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Technological Assessment of Autochthonous Lactic Acid Bacteria and their Antibacterial Activities Against Foodborne Pathogens in Goat Milk Lactic Cheese

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Abstract

Background and Objective: Lactic cheese is a highly consumed dairy product that is of great nutritional values. However, great concerns are reported regarding their safety, microbial quality and short shelf life. Addition of antimicrobial compound-producing lactic acid bacteria as protective cultures is known to improve safety and ensure quality of food products. The current study was carried out to assess antibacterial and several technological characteristics of autochthonous Lactic acid bacterial isolates for their use in goat milk lactic cheese to control *Staphylococcus aureus* and *Listeria monocytogenes.*

Material and Methods: Antibacterial and other technological characteristics, including proteolytic activity, diacetyl production, autolytic activity and survival, in various NaCl concentrations and at suboptimal temperatures of several Lactic acid bacteriasolates were assessed. The potent isolates were identified to species level by phenotypic and genotypic methods (16 srRNA gene sequencing) and finally they were assessed for their ability to inhibit *Staphylococcus aureus* and *Listeria monocytogenes* in prepared goat milk lactic cheese.

Results and Conclusion: Lactic acid bacterial isolates, showing significant antibacterial action, proteolytic activity and diacetyl production, were identified as *Lactiplantibacillus plantarum* RTCC 1290-1, *Lactobacillus acidophilus* RTCC 1299 and *Lacticaseibacillus casei* RTCC 1296-1. These isolates survived at 25 and 45 ºC, tolerated up to 8% NaCl and 55 ºC for 10 min ($p \leq 0.01$). *Lactobacillus acidophilus* demonstrated the highest autolytic activity (38.69%) followed by *Lacticaseibacillus casei* (28.44%) and *Lactiplantibacillus plantarum* (18.38%), respectively. Cocultures of the selected lactic acid bacterial isolates in goat milk lactic cheese resulted in a 5 log CFU g^{-1} decrease in *Listeria monocytogenes*, while, *Staphylococcus aureus* counts decreased by only 3 Log CFU g^{-1} ($p \leq 0.05$) during a month of storage at room temperatures. However, lactic acid bacterial counts increased 2–4 Log CFU g^{-1} from Day 15, which was reported stable up to Day 30. In conclusions, *Lacticaseibacillus casei*, *Lactiplantibacillus plantarum* and *Lactobacillus acidophilus* bears promising biopreservative effect for the control of foodborne pathogens.

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1. Introduction

Lactic cheese is prepared by coagulation of milk using edible acids and the obtainedsoft coagulated curd-like cheese is ready for consumption without the need of long ripening period [1]. To avoid bacterial contaminants, usually a 7-12% brine solution is added to control the growth of contaminants and prolong the shelf-life of lactic cheeses. However, inappropriate storage without refrigeration can easily contaminate the prepared lactic cheese by bacterial food contaminants such as *Listeria* (*L.*) *monocytogenes* and *Staphylococcus* (*S.*) *aureus* [2]. *L. monocytogenes*, a Gram-positive pathogen is considered the major contaminant of dairy and meat products and the agent of listeriosis outbreaks worldwide [3]. Similarly, *S. aureus*, a Gram-positive opportunistic pathogen, is an important contaminant in meat and dairy products. Some Species of *S. aureus* produce staphylococcal enterotoxins in foods and are responsible for staphylococcal food poisoning outbreaks [4].

Lactic acid bacteria (LAB), including several species of *Lactococcus*, *Lactobacillus*, *Enterococcus*, *Streptococcus* and *Leuconostoc*, are well studied for their antimicrobial potential especially in food matrices, where they are able to control the growth of undesirable pathogens and often considered as natural biopreservative agents [5,6]. Lactobacill species are the dominant group of LAB and well-known for their beneficial health effects [7]; for which, they are often termed as probiotics. These bacteria have been used for decades in production of fermented food products and are well studied as targeted interventions for the control of foodborne pathogens such as *L. monocytogenes* and *S. aureus* [2,8].

Antimicrobial activity of LAB species is considered as a vital function that allows them to prevent overgrowth of pathogens and control infections in humans and animals, being safe alternatives to antibiotics [9]. Antimicrobial activities demonstrated by this group of bacteria are linked to their abilities to produce various bioactive metabolites such as organic acids, aldehydes, alcohols, esters, peptides, amino acids and short chain fatty acids [10]. In addition to these metabolites, the protein-like inhibitory substance produced by a majority of LAB species referred to as bacteriocins or bacteriocin-like inhibitory substances are suggested to be responsible for their antimicrobial potentials [11,12]. These bioactive metabolites not only include roles in development of taste, aroma and texture in fermented food products, but also are most importantly responsible for preventing and inhibiting growth of undesirable bacterial contaminants, leading to extended shelf-life of foods [13]. Overall, LAB species are often used as adjunct cultures during food manufacturing processes owing to their safety, functional and technological characteristics, where they confer desirable effects on the functional and sensory attributes of the food matrices, enhance the shelf life and help maintain the host health [5,12].

Traditionally made Iranian goat milk lactic cheese is a highly nutritious dairy product, popular within the locals owing to its distinct taste, odor and soft spongy texture. Since this type of cheese is prone to microbial contaminants, hence concerns exists regarding its safety and prolong storage time. Based on our knowledge, this is the first report that addresses roles of indigenous LAB isolates in control of two most important dairy pathogens in traditionally-made goat milk lactic cheese during storage at room temperature (RT).

2. Materials and Methods

2.1. Culture media and growth conditions

The LAB isolates used in this study were previously isolated from various sources (Table 1). These isolates were cultured anaerobically in De Man Rogosa and Sharpe (MRS) media (Merck, Germany) at 37 ºC for 24 h. The *S. aureus* ATCC 25923 and *L. monocytogenes* ATCC 7466 were cultured in brain heart infusion (BHI) media (Oxoid, UK) at 37 ºC for 24 h in aerobic condition. For detection and enumeration of the respective pathogens in cheese samples, Baird-Parker agar (HiMedia, India) and polymyxin acriflavin lithium-chloride ceftazidime esculin mannitol (PALCAM) *Listeria* agar (BD, Germany) were used. All the bacterial isolates were stored at -80 ºC in BHI broth with 25% v v^{-1} sterile glycerol (Merck, Germany) for long-term preservation.

2.2. Antimicrobial activity against *Listeria monocytogenes* and Staphylococcus aureus

In this study, all the LAB isolates were assessed for their inhibitory activities against *S. aureus* and *L. monocytogenes* using agar well diffusion method [14]. In brief, 100 µl of the overnight cultured LAB (1×10^8 CFU ml^{-1}) and/or their filter sterilized supernatant fluid (achieved by centrifugation of overnight cultures at 6000 rpm for 15 min and filtration of the collected supernatant using 0.45-µm Millipore membranes) were added into 4-mm wells in agar plates that were previously overlaid with 5 ml of semisolid (0.7%) agar seeded with the indicator microorganisms (10^6 CFU ml^{-1}). Antimicrobial potentials of the isolates were assessed based on the inhibitory zone diameters (mm) formed around the wells after incubation at 37 ºC for 24 h. Isolates, showing significant antimicrobial actions against the highlighted pathogens, were selected for further investigations.

2.3. Phenotypic and genotypic identifications

Pure LAB colonies cultured on MRS agar plates were assessed for Gram reaction, morphology, catalase reaction, spore forming ability and hemolysis on sheep blood agar plates.

Table 1. Antagonistic actions, proteolytic activity and Diacetyl production by LAB isolates collected from different sources

No	LAB Isolates	Isolation source	Antibacterial activity (mm)		Proteolytic activity (mm)	Diacetyl production
			S. aureus	*L. monocytogenes*		
1	TA030	Infant feces	17.7 ± 2.3	0	27 ± 0.02[a]	Weak
2	TA070	yoghurt	19.0 ± 1.7	17.7 ± 0.9	21 ± 0.12[b]	Weak
3	TA081	Infant feces	20.0 ± 1.9	19.3 ± 1.2	28 ± 0.08[a]	Weak
4	TA078	Ewe milk	15.0 ± 2.2	15.0 ± 1.7	18 ± 0.0[c]	Medium
5	TA042	Traditional cheese	30.0 ± 1.0	27.3 ± 1.3	29 ± 0.06[a]	Strong
6	TA0209	Traditional yogurt	14.3 ± 1.3	0	26 ± 0.09[ad]	Weak
7	TA0181	Lactic cheese	13.0 ± 2.5	12.0 ± 3.8	25 ± 0.08[d]	Strong
8	TA0185	Traditional yogurt	17.3 ± 1.6	16.4 ± 2.7	28 ± 0.02[a]	Weak
9	TA068	Traditional yogurt	18.0 ± 2.0	0	24 ± 0.14[d]	Weak
10	TA067	Traditional yogurt	19.7 ± 1.3	18.7 ± 1.1	23 ± 0.11[d]	weak
11	TA0179	Breast milk	16.7 ± 2.2	14.3 ± 1.8	27 ± 0.05[a]	Weak
12	TA0142	Poultry intestine	18.7 ± 0.7	0	27 ± 0.02[a]	Medium
13	TA0144	Poultry intestine	27.7 ± 1.4	26.5 ± 1.3	29 ± 0.04[a]	Strong
14	TA0183	Traditional yogurt	0	15.0 ± 3.2	18 ± 0.09[c]	Weak
15	NBT188	Ewe milk	28.3 ± 1.3	25.7 ± 0.8	28 ± 0.15[a]	Strong
16	NBT179	Traditional yogurt	13.3 ± 2.5	13.7 ± 1.7	27 ± 0.04[a]	Medium
17	TA021	Cow milk	17.7 ± 1.4	0	24 ± 0.01[d]	Strong
18	NBT190	Traditional yogurt	0	12.2 ± 2.4	25 ± 0.07[d]	Medium
19	NBT189	Traditional yogurt	16.3 ± 2.6	12.2 ± 1.1	20 ± 0.05[b]	Weak
20	ZA007	Honeybee	17.0 ± 1.2	16.1 ± 1.7	19 ± 0.12[bc]	Weak

Results are mean values of triplicate determinations ± sd. Mean values followed by different uppercase letters in each row shows significant differences among the isolates.
Proteolytic activity was determined by measuring halo zone diameters (mm) appearing around the colonies. Antagonistic activity was determined measuring zone diameters (mm) around the wells. Diacetyl production was recorded as weak, medium and strong based on the intensity of red color ring. *S. aureus*= *Staphylococcus aureus, L. monocytogenes*= *Listeria monocytogenes*

Pure non-hemolytic colonies appearing Gram positive, non-spore forming and catalase negative were selected for further analyses. Selected LAB were identified genotypically using 16S rRNA sequencing. A pair of universal primers, including 27F (5′-AGAGTTTGATCMTGGCTCAG-3′) and 1492R (5'-CGGTTACCTTGTTACGACTT-3'), were used as previously described [15]. Amplified DNA bands, corresponding to 1500 bp, were purified using QIAquick PCR purification kit (QIAGEN, Maryland USA) according to the manufacturer's instruction and sequenced commercially (Macrogen, South Korea). Sequence similarity searches were carried out using GenBank data library and BLAST online tool (https://blast.ncbilm.nih.gov/BLAST .cgi). Respective isolates were identified as *Lacticaseibacillus casei* TA0144, *Lactiplantibacillus plantarum* TA0042 and *Lactobacillus* (*L.*) *acidophilus* NBT188 based on ≥ 98% similarity with the 16S rRNA sequences of other similar species in GenBank database. Nucleotide sequences for the strains TA0144, TA0042 and NBT188 were deposited in GenBank under accession nos of KY550668, KY550664 and MN888745.1, respectively.

2.4. Technological assessments

The selected LAB isolates were analyzed for their proteolytic activity and diacetyl production using previously described methods [12]. For assessing the microbial proteolytic activity, respective bacteria were freshly cultured on skim milk agar and anaerobically incubated for 24 h. Later, the plates were observed for transparent halo areas around the bacterial colonies. Diameters of the halos were recorded in mm. Diacetyl production was assessed as described by Agostini et al. [12]. In brief, 1% (v v^{-1}) of the fresh LAB cultures were inoculated in 10 ml of sterilized whole milk. Following 24 h of incubation at 30 °C, 1 ml of the suspension was added to a tube containing 0.5 ml of α-naphthol (1% w v^{-1}) and KOH (16% w v^{-1}). After incubating the tube at 30 °C for 20 min, it was inspected for diacetyl generation indicated by occurrence of a red ring on the top of the tube. Results were reported as weak, medium or strong, based on the color intensity and depth of the ring. Growth of the selected LAB isolates at suboptimal temperatures (4, 25 and 45 ºC) and in presence of various NaCl concentrations (0, 2, 4 and 8% w v^{-1}) was assessed as described previously [16]. After 24 h of incubation, optical density (OD_{560nm}) was recorded using spectrophotometer and results were represented as relative abundance (%) using the Eq. 1[17]:

Relative abundance (%) = OD_t (test sample)/OD_c (positive control) × 100% Eq.1

Thermal resistance of the isolates at high temperatures of 55 and 65 ºC was assessed using slight modifications in a method described previously [18]. In brief, the cell suspension collected via centrifugation was washed twice and adjusted to 0.5 McFarland index (1.5×10^8 CFU ml^{-1}). The prepared bacterial suspension was incubated for 10 min using water bath adjusted to 55 and 65 ºC, cooled down on ice for 5 min and serially diluted for assessing the viable cell count using standard plate count method. Results were recorded in CFU ml^{-1}. Autolytic activity of the selected

isolates was assessed using a method described previously [19]. The LAB cultures in MRS broth with an OD_{600nm} of approximately 0.7-0.8 were centrifuged at 6000g for 15 min at 4 ℃. Pellets were washed with 20 mM sodium phosphate monobasic buffer (pH 6.8) and resuspended in a similar buffer. After 4 h of incubation at RT, OD_{600nm} was recorded and autolytic activity was assessed using Eq. 2:

Autolytic activity = 100 - (A1/A2) × 100 Eq.2

Where, A_1 and A_2 were the minimum and the maximum OD_{600nm}.

2.5. In situ analysis

2.5.1 Preparation of goat milk lactic cheese

Fifteen liters of raw goat milk samples from a commercial dairy farm in Tehran Province, Iran was used to prepare lactic cheese. Lactic cheese was prepared according to the process described in Fig 1. Prepared lactic cheese was distributed in sterile containers (50 g each) and stored at -20 ℃ until use. The cheese samples were assured to be free of bacterial and mold contaminants based on the Iranian Standard Organization INSO 1011 (data not included). Cheese samples free of contaminants were divided into six treatment groups with five replicates in each group. Selected LAB isolates, including *L. plantarum* RTCC 1290-1, *L. acidophilus* RTCC 1299 and *L. casei* RTCC 1296-1, were inoculated at concentrations of 2.3×10^8 CFU ml^{-1}, while the two pathogens were added at 10^6 CFU ml^{-1} to the milk prior to its coagulation and curd draining. Treatments included cheese samples T1 (only *S. aureus*), T2 (*S. aureus* cocultured with LAB), T3 (*L. monocytogenes*), T4 (LAB cocultured with *L. monocytogenes*) and T5 (only LAB). Cheese samples with no added cultures served as control (T6). All samples were analyzed at variable time intervals during storage at RT (28-30 °C) for 30 days.

2.5.2 Survival of lactic acid bacteria and pathogenic bacteria in cheese samples

Cheese samples from various treatment groups were analyzed for total viable counts of *L. monocytogenes*, *S. aureus* and LAB on Days 0, 1, 7, 15 and 30. Briefly, 25 g of the cheese from respective treatment groups were aseptically collected and homogenized with 225 ml of 2% sterile sodium citrate, making 1/10 dilutions. Further dilutions were prepared by mixing 10 ml of the dilutions with 90 ml of buffered peptone water (Merck, Germany). Then, 1 ml of each dilution was used to enumerate *Lactobacillus* counts using standard plate count and pour plate method. For enumerating *Staphylococcus* and *Listeria* colonies, surface plate method using 0.1 ml of each dilution was used. Respective dilutions were plated on each medium in triplicate. After 48 h of incubation, colonies were enumerated and the average value expressed as Log 10 CFU g^{-1} cheese. Typical gram positive *Staphylococcus* colonies (dark-gray to black shiny convex colonies) on Baird-Parker agar plates with catalase and coagulase-positive reactions were verified [20].

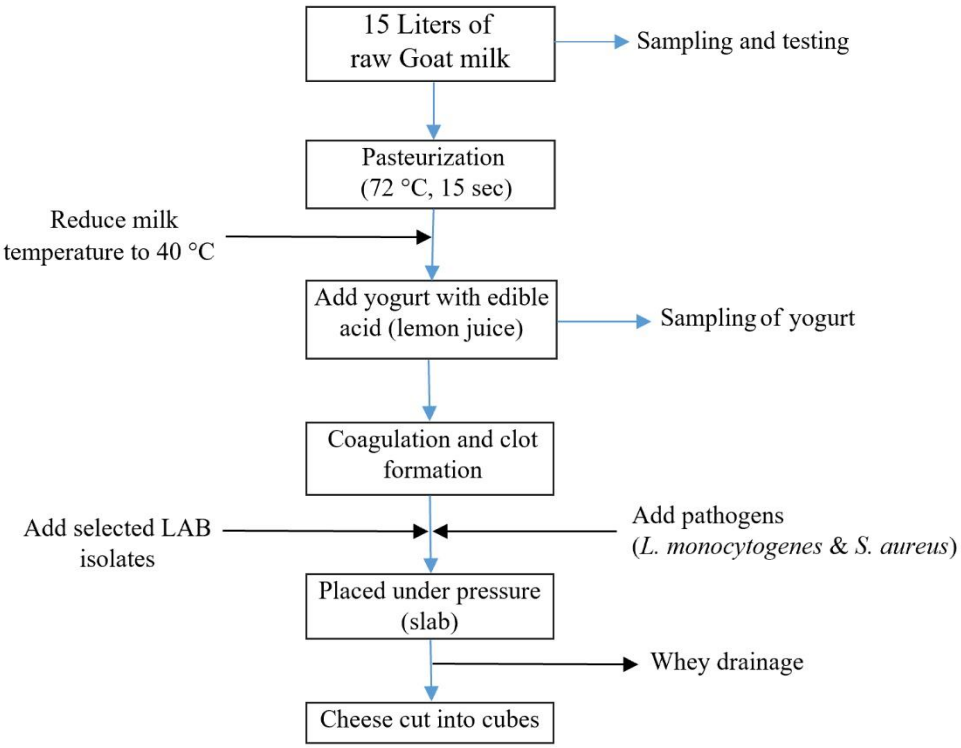

Figure 1. Schematic flow chart showing production of goat milk lactic cheese

Listeria colonies (gray-green colonies surrounded by dark-brown to black halos) on the selective media were further verified as small Gram-positive rods, showing catalase-positive reaction and motile on sulfide indole motility media (HiMedia, India). Typical creamy-white colonies on MRS agar plates with Gram-positive and catalase-negative reactions were tentatively considered as LAB.

2.6. Statistical analysis

All assessments were repeated in triplicate and results were expressed as mean ± SD (standard deviation). Data were statistically analyzed in completely randomized design using SPSS Software v.20 and ANOVA. Means were compared using Tukey's test ($\alpha = 0.05$) and differences were considered statistically significant at $p<0.05$.

3. Results and Discussion

3. Results and Discussion

With technology advancements, high interests have reported on use of safe biological preservatives for extending shelf-life of dairy food products and avoiding growth of bacterial contaminants with no health concerns. Use of LAB and their bioactive metabolites as natural biopreservatives has been addressed as a promising strategy for the control of undesirable microbial population, majorly in dairy products [5]. Enhanced use of LAB as an adjunct culture in dairy products majorly owes to their approved safety and health promoting effects [21].

3.1 Antibacterial activity

In this study, LAB isolates were screened for their antibacterial effects against two most important foodborne pathogens including*L. monocytogenes* and *S. aureus*. As shown in Table 1, several LAB isolates demonstrated antibacterial actions against the tested bacterial pathogens. Similar to these findings, the antagonistic potential of LAB against these pathogens has been documented [22]. The ability of LAB species to produce antimicrobial agents during fermentation processes can improve safety and quality of the food products and most importantly extend the food shelf-life [23]. However, the tested LAB isolates showed significant differences in extent of the antagonistic effects, apparent by the differences observed in the inhibitory zone diameters ($F_{19,\ 97} = 10.633$; $p<0.001$). Levels of the sensitivity of the two pathogens to the LAB isolates differed as well ($F_{1,97} = 16.008$; $p<0.001$). Results indicated enhanced sensitivity of *S. aureus* to the actions of LAB, compared to *L. monocytogenes*. Variations observed in the inhibitory effects of various LAB species against the two pathogens indicated that the inhibitory potential of LAB against the pathogens were strain-specific [24]. Kumar and Kumar [25], reported that various antibacterial activities by LAB could be attributed to different antimicrobial metabolites secreted by the LAB strains. Cotter and Hill [26] reported that active factors responsible for enhanced resistance of several *L. monocytogenes* to LAB metabolites might be due to their inducible cellular resistance against low pH, weak acids and hydrogen peroxide.

3.2. Technological characteristics of the LAB isolates

All LAB isolates were assessed for their proteolytic activity. As seen in Table 1, all isolates were proteolytic, while their extents of activities were non-significantly variable ($F = 1$; $p>0.05$), as apparent by their halo zone diameters. Size of the transparent halos around the bacterial colonies ranged 18-29 mm with the highest proteolytic activity demonstrated by TA0144 strain isolated from poultry intestines and the least proteolytic activity shown by ZA007 strain isolated from honeybees ($p\leq0.05$). Based on the reports, proteolytic activity and diacetyl production are important attributes of isolates especially during industrial processes, where they are known to contribute in flavor and aroma developments [27], playing roles in consistency of the fermented foods [30]. As reported by Duan et al. [28], proteolytic activities of LAB are linked to the formation of peptides and free amino acids during the ripening of cheese, which contributes to flavors and off-flavors. These researchers were able to show that use of proteolytic LAB cultures (*L. plantarum*) as adjunct cultures in cheese resulted in higher contents of water-soluble nitrogen during ripening.

Diacetyl is a flavor compound generated as an end product of the citrate metabolism by LAB species and reported as a strain-specific trait [29]. During studies, the authors detected that only five of the isolates showed strong diacetyl production ability as indicted by the extent of red ring formed in the experimental tubes (Table 1). This characteristic of the isolates might be an interesting technological potency for the production of fermented foods. Studies have shown that LAB isolates, showing significant proteolytic activities and high diacetyl production, are capable of hydrolyzing milk proteins; thereby, increasing food digestibility and contributing to the production of desirable flavors [29].

Selected LAB isolates were screened for their tolerance under various environmental stress conditions to assess their functionality during industrial processing when exposed to condition such as suboptimal temperatures and high-salt concentrations. Significant differences in the growth rates of the three isolates at 25, 37 and 45 ºC was recorded ($p\leq0.05$). As seen in Fig. 2a, the optimal temperature allowing maximum growth of the three isolates was 37 °C (Fig. 2a); however, isolates showed weaker growth rates at 25 °C. Barakat et al. reported that *Lactobacillus* strains isolated from cheese maximally grew at 37 ºC [16]. In this study, *L. plantarum* showed the highest relative abundance percentage (60%) at the saidtemperatures, followed by *L. casei* (≥ 50%) and *L. acidophilus* (≥40%), pectively compared to other tested isolates. However, at a higher temperature (45 °C),

isolates demonstrated the minimum growth rate (20%) with no significant differences between the isolates (p>0.05). Suboptimal temperatures, heat treatments and high salt concentrations are critical factors that might affect the survival of LAB during food processing [30].

Salt tolerance of the LAB isolates is shown in Fig 2b. Overall, isolates tolerated 2, 4 and 6% NaCl with no significant differences (p>0.05). At higher salt concentrations (8%), isolates showed the minimum survival rate (≥15%). NaCl is widely used as additive in food products, where it confers antibacterial effects due to its water reducing activity. Presence of salt can not only enhance preservative and antimicrobial effects but also is responsible for flavor enhancement by modulating enzymatic activity of the enzymes that are involved in organoleptic characteristics [31]. Ability of the isolates to tolerate 1-8% NaCl has previously been reported for several LAB isolates [32].

Thermophilic nature of LAB is considered as a highly desirable characteristic, especially during various cheese manufacturing processes [33]. Figure 2c shows that the LAB isolates possessed reasonable heat tolerance and approximately 7 Log CFU ml^{-1} survival was recorded for the three isolates after 10 min of exposure to 55 ºC (F=1;p>0.05). However, variations were seen in the survival rates between the three isolates at 65 ºC ($p \leq 0.05$). *L. casei* showed 3 log decreases within 10 min of incubation, while *L. acidophilus* showed approximately a 4 log CFU ml^{-1} decrease and *L. plantarum* seemed the least heat tolerant strain, showing more than 6 log CFU ml^{-1} decrease at the saidtemperature. Similarly, other findings have demonstrated that 65 ºC could be lethal to the growth of LAB [34]. Nevertheless, conflicting findings have been reported and Minervini et al. [8] delineated thermophilic nature of probiotic strains, stating that the LAB strains in their study tolerated 65 ºC for 10 min.

In the present study, LAB isolates showed significant autolytic activity (p≤0.05) (Fig. 3) with *L. acidophilus* NBT188 presenting the maximum autolytic activity (38.69%) in contrast to *L. plantarum* TA0042, which included the least autolytic activity (18.38%). Autolytic activity is another desirable technological trait of LAB species that determines sensory and textural characteristics of cheeses [35].

Autolytic LAB species are able to bring enzymatic reactions, leading to the release of lipase and protease enzymes, which subsequently leads to improvement in sensory and textural characteristics of cheeses [19].

3.3. Phenotypic and genotypic identifications of the lactic acid bacterial isolates

The LAB isolates collected from different sources were identified using morphological, biochemical and and 16S rRNA gene sequencing. Gram morphology of the isolates categorized them as cocobacilli (n = 11), rods (n = 5) and cocci (n = 4). Gram-positive isolates that were catalase-negative, non-motile, non-spore forming and non-hemolytic were then identified to species level using 16S rRNA gene sequencing [27]. The PCR amplification of 16S RNA genes of the LAB isolates resulted in a DNA fragment of approximately 1500 bp that were sequenced commercially.

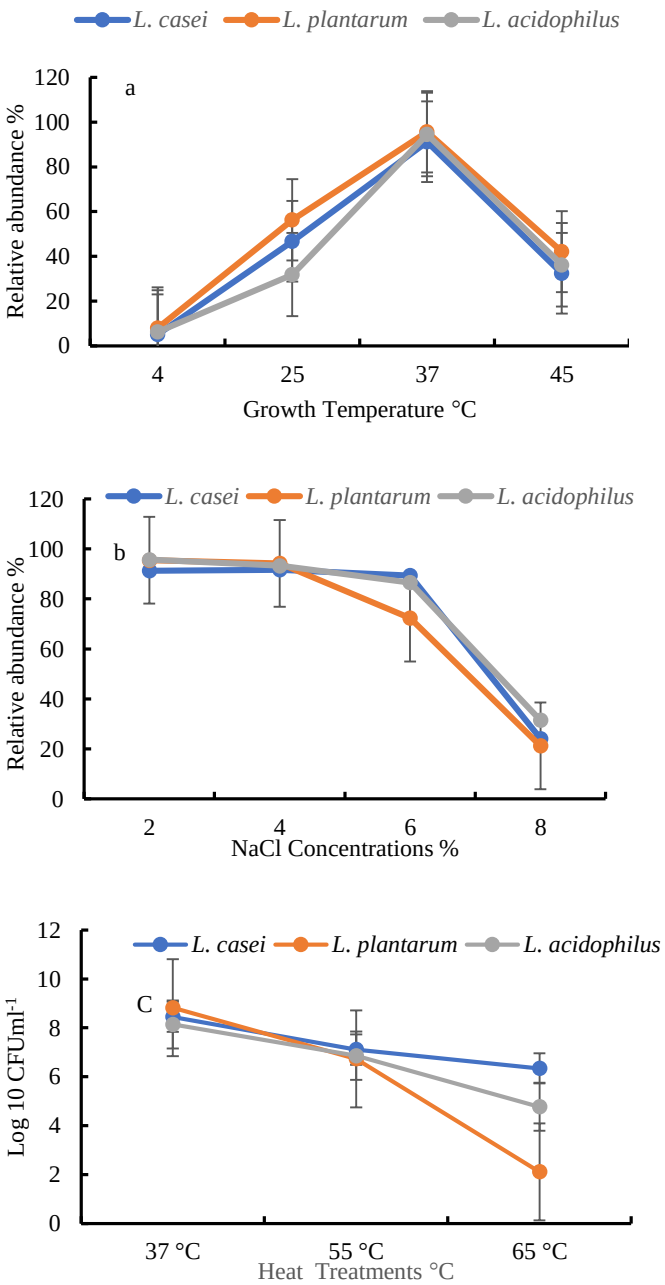

Figure 2. Technological characteristics of LAB isolated from different sources. (a) Temperature tolerance (b) NaCl tolerance (c) heat treatment tolerance. Relative abundance % was determined according to the provided formula. While, heat resistance was determined by recording survival rate in CFU ml^{-1}. Results are mean values of triplicate determinations. Error bars indicate standard deviations.

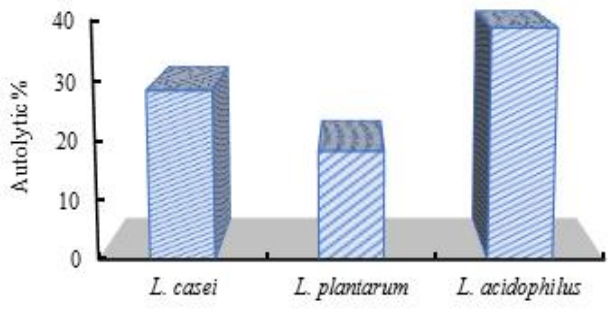

Figure 3. Autolytic percentage of three selected LAB isolates.

The 16S rRNA sequences were edited using BioEdit Software and the consensus sequences were analyzed using BLAST online tool of GenBank. Identified isolates included *L. casei* TA0144, *L. plantarum* TA0042 and *L. acidophilus* NBT188 with similarity indices of ≥ 98% with other related species. Isolates were further deposited in the local cell culture bank of Razi Type Culture Collection (RTCC), Razi Vaccine and Serum Research Institute, Karaj, Iran. Submitted isolates were allocated with RTCC codes of 1296-1, 1290-1 and 1299, respectively.

3.4. Growth pattern of LAB and pathogenic bacteria in cheese samples

The most important criterion for the use of LAB in dairy products includes their viability during processing, storage and temperature. The growth pattern of LAB and the pathogens in lactic cheese samples stored at RT were recorded on Days 1, 7, 15 and 30. As demonstrated in Fig. 4, significant survival and growth of the LAB isolates during the storage were observed (p<0.05). A decrease of 1 log CFU g^{-1} in LAB counts was seen immediately after addition to the cheese samples that might be explained by the fact that the bacteria were drained off with whey during processing. However, increases in total LAB counts were reported in all the treatment groups since Day 15. As cheese hasa solid network, it could better prese-rve the lactic flora during storage [36]. Comparatively, LAB count in the control cheese sample (T5) containing LAB alone (10 Log CFU g^{-1}) was significantly higher than that in treatment groups (T2 and T4), where LAB was cocultured with the pathogens (8 Log CFU g^{-1}) on Day 30. The growth pattern of pathogens in LAB-added lactic cheese samples was compared with that of the control groups at different time intervals (Fig 5).

The growth rates of the pathogens in treatment samples containing LAB cultures were significantly lower than those in the control samples with no added LAB cultures (p<0.05). Immediate decreases in the survival of *L. monocytogenes* were recorded in the LAB added cheese samples on Day 1 and their counts decreased approximately 1 Log CFU g^{-1} cheese in the respective treatment groups. However, this enhanced drastically on Day 30 and approximately 3 and 5 log CFUg^{-1} decreases were recorded for *S. aureus* and *L. monocytogenes*, respectively.

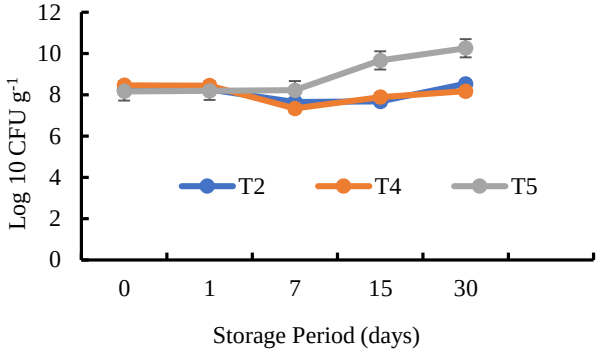

Figure 4. Total viable counts of LAB in three treatments groups during different storage period
T2: LAB co-cultured with *S. aureus*; T4: LAB co-cultured with *L. monocytogenes*; T5: LAB alone without addition of the respective pathogens; Results are mean values of triplicate determinations. Error bars indicate standard deviations

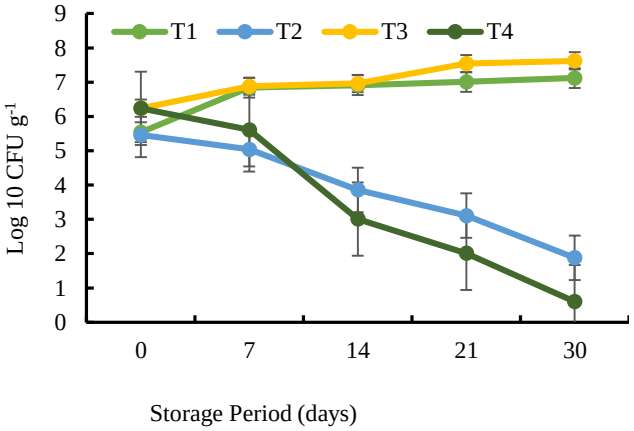

Figure 5. Total viable counts of *S. aureus* and *L. monocytogenes* counts in treatment groups during different storage period. T1: Cheese containing only SA; T2: LAB co-cultured with SA; T3: LM only; T4: LAB co-cultured with LM. Results are mean values of triplicate determinations. Error bars indicate standard deviations

Antibacterial effects of LAB against foodborne pathogens in various types of cheeses have been analyzed previously. In a report, LAB isolated from traditional Italian soft cheeses were capable of decreasing 0.5-1.0 Log unit of *L. monocytogenes* [37,38]. Similarly, Olea-rodriguez et al. showed that *L. monocytogenes* and *S. aureus* counts decreased significantly from Days 15 to 60 of Cotija cheese ripening. While *L. monocytogenes* viability was completely inhibited, *S. aureus* was still present until the end of storage [39]. Similar to these reports, the growth of *L. monocytogenes* was almost negligible (0.2 Log CFU g^{-1} on Day 30) in LAB added cheese samples, compared to *S. aureus* (1.8 Log CFU g^{-1}), in this study. Contrasting results

were reported by Prezzi et al., who reported that addition of *L. rhamnosus* to Mina's frescal cheese resulted in 1.1-1.6 Log CFU g^{-1} decreases of *L. monocytogenes* with no inhibitory effects on *S. aureus* counts [8]. Although, in this study a decrease was observed in *S. aureus* count, but *L. monocytogenes* inhibition was more pronounced and approximately 90% of its viability was lost within 30 days of storage. Most interestingly, increases in pathogen inhibitions with increasing storage were observed. Similar findings have been reported by Adhikari et al., who reported that storage has direct effects on viability of the pathogens [20]. Based on the results, selected LAB isolates seem to have potentials to tolerate cheese manufacturing process, storage temperature and storage time.

4. Conclusion

Lactic cheese is often consumed immediately after preparation and includes a low shelf-life due to microbial contaminants. With increased consumer awareness regarding safer and healthier food products, it seems that indigenous LAB can play significant roles in enhancing safety and nutritious values of this dairy product as well as extending its shelf life. The current study demonstrated antibacterial potentials of indigenous LAB isolates against *S. aureus* and *L. monocytogenes* in goat milk lactic cheese samples. The indigenous LAB isolates seemed potential producers of antimicrobial metabolites and hence could be used as biopreservative agents for the control of spoilage bacteria in various dairy products. It is noteworthy that by merging studies, the authors have characterized the LAB isolates for their probiotic characteristics and assessed the probiotic goat milk lactic cheese for phsicochemical and sensory parameters (data submitted for publishing elsewhere). Results from the present study suggest approp[riateness of the probiotic goat milk lactic cheese as a functional dairy product.

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6. Conflict of Interest

The authors report no conflicts of interest.

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ارزیابی خصوصیات تکنولوژیکی باکتری‌های لاکتیک اسید بومی و اثرات ضد باکتری آن‌ها در برابر بیماریزاهای غذایی در پنیر لاکتیکی تهیه شده با شیر بز

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چکیده

سابقه و هدف: پنیر لاکتیکی فرآورده لبنی پر مصرف با ارزش غذایی بالا است. با این حال، نگرانی‌های زیادی در ارتباط با ایمنی کیفیت میکروبی و زمان ماندگاری کوتاه آن وجود دارد. افزودن باکتری‌های لاکتیک اسید تولیدکننده ترکیبات ضد میکروبی به‌عنوان کشت محافظ در مواد غذایی می‌تواند به بهبود ایمنی و اطمینان از کیفیت مواد غذایی منجر شود. مطالعه حاضر جهت بررسی خصوصیات تکنولوژیکی باکتری‌های لاکتیک اسید بومی برای استفاده در پنیر لاکتیکی تهیه شده با شیر بز و کنترل *استافیلوکوکوس اورئوس* و *لیستریا مونوسایتوژنز* انجام شده است.

مواد و روش ها: خصوصیات ضد میکروبی و تکنولوژیکی از جمله فعالیت پروتئولیتیک، تولید دی استیل، فعالیت اتولیتیک و زنده مانی در دماها و غلظت‌های گوناگون نمک چندین جدایه باکتری‌های لاکتیک اسید مورد ارزیابی قرار گرفت. جدایه‌های قوی با استفاده از روش های فنوتیپی و ژنوتیپی (16 SrRNA) در سطح جنس و گونه شناسایی شدند و سرانجام، اثرات ضد باکتریایی آنها بر علیه *استافیلوکوکوس اورئوس* و *لیستریا مونوسایتوژنز* در پنیر لاکتیکی تهیه شده با شیر بز مورد بررسی قرار گرفت.

یافته‌ها و نتیجه‌گیری: جدایه‌های باکتری‌های لاکتیک اسید که فعالیت ضدمیکروبی و پروتئولیتیکی و تولید دی‌استیل قابل توجهی داشتند به‌عنوان *لاکتی‌پلانتی‌باسیلوس پلانتاروم* RTCC 1290-1، *لاکتوباسیلوس اسیدوفیلوس* RTCC 1299 و *لاکتیک‌اسی‌وباسیلوس کازئی* RTCC 1296-1 شناسایی شدند. جدایه‌هایی که در دمای ۲۵ و ۴۵ درجه سلسیوس زنده ماندند، تا غلظت ۸ درصد NaCl را تحمل و در دمای ۵۵ درجه سلسیوس به مدت ۱۰ دقیقه زنده ماندند ($p\leq0.01$). *ل. اسیدوفیلوس* بیشترین فعالیت اتولیتیک (۳۸/۶۹٪) و پس از آن *ل. کازئی* (۲۸/۴۴٪) و *ل. پلانتاروم* (۱۸/۳۸٪) قرار داشتند. تلقیح مخلوط هر سه باکتری در پنیر لاکتیکی شیر بز منجر به کاهش $\log CFU\ g^{-1}$ ۵ *لیستریا مونوسایتوژنز* بود ولی *استافیلوکوکوس اورئوس* تنها $\log CFU\ g^{-1}$ ۳ کاهش را در مدت یکماه نگهداری نشان داد ($p\leq0.05$). از روز پانزدهم، باکتری لاکتیک اسید افزایش $\log CFU\ g^{-1}$ ۲ تا ۴ داشتند و تا روز سی‌ام پایدار ماندند. می‌توان نتیجه‌گیری کرد که *ل. کازئی*، *ل. پلانتاروم* و *ل. اسیدوفیلوس* اثرات امیدوار کننده‌ای به‌عنوان ماده نگهدارنده زیستی برای کنترل پاتوژن‌های بیماری‌زا دارند.

تعارض منافع: نویسندگان اعلام می‌کنند که هیچ نوع تعارض منافعی مرتبط با انتشار این مقاله ندارند.

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