a probiotic bacterium [42], and thus has been taken into account in the majority of studies investigating the probiotic properties of LAB bacteria [10,12]. Furthermore, it is believed that probiotics' ability to attach to pathogenic bacteria in the intestine can stop them from adhering to the epithelial cells, acting as a defense mechanism against the pathogens' invasion into the gut epithelium [5].

Table5. Aggregation activities of selected LAB isolates

Isolates	Auto-aggregation (%)	Co-aggregation (%) with	
		E. coli	*S. enterica*
LS6	78.60±0.49	88.33±0.18	80.07±0.35
LS9	38.46±0.13	40.30±0.22	50.85±0.40
LS18	40.26±0.24	36.49±0.66	39.57±0.59
LS33	79.53±0.45	59.46±0.36	69.19±0.54
LS37	51.43±0.30	44.46±0.24	30.14±0.39
LS59	21.22±0.39	31.49±0.49	42.44±0.61
LS71	67.57±0.41	87.52±1.33	70.86±0.10
LS85	40.78±0.51	47.87±0.20	35.75±0.40
LS106	56.52±0.37	76.65±0.52	70.43±0.19

Data are mean ± standard deviation of three independent experiments.

3-2-4- Antibiotic resistance

Table 6 shows the susceptibility of selected LAB isolates to 11 commonly used antibiotics. Overall, the isolates showed high susceptibility to the antibiotics used, with all isolates being sensitive to tetracycline and ampicillin. Resistance of 4 isolates to clindamycin and 3 isolates to both vancomycin and ceftriaxone was also recorded as the highest antibiotic resistance of the isolates. The resistance of LAB isolates to vancomycin in present study is consistent with prior research indicating that many Lactobacillus species are highly resistant to vancomycin [41]. Contrary to our findings, Kouhi et al. (2022) reported that none of the enterococci isolated from Motal cheese were resistant to vancomycin. The susceptibility of probiotic microorganisms to typical medicinal antibiotics is significant in two respects. First, certain LAB bacteria, such as enterococci, are suspected of being harmful; vancomycin-resistant enterococci, for instance, are one of the major problems in the field of hospital infections [43]. Furthermore, the need to evaluate and ensure probiotics' susceptibility to common clinical antibiotics is underscored by the potential for horizontal gene transfer (HGT) of antibiotic resistance between probiotics and other gastrointestinal tract bacteria, including pathogenic bacteria [44].

Table6. Antibiotic resistance in selected LAB isolates

Antibiotics	Inhibition zone diameter (mm) for LAB isolates			
	LS106	LS71	LS33	LS6
Vancomycin	13.5±0.36 (R)	10.6±0.11 (R)	15.4±0.08 (I)	7.3±0.09 (R)

[Downloaded from fsct.modares.ac.ir on 2025-04-14]

[DOI: 10.22034/FSCT.22.158.270]

Cefixime	15.5±0.14 (R)	27.4±0.14 (S)	18.1±0.05 (S)	16.3±0.09 (S)
Penicillin	30.6±0.36 (S)	29.4±0.08 (S)	27.3±0.16 (I)	31.1±0.17 (S)
Clindamycin	0 (R)	10.8±0.09 (R)	0 (R)	0 (R)
Tetracycline	31.3±0.11 (S)	25.5±0.15 (S)	23.4±0.09 (S)	29.3±0.15 (S)
Chloramphenicol	22.1±0.38 (S)	26.5±0.18 (S)	18.6±0.15 (I)	29.4±0.23 (S)
Ampicillin	25.2±0.39 (S)	27.4±0.14 (S)	18.5±0.14 (S)	22.3±0.14 (S)
Erythromycin	26.4±0.22 (S)	20.7±0.33 (I)	24.3±0.22 (S)	21.3±0.39 (S)
Azithromycin	13.4±0.08 (I)	23.5±0.21 (S)	13.7±0.08 (I)	17.4±0.09 (I)
Gentamicin	18.7±0.09 (S)	18.5±0.13 (S)	17.4±0.08 (S)	14.5±0.06 (I)
Ceftriaxone	4.3±0.06 (R)	28.3±0.28 (S)	21.2±0.16 (R)	15.1±0.19 (R)

Data are mean ± standard deviation of three independent experiments.
(I) indicates intermediate sensitivity (R) resistance and (S) susceptibility to the antibiotics based on CLSI, 2018 guideline.

3-2-5- Hemolytic activity

The evaluation of the hemolytic activity of four selected isolates (LS106, LS71, LS33, and LS6) revealed that none of them formed a clear or green hemolytic halo and thus lacked alpha or beta hemolysis. The absence of hemolysis, which prevents intestinal epithelium from being destroyed and renders them vulnerable to the entry and colonization of harmful bacteria, is one of the key characteristics pertaining to the safety of probiotic bacteria (26). Similar to the present study, other researches on lactic acid bacteria isolated from fermented foods have also found no evidence of hemolysis [5, 45].

4- Conclusion

Two strains of the Enterococcus genus LS106 and LS6), and two Lactobacillus strains (LS71, LS33) that were isolated from Lighvan cheese demonstrated in the present study had the ability to survive in simulated human gastrointestinal conditions. Also, these isolates had other probiotic properties such as antimicrobial activity and accumulation and colonization in the intestine. Thus, these isolates can be suitable choices for use in food products and benefit from their health-promoting properties, due to the lack of hemolytic activity and sensitivity to widely used therapeutic antibiotics. Evaluation of the presence of virulence factor genes, particularly in Enterococcus isolates, and conducting *in-vivo* experiments on animal models to further guarantee their safety are recommended for future studies.

5- References

[1] Nami, Y., Vaseghi Bakhshayesh, R., Mohammadzadeh Jalaly, H., Lotfi, H., Eslami, S. & Hejazi, M.A. (2019). Probiotic properties of Enterococcus isolated from artisanal dairy products. *Frontiers in microbiology*, 10:300.

[2] Salek, F., Mirzaei, H., Khandaghi, J., Javadi, A. & Nami, Y. (2023). Identification of enterocins and in vitro characterization of their antimicrobial and anticancer activity in Enterococcus strains isolated from traditional fermented products. Food Hygiene, 13(2), 17-32 (In Persian).

[3] Johnson, M.A. (2017). 100-Year Review: Cheese production and quality. *Journal of Dairy Science*,100(12), 52-65.

[4] Rashtchi, P., Bazmi, A., Noshirvani, N. & Moosavy, M.H. (2021). Comparison of the microbial, physicochemical, and sensorial properties of raw and pasteurized Lighvan cheeses during ripening time. *Food Science & Nutrition*, *9*(10), 5527-5535.

[5] Soleimani, H., Shokri, R., Nami, Y., Khandaghi, J. & Panahi, B. (2023). Potential probiotic characterization of lactic acid bacteria isolated from Duimaj, an Iranian traditional snack food, using biochemical, molecular and computational approaches. *LWT*, *184*, 115091.

[6] Soltan Dallal, M.M., Hosseini, M., Davoodabadi, A., Rajabi, Z. & Zamani, S. (2016). Isolation and Identification of Lactic Acid Bacteria in traditional pickles and salted pickles from Tehran, Iran. *Razi Journal of Medical Sciences,* 23(143),81-90 (In Persian).

[7] Koohsari, H., Rashti, Z. & Arab, S. (2019). The Isolation of lactic acid bacteria from local dairy products of Gorgan township with the ability to inhibit the growth of some gastrointestinal pathogens. *Journal of Food Microbiology*, 6(3),22-36 (In Persian).

[8] Nasrollahzadeh, A. ,Khomeiri, M. & Mahmoudi, M. (2019). Genetic identification and evaluation of antibacterial activity of lactic isolates derived from Masske butter against pathogenic bacteria *Staphylococcus aureus* and *Salmonella enterica*. *Journal of food science and technology (Iran), 16*(93), 23-33. (In Persian).

[9] Moraes, P.M., Perin, L.M., Silva Júnior, A. & Nero, L.A. (2013). Comparison of phenotypic and molecular tests to identify lactic acid bacteria. *Brazilian Journal of Microbiology*, 44,109-112.

[10] Kouhi, F., Mirzaei, H., Nami, Y., Khandaghi, J. & Javadi, A. (2022). Potential probiotic and safety characterisation of Enterococcus bacteria isolated

[Downloaded from fsct.modares.ac.ir on 2025-04-14]

[DOI: 10.22034/FSCT.22.158.270]

from indigenous fermented Motal cheese. *International Dairy Journal*,126,105247.

[11] Mathur, H., Beresford, T.P. & Cotter, P.D. (2020). Health benefits of lactic acid bacteria (LAB) fermentates. *Nutrients,*12(6),1679.

[12] Aali, N. & Khandaghi, J. (2022). Evaluation of probiotic properties of *Enterococcus faecalis* and *Enterococcus faecium* species isolated from Pousti cheese of Gilan province in 2020. *Journal of Food Microbiology*, *9*(2), 60-70. (In Persian).

[13] Saarela, M., Mogensen, G., Fonden, R. , Mättö, J. & Mattila-Sandholm, T. (2000). Probiotic bacteria: safety, functional and technological properties. *Journal of biotechnology*, 84(3),197-215.

[14] Azizi, F., Najafi, M.B.H. & Edalatian Dovom, M.R. (2017). The biodiversity of Lactobacillus spp. from Iranian raw milk Motal cheese and antibacterial evaluation based on bacteriocin-encoding genes. *AMB Express*, 7(1),176-183.

[15] Edalatian Dovom, M.R., Habibi Najafi, M.B. & Mortazavi, S.A. (2014). Identification of Anti-microbial Producing Enterococci Isolated from Iranian Raw Milk Cheeses Using Polyphasic Approach. *Applied Food Biotechnology*, 1(2), 19-24.

[16] Hassanzadazar, H., Mardani, K., Yousefi, M. & Ehsani, A. (2017). Identification and molecular characterisation of lactobacilli isolated from traditional Koopeh cheese. *International Journal of Dairy Technology*, *70*(4), 556-561.

[17] Ahmadi, S., Khomiri, M., Khosroushahi, A. & Kashaninezhad, M. (2009). Isolation and identification of lactic acid bacteria (LAB) from Iranian traditional Lighvan cheese. *Journal of Agricultural Sciences and Natural Resources*,16(3),136-146 (In Persian).

[18] Carr, F.J., Chill, D. & Maida, N. (2002). The lactic acid bacteria: a literature survey. *Critical reviews in microbiology*, 28(4), 281-370.

[19] Dubernet, S., Desmasures, N. & Guéguen, M.A. (2002). PCR-based method for identification of lactobacilli at the genus level. *FEMS microbiology letters*, 214(2), 271-275.

[20] Nasiri, M. & Hanifian, S. (2022). *Enterococcus faecalis* and *Enterococcus faecium* in pasteurized milk: Prevalence, genotyping, and characterization of virulence traits. *LWT*,153:112452.

[21] Cousin, F.J., Le Guellec, R., Chuat, V., Dalmasso, M., Laplace, J.M. & Cretenet, M. (2019). Multiplex PCR for rapid identification of major lactic acid bacteria genera in cider and other fermented foods. *International journal of food microbiology*, 291:17-24.

[22] Turková, K., Mavrič, A., Narat, M., Rittich, B., Španová, A., Rogelj, I. & Matijašić, B.B. (2013). Evaluation of Lactobacillus strains for selected probiotic properties. *Folia microbiologica*, 58(4), 261-267.

[23] Wang, H., Yan, Y., Wang, J., Zhang, H. & Qi, W. (2012). Production and characterization of antifungal compounds produced by *Lactobacillus plantarum* IMAU10014. *PloS one*, 7(1), e29452.

[24] Collado, M.C., Surono, I., Meriluoto, J. & Salminen, S. (2007). Indigenous dadih lactic acid bacteria: cell-surface properties and interactions with pathogens. *Journal of food Science*, 72(3), M89-M93.

[25] National Committee for Clinical Laboratory Standards. (2018). Performance standards for antimicrobial disk susceptibility tests, 9th ed. Approved standard M11. National Committee for Clinical Laboratory Standards, Wayne, Pa.

[26] Angmo, K., Kumari, A. & Bhalla, T.C. (2016). Probiotic characterization of lactic acid bacteria isolated from fermented foods and beverage of Ladakh. *LWT-food Science and Technology*, 66, 428-35.

[27] Ljungh, A. & Wadstrom, T. (2006). Lactic acid bacteria as probiotics. *Current issues in intestinal microbiology*, 7(2), 73-90.

[28] Munekata, P.E., Pateiro, M., Tomasevic, I., Domínguez, R., da Silva Barretto, A.C., Santos, E.M. & Lorenzo, J.M. (2022). Functional fermented meat products with probiotics—A review. *Journal of Applied Microbiology*, 133(1), 91-103.

[29] Afshari, A., Hashemi, M., Tavassoli, M., Eraghi, V. & Noori, S.M.A. (2022). Probiotic bacteria from 10 different traditional Iranian cheeses: Isolation, characterization, and investigation of probiotic potential. *Food Science & Nutrition,* 10(6), 2009-2020.

[30] Nami, Y., Haghshenas, B., Bakhshayesh, R.V., Jalaly, H.M., Lotfi, H., Eslami, S. & Hejazi, M.A. (2018). Novel autochthonous lactobacilli with probiotic aptitudes as a main starter culture for probiotic fermented milk. *LWT*, 98, 85-93.

[31] Vidhyasagar, V. & Jeevaratnam, K. (2013). Evaluation of Pediococcus pentosaceus strains isolated from Idly batter for probiotic properties in vitro. *Journal of Functional foods*, 5(1), 235-243.

[32] Maragkoudakis, P.A., Zoumpopoulou, G., Miaris, C., Kalantzopoulos, G., Pot, B. & Tsakalidou, E. (2006). Probiotic potential of

[Downloaded from fsct.modares.ac.ir on 2025-04-14]

[DOI: 10.22034/FSCT.22.158.270]

Lactobacillus strains isolated from dairy products. *International Dairy Journal*, 16(3), 189-199.

[33] Dunne, C., O'Mahony, L., Murphy, L., Thornton, G., Morrissey, D., O'Halloran, S., et al. (2001). In vitro selection criteria for probiotic bacteria of human origin: correlation with in vivo findings. *The American journal of clinical nutrition*, 73(2), 386s-392s.

[34] Haghshenas, B., Nami, Y., Almasi, A., Abdullah, N., Radiah, D., Rosli, R., et al. (2017). Isolation and characterization of probiotics from dairies. *Iranian journal of microbiology*, 9(4), 234-243.

[35] Mulaw, G., Sisay Tessema, T., Muleta, D. & Tesfaye, A. (2019). In vitro evaluation of probiotic properties of lactic acid bacteria isolated from some traditionally fermented Ethiopian food products. *International journal of microbiology*, 2019, 7179514.

[36] Nigatu, J.M., Tuji, F.A. & Tefera, A.T. (2015). Evaluation of the antagonistic effect of six mixed cultures of lactic acid bacteria, isolated from the Ethiopian fermented milk ergo, against some foodborne pathogens inoculated into the Ethiopian cottage cheese ayib. *African Journal of Microbology Researches*, 9(29),1789-1797.

[37] De Vuyst, L. & Leroy, F. (2007). Bacteriocins from lactic acid bacteria: production, purification, and food applications. *Microbial Physiology*, 13(4),194-199.

[38] Sami, A., Khandaghi, J. & Abbasgholizadeh, N. (2022). Evaluation of Safety Aspects and Antagonistic Activity of Enterococcus Strains Isolated from Traditional Pot Cheese. *Journal of Health*, 13(1),7-16 (In Persian).

[39] Sjögren, J., Magnusson, J., Broberg, A., Schnürer, J. & Kenne, L. (2003). Antifungal 3-hydroxy fatty acids from *Lactobacillus plantarum* MiLAB 14. *Applied and environmental microbiology*, 69(12), 7554-7557.

[40] Prema, P., Smila, D., Palavesam, A. & Immanuel, G. (2010). Production and characterization of an antifungal compound (3-phenyllactic acid) produced by *Lactobacillus plantarum* strain. *Food and Bioprocess Technology*, 3, 379-386.

[41] Mahjoory, Y., Mohammadi, R., Hejazi, M.A. & Nami, Y. (2023). Antifungal activity of potential probiotic *Limosilactobacillus fermentum* strains and their role against toxigenic aflatoxin-producing aspergilli. *Scientific Reports*, 13(1), 388.

[42] Del Re, B., Sgorbati, B., Miglioli, M. & Palenzona, D. (2000). Adhesion, autoaggregation and hydrophobicity of 13 strains of *Bifidobacterium longum*. *Letters in applied microbiology*, 31(6), 438-442.

[43] Harbarth, S., Cosgrove, S. & Carmeli, Y. (2002). Effects of antibiotics on nosocomial epidemiology of vancomycin-resistant enterococci. *Antimicrobial agents and chemotherapy*, 46(6),1619-1628.

[44] Frieri, M., Kumar, K. & Boutin, A. (2017). Antibiotic resistance. *Journal of infection and public health*, 10(4), 369-378.

[45] Chouraddi, R., Kumar, S., Kumar, B., Bhatia, M., Varada, V.V., Tyagi, N. & Mallapa, R.H. (2023). Techno-functional characterization of fecal lactobacilli isolates of *Bos indicus* calves for probiotic properties. *Veterinary Research Communications*, *47*(3), 1285-1302.

[Downloaded from fsct.modares.ac.ir on 2025-04-14]

[DOI: 10.22034/FSCT.22.158.270]

مجله علوم و صنایع غذایی ایران

سایت مجله: www.fsct.modares.ac.ir

مقاله علمی_پژوهشی

بررسی پتانسیل پروبیوتیکی باکتری‌های اسید لاکتیک جدا شده از پنیر لیقوان

مهدی حسین‌زاده[1]، صمد وجدی حکم آباد[2] و جلیل خندقی[3و4]*

۱. دانش‌آموخته کارشناسی ارشد میکروبیولوژی، واحد سراب، دانشگاه آزاد اسلامی ، سراب، ایران.

۲. استادیار گروه علوم دامی، واحد سراب، دانشگاه آزاد اسلامی، سراب، ایران.

۳. استادیار گروه علوم و صنایع غذایی، واحد سراب، دانشگاه آزاد اسلامی، سراب، ایران.

۴. گروه بیوتکنولوژی مواد غذایی، مرکز تحقیقات بیوتکنولوژی، واحد تبریز، دانشگاه آزاد اسلامی، تبریز، ایران.

اطلاعات مقاله

تاریخ های مقاله :

تاریخ دریافت:۱٤۰۳/۳/۳

تاریخ پذیرش: ۱٤۰۳/۹/٤

کلمات کلیدی:

پروبیوتیک،

پنیر لیقوان،

لاکتوباسیلوس،

انتروکوکوس

DOI:10.22034/FSCT.22.158.270.

* مسئول مکاتبات:

khandaghi@iausa.ac.ir

چکیده

عامل اصلی حضور میکرفلور خاص مانند باکتری‌های لاکتیک اسید در محصولات لبنی تهیه شده از شیر خام مانند پنیر لیقوان، روش‌های سنتی آماده‌سازی این محصولات است. تعداد زیادی از این باکتری‌ها میکروارگانیسم‌های پروبیوتیک بوده و بعنوان مثال گونه‌های بسیاری از باکتری‌های لاکتوباسیلوس، انتروکوکوس و پدیوکوکوس که در غذاها یافت شده‌اند، ویژگی پروبیوتیکی نشان داده‌اند. در تحقیق حاضر پس از جداسازی فنوتیپی باکتری‌های لاکتیک اسید از ۲۵ نمونه پنیر سنتی لیقوان، جدایه‌ها با استفاده آزمون PCR اختصاصی جنس‌های لاکتوباسیلوس، انتروکوکوس و پدیوکوکوس تایید شدند. سپس ویژگی‌های پروبیوتیکی شامل مقاومت به شرایط شبیه‌سازی شده دستگاه گوارش انسان، خواص ضدمیکروبی، خاصیت خودتجمعی و تجمع مشترک، مقاومت آنتی‌بیوتیکی و قدرت همولیزکنندگی سویه‌های منتخب مورد ارزیابی قرار گرفت. بر اساس آزمون‌های تشخیص بیوشیمیایی و مولکولی، از بین ٨٤ سویه‌ی منتخب، تعداد ٥٨ جدایه به‌عنوان لاکتوباسیلوس (۲۹ جدایه)، انتروکوکوس (۱۸ جدایه) و پدیوکوکوس (۱۱ جدایه) شناسایی شدند. غربال‌گری این جدایه‌ها برای زنده‌مانی در شرایط شبیه‌سازی شده دستگاه گوارش انسان نشان داد که ٢٦/١ درصد جدایه‌ها (١٤ جدایه) مقاومت بالای ٥٠ درصد داشتند. همچنین، تعداد نه جدایه از سویه‌های منتخب اثر ضدمیکروبی قوی روی *لیستریا مونوسایتوجنز* و *استافیلوکوکوس ارئوس* و همچنین کپک‌های *آسپرژیلوس فلاووس* و *پنی‌سیلیوم سیترینوم* نشان دادند. به‌علاوه، بر اساس قابلیت خودتجمعی و تجمع مشترک، تعداد چهار جدایه‌ی LS106، LS71، LS33 و LS6 (متعلق به دو جنس لاکتوباسیلوس و انتروکوکوس) انتخاب شدند. در نهایت، بررسی مقاومت آنتی‌بیوتیکی حاکی از حساسیت این جدایه‌ها به اکثر آنتی‌بیوتیک‌های به‌کار رفته بود بطوریکه تمام جدایه‌ها نسبت به تتراسایکلین و آمپی‌سیلین حساس بودند. همچنین خاصیت همولیتیکی در هیچ‌یک از این چهار جدایه مشاهده نشد. بنابراین، این جدایه‌ها به‌عنوان گزینه‌های مناسب برای استفاده در محصولات غذایی تخمیری و بهره‌گیری از خواص سودمند آن‌ها معرفی می‌شوند.

[Downloaded from fsct.modares.ac.ir on 2025-04-14]

[DOI: 10.22034/FSCT.22.158.270]