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(Research Paper)

Cheese Starter Production Using Lactic Acid Bacteria Isolated from Iranian Traditional Cheeses

Zohreh Harsij
Department of Biotechnology, Faculty of Advanced Sciences and Technologies, University of Isfahan, Isfahan, Iran.
zohrehharsij@gmail.com
Asghar Taheri- Kafrani*
Department of Biotechnology, Faculty of Advanced Sciences and Technologies, University of Isfahan, Isfahan, Iran.
a.taheri@ast.ui.ac.ir

Abstract

Introduction: Lactic acid bacteria (LAB), as the main dairy starters, play an essential role in the production of volatile compounds such as proteolytic and lipolytic enzymes involved in cheese processing, suppression of pathogenic microorganisms, formation of curdling tissue, and creation of a fresh acidic flavor.

Materials and Methods: In this study, the LABs were isolated from fourteen samples of traditional cheeses in different part of Iran. The isolates with the highest cheese production capacities were selected and subsequently various factors including pH and temperature were evaluated on their activity. Besides, the viability of strains, which were used to produce traditional cheeses, under microencapsulation and gastric simulation conditions was also investigated. Finally, the best LAB strains (BCC7 and BCC10), with desirable characteristics for cheese production, were molecularly identified by using the 16srDNA test.

Results: This study showed that the survival rate of BCC7 and BCC10 isolates microencapsulated with sodium alginate/chitosan were higher than free cells in the storage condition of -80 °C after six weeks by 23.50% and 20.78%, respectively. Moreover, the quantification of the activity of

* Corresponding Author

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BCC7 and BCC10 strains at a temperature of 4 °C yielded 11×10^7 CFU/ml and 9×10^7 CFU/ml, respectively; exhibiting a greater activity than that observed at a temperature of 28 °C. Furthermore, the preservation of their morphological and biochemical properties was observed.

Discussion and Conclusion: The present study determined the sequences of the most efficient lactic acid strains, and subsequent analyses indicated that the BCC7 and BCC10 strains isolated from native cheese samples, were corresponded to *Enterococcus faecium* strains *SKL-4* and *276-18*, respectively. Therefore, these two strains are good candidate to introduce to the market as high-quality starters for dairy industries.

Key words: Cheese starter, *Enterococcus faecium*, Lactic acid bacteria, Proteolytic activity, Viability

Introduction

Cheese is the most diverse product of the dairy group and almost the most challenging part of academic research. While most dairy products are biologically and biochemically stable, cheese is unstable in this respect. World annual cheese production is about 19×10^6 tons. In general, cheese consumption is constantly increasing [1]. Fox et al. described a similar protocol for producing different types of cheese. Its common steps involve standardization, acidification, dehydration of coagulated milk, curd texture formation, and ripening [2]. Lactic acid production is one of the most essential processes in cheese production. Therefore, adding lactic acid bacteria, to initiate lactic acid production in milk before curdling is very important [3].

Several studies demonstrated that adding a starter does not affect all the physicochemical properties of white cheese; however, it has the most significant effect on the microbiological quality of cheese. By adding starter, the total number of live bacteria *Staphylococcus aureus*, yeast, and mold has decreased. Besides, the shelf life has affected all the properties of the cheese except its fat content. Therefore, adding a starter to the cheese has a more favorable effect on the color, smell, and taste of the cheese [4-5].

Industrial starters are natural and traditional species, while commercial starters are specific and defined species that provide fewer flavors to consumers. Selecting native starters with desirable properties can be the first tool to achieve cheese production with better quality traits [6]. Native lactic acid bacteria from cheese have complex profiles and better sensorial attributes than commercial strains. Traditional cheeses fight pathogens using antipathogenic strains or groups of microbes. The preservation of microbiota richness in individual cheeses, as well as between cheeses throughout processing, is facilitated by utilizing conventional expertise spanning from farming to cheese production [7]. The influence of *enterococci* from Lighvan (an Iranian ultra-filtered (UF) white cheese) has been studied during ripening. Lipolysis rates and flavor were improved by *Enterococcus faecalis* and *Enterococcus faecium* strains. Proteolysis assay showed a higher proteolysis rate in cheese by using these strains vs. control cheeses [8].

All lactic acid bacteria used in cheese industries are Gram-positive, catalase-negative, immobilized, and spore-free starters. They are classified into five groups including *Lactococcus*, *Enterococcus*, *Streptococcus*, *Leuconostocus*, and *Lactobacillus*. Starter cultures are generally divided into mesophilic and thermophilic categories, with the optimum temperature of 30 °C and 42 °C, respectively [2].

Tolerance to acidic environments in lactobacilli depends on the ability to maintain a stable pH gradient between their moderate and cytoplasmic pH. The buffering capacity and pH of the culture medium are important because products such as yogurt, cheese, and milk have pH values of 3.5-4.5 and a high buffering capacity, which increases the pH of the gastrointestinal tract, thereby increasing the survival of probiotic bacteria [9]. LABs are valuable candidates for dairy industries [10]. LABs are vulnerable to various hard conditions during fermentation, subsequently, the viability of lactic acid bacteria is also essential in dairy industries. The encapsulation might be toward developing the survival of lactic acid bacteria including diverse nourishment networks [11]. Microencapsulation is a technology that protects and stabilizes the encapsulated materials, allowing controlled encapsulation of the material until reaching the desired location. Moreover, the release of materials occurs when the capsule is exposed to particular conditions [12-13]. Various methods such as spray drying, spray cooling, fluidized bed coating, coagulation, complex co-deposition, solvent evaporation, in situ polymerization, and intermediates have been used as microencapsulation technologies [14-17].

Many gelling biopolymers such as alginate, guar gum, xanthan gum, locust gum, gellan gum, and carrageenan gum have been studied as capsule constituents for microencapsulation, among which it seems that the alginate and carrageenan coating are protected. Lactic acid bacteria are reliable under adverse conditions such as acidic conditions and bile salts [15, 18-19]. Alginate is a linear heteropolysaccharide derived from a variety of algae. It is principally used as a basis for coating in the microencapsulation method due to its low cost, non-toxicity to the human body, and being beneficial to engineering applications. Many scientists have found that alginate can be effective as a microencapsulation ingredient that protects probiotics in a safe environment against external influences [20-22]. Alginate microencapsulations have the advantage of safety and non-toxicity, and can safely transmit live bacteria to the intestine as a protective transmitter [23-28].

The variety of tastes, diets, and nutritional values in the world leads to the production of many types of cheeses. In this study, an attempt was made to produce a suitable starter by using LABs from traditional Iranian cheeses. By increasing the survival rate of LABs in native Iranian cheeses, long-term storage achievement, and overcoming the high acidity of starters, the production of a high-quality product was obtained. The purpose of this study was to isolate lactic acid bacterial strains from native Iranian cheese. In addition, influential factors such as pH and temperature on the isolated strains were considered. Moreover, to increase the survival rate of starters, lactic acid bacteria were microencapsulated with sodium alginate and chitosan. Finally, molecular identifications of the best strain were performed through 16srDNA amplification and sequencing.

Materials and Methods

Reagents: Culture media were obtained from Ibresco Laboratories (Iran). High-quality reagents of paramount purity were acquired from Merck, Ibresco, and Sinaclon chemical companies. Whey and renin powder were prepared by Iran Dairy Industries Co. (PEGAH). Sequencing of unknown PCR products was carried out by the Niagen Noor Genomic Service Center.

Preparation of starter culture: To prepare 100 ml of starter culture, 5.66 g of whey powder was poured into 100 ml of distilled water, mixed for 10 min, and autoclaved at 110 °C for 10 min. After centrifugation at 4000 rpm for 10 min within another step, 0.5 g of yeast extract and 2 g of

lactose were added to the supernatant. Finally, to re-sterilize the culture medium, an autoclave was performed at 110 °C for another 10 min [29].

Isolation and Identification of Lactic Acid Bacteria: A Sampling was performed from fourteen different types of cheeses native to Mazandaran and Isfahan provinces, Iran. These samples did not have industrial starters and were produced in a completely traditional way. Collected samples were transferred to the laboratory under sterile conditions and stored at 4 °C. The samples (0.1 ml diluted with normal saline solution) were poured on MRS-agar medium and cultured by four quadrant streak method. To create microaerophilic conditions, the plates were placed in desiccators that preserve an environment characterized by a reduced humidity level. The cultured plates were incubated at 37 °C for 24 to 48 hours. In the last step, the isolated colonies were isolated and initially detected by catalase test and Gram staining [30-31]. Finally, the isolated bacteria were conserved in MRS broth incrementally with 30% (w/v) glycerol to freezing. The selected strains were streaked every four weeks.

Growth Curve of LABs Isolated from Native Cheeses: Determining turbidity is very important to plot the growth curve of bacteria in a liquid medium. In this method, changes in bacterial growth over time are easily visible. For this purpose, several colonies were first isolated from MRS-agar culture, transferred to MRS-broth culture, and shaken at 37 °C for 48 hours. Samples were poured into a clean jar, and their absorbance was read at 600 nm in a 10-hour period every two hours until two days.

Measurements of PH Changes by Starter Cultures: To measure the pH changes by LABs, 0.01 g of starter culture and 1 ml of normal saline solution were mixed and added to a 9 ml skim milk culture. The pH of the solution was measured at different times.

Cheese Production from Isolated LAB Strains: High-fat milk was pasteurized at 72 °C for 10 min. Then, 200 ml of raw milk was considered for each starter culture. In the next step, at 35 °C, 1% starter, 0.045% $CaCl_2$, and 0.003% rennin were added to pasteurized milk at 35 °C. Milk was coagulated at 35 °C for 90 min, and curdled milk was formed. The curdled milk was poured into clean textiles for 35 min until their water was completely drained. Molding was then performed, and the cheese texture was completely created after 2 hours. Finally, some salt was added to the cheeses and were kept at 4 °C [32].

The Effect of Temperature on the Survival Conditions of LABs: Purified colony samples were placed at 4 and 28 °C. The number of colonies was counted at 0, 7, 14, 21, and 28 days. The pour plate method was used to evaluate the number of living cells of LABs [33].

Encapsulation of LABs with Sodium Alginate and Chitosan Functionalized Sodium Alginate: Sodium alginate and sodium alginate/chitosan solutions were prepared [34]. The 1:9 ratios of sodium alginate solution and lactic acid bacteria were mixed and stirred to distribute the material evenly. The mixture was then transferred into a syringe and poured into the calcium chloride solution (0.05 M, 50 mL) dropwise at appropriate intervals. The materials were allowed for 40 min to form microcapsules [35]. To prepare chitosan functionalized microcapsules, after preparing sodium alginate capsules, 12 g of sodium alginate microcapsules were mixed with 100 ml of chitosan solution at 0.002 mg/ml. The mixture was stirred gently for 40 min. The chitosan-coated alginate microcapsules were separated and finally washed with a normal sterile saline solution. To evaluate the survival rate of lactic acid bacteria encapsulated into sodium alginate and chitosan functionalized sodium alginate microcapsules, the capsules were first

placed at 4 °C and then poured into tubes and placed in a freezer at -80 °C. The number of colonies was counted by the pour plate method every two weeks until six weeks [36]. The survival of lactic acid bacteria was measured as follows:

Percent of Lactic acid bacterial viability: (number of bacterial colonies at the initial time/number of bacterial colonies at the specified time) ×100

Release of Microencapsulated LABs: To evaluate the survival of microencapsulated LABs, 1 g of the microcapsules were poured into 9 ml of phosphate buffer [37] and then centrifuged at 37 °C at 180 rpm for one hour. Finally, the number of colonies was counted by the pour plate method every two weeks until 6 weeks [38].

Determining the Survival of LABs in Gastric Simulation Fluid Conditions: The gastric simulation fluid was prepared via the Randhira method [39], by adding 300 mg of pepsin enzyme to a sterile saline solution to attain a 0.5 % w/v concentration. This implies that the solution contains 0.5 g of solute per 100 ml. The pH was balanced to 2.0 using 0.1 M HCl. The purified colonies in MRS-agar culture were transferred to MRS-broth culture under sterile conditions to be incubated for 24 to 48 hours at 37 °C. The culture was then centrifuged for 10 min at 2000 rpm and 4 °C. Then, the harvested bacteria were suspended in 200 ml of sterile peptone water at a concentration of 2% w/v. Next, the 1:9 ratios of prepared bacterial samples and gastric simulation fluid were combined and put away at 37 °C. Finally, the survival rate of lactic acid bacteria was evaluated by the pour plate method every two weeks until six weeks.

16SrDNA Amplification, Identification, and Sequencing of LABs: NucleoSpin Microbial DNA kit (Macherey–Nagel, Düren, Germany) was used to extract the genomic DNA of the target LABs strains according to the manufacturer's instructions and utilized as a template for 16S rDNA gene amplification. In the initial stage, some bacterial cells were separated from the MRS culture by supernatant centrifugation. About 40 mg of each sample were weighted for extraction [40]. A total DNA of the LABs strain was used as a template for the amplification of the 16S rDNA gene by PCR with universal primers 27 F **(**5′-AGAGTTTGATCMTGGCTCAG-3′) and 1525R (5′-AAGGAGGTGWTCCARCC-3′). To perform the PCR for the BCC10 sample, 6 µl of DNA, 1 µl of 10 µM forward primer, 1 µl of 10 µM reverse primer, 10 µl of 1X master mix, 0.8 µl of 50 mM $MgCl_2$, and 6.2 µl of distilled water were poured into a microtube. In addition, for the BCC7 sample, the initial concentration of primers is the same as before, with the difference that 1.7 µl of 50 mM $MgCl_2$ and 3.5 µl of distilled water were used to PCR in the microtube. PCR was adjusted for 5 minutes at 95 °C, 30 seconds at 94 °C, 1 minute at 68 °C, 1 minute at 72 °C, and finally 10 minutes at 72 °C. Amplicons were evaluated on 1% (w/v) agarose gel with DNA-safe stain (2 µl/ml) in 1x TAE buffer pH 8.0 (40), for 50 min at 80 V and prepared visible by UV trans-illumination. Volumes of 30 µl of unknown PCR products were carried out by the Niagen Noor Company for sequencing. Samples of lactic acid bacteria were identified through the alignment in GenBank (http://www.ncbi.nlm.nih.gov/BLAST) by using the NCBI BLAST search tool.

Results

Isolation and Identification of LABs: LABs were isolated from fourteen samples of native Iranian cheeses by using MRS-agar culture at 37 °C for 24 to 48 hours. Gram staining and the catalase test were performed on the colonies formed in MRS-agar culture. On samples that were negative for the catalase test, Gram staining was performed to investigate the morphological properties of the isolates. The isolates were cocci forms

of lactic acid bacteria. Seven out of 14 isolates from native cheeses were Gram-positive and catalase-negative. Then, once every two weeks, re-cultures were performed from single colonies to purify LABs, and the isolated strains were stored with glycerol at -80 °C.

The Growth Curves of LAB Samples: To compare the activity of LABs, the growth curves of LABs at an optical density of 600 nm were achieved. Because acid production is directly related to the growth of lactic acid bacteria, it is imperative to assess the growth of LABs. The higher of lactic acid produced, the better the bacterial growth. Fig. 1A and 1B illustrated the growth curves of LABs isolated from the MRS-broth culture by measuring OD_{600} of the samples and the pH of the MRS-broth medium for up to 48 hours. As shown in Fig. 1A, the sigmoid growth curve showed the highest growth rate for the BCC7 isolate and the lowest growth rate for the BCC9 isolate. However, as shown in Fig. 1B, the pH of the media was decreased as time increased due to lactic acid production, and subsequently after 48 hours, the lowest growth rate was related to BCC8 isolate with a pH of 4.9, and the highest growth rate was associated with the BCC7 isolate with pH 4.5. Based on these results, BCC7 isolate was the best candidate for cheese starter.

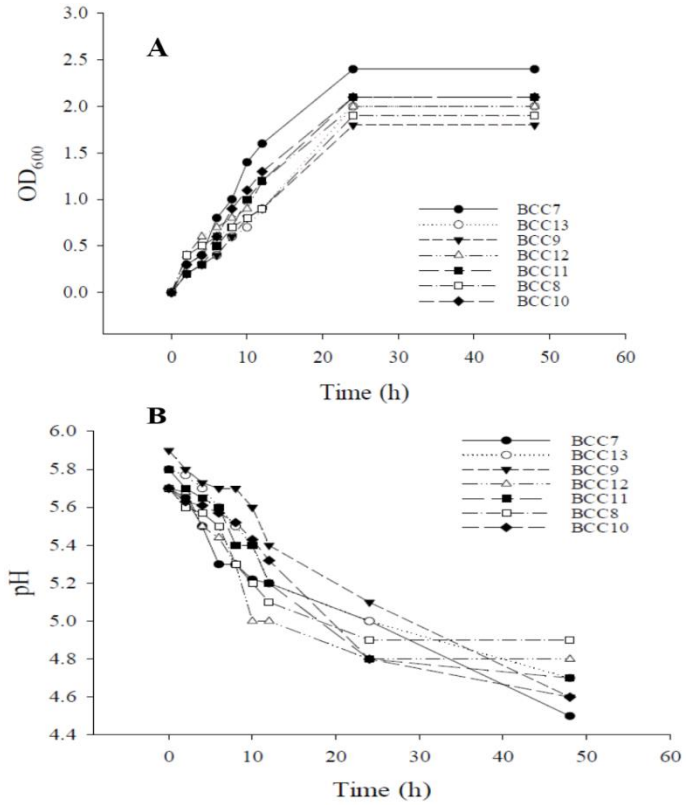

Fig 1- The growth curve of the isolated samples at regular intervals after the start of the incubation time at 37 °C is plotted in terms of (A) light absorption at 600nm and (B) the pH of the culture medium

Cheese Production Using Lactic Acid Starters: Out of fourteen types of isolated bacteria, seven types of LABs successfully produced different kinds of cheese in the laboratory. Since LABs play a significant role in the production of flavor and texture, seven different flavors of white cheese have been produced in this study. The cheeses were uniformly free of cavities or circular hollows. They were also slightly different in texture and seemed semi-hard. Due to the fact that isolates BCC12 and BCC9 were of

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lower quality to measure the survival of lactic acid bacteria in microcapsule conditions, they were simply removed from this study. Finally, five LAB isolates named BCC7, BCC11, BCC10, BCC8, and BCC13 were selected for further studies.

Measurement of Resistance of LABs to Gastric Simulation Conditions: Although the pH of the stomach rises to a higher level depending on the buffer capacity after eating, it generally stabilizes after a while. Therefore, lactic acid bacteria must first survive in the gastrointestinal tract to function properly in the gut [9]. Consequently, bacterial survival, which is one of the most critical factors of starters, was studied. In this regard, the survival of seven LAB strains was studied *in vitro* under gastric simulation conditions. The number of LAB colonies present in acidic gastric conditions at specified time intervals is shown in Table 1. The results demonstrated a significant reduction in the survival of LABs in all isolates after one hour. Counting the number of colonies of the BCC13 isolate with 12×10^9 CFU / ml showed that the isolate had the lowest survival rate while the BCC10 and BCC7 isolates with 22×10^9 CFU / ml, and 17×10^9 CFU / ml, respectively, had the highest survival rates and higher tolerances than the other isolates. This reduction in survival is due to the pepsin enzyme activated under gastric simulation conditions, which destroys the peptide, amino acid bonds, and cell membrane of lactic acid bacteria.

Table 1- Resistance of Lactic Acid Bacteria to Gastric Conditions after 28 days

	Number of colonies (CFU/ml) available on specified storage days				
LABs	0	7	14	21	28
BCC7	28×10^9	26×10^9	26×10^9	24×10^9	22×10^9
BCC13	30×10^9	25×10^9	18×10^9	15×10^9	12×10^9
BCC11	33×10^9	23×10^9	21×10^9	19×10^9	15×10^9
BCC10	31×10^9	27×10^9	22×10^9	21×10^9	20×10^9
BCC8	29×10^9	25×10^9	22×10^9	19×10^9	17×10^9

The Effect of Temperature on the Survival of LABs: The temperature has a significant effect on the growth of lactic acid bacteria. The ripeness and quality of the cheese are strongly influenced by the temperature. The storage conditions at different temperatures may affect the survival of lactic acid bacteria. The results presented in Table 2 demonstrated that the survival rate of lactic acid bacteria at 4 °C was higher than at 28 °C. The highest resistance was related to the BCC7 sample with 11×10^7 CFU/ml at 4 °C and 9×10^7 CFU/ml at 28 °C. However, the lowest resistance was associated with the BCC13 sample at 6×10^7 CFU/ml at 4 °C and 3×10^7 CFU/ml at 28 °C. Therefore, it can be concluded that at lower temperatures, the activity of LABs and their survival rate increase.

Table 2- The Effect of Storage Temperatures of 4 °C and 28 °C on the Survival of Lactic Acid Bacteria after 28 Days

	Number of colonies available on selected storage days (CFU / ml) at 4°C					Number of colonies available on selected storage days (CFU / ml) at 28°C				
LABs	0	7	14	21	28	0	7	14	21	28
BCC7	11×10^7	12×10^7	12×10^7	10×10^7	11×10^7	11×10^7	11×10^7	10×10^7	10×10^7	9×10^7
BCC13	6×10^7	5×10^7	5×10^7	6×10^7	6×10^7	6×10^7	4×10^7	5×10^7	4×10^7	3×10^7
BCC11	8×10^7	8×10^7	8×10^7	7×10^7	8×10^7	8×10^7	8×10^7	7×10^7	6×10^7	6×10^7
BCC10	9×10^7	9×10^7	8×10^7	9×10^7	9×10^7	9×10^7	9×10^7	8×10^7	8×10^7	7×10^7
BCC8	8×10^7	8×10^7	7×10^7	8×10^7	7×10^7	9×10^7	9×10^7	8×10^7	7×10^7	6×10^7

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The Survival of LABs Microencapsulated with Sodium Alginate and Chitosan: The microencapsulation of LABs can increase their survival rate. Microencapsulation knowledge is recognized as one of the foremost valuable ways to increase the constancy and persistence of LABs in cruel ecological conditions and facilitate working with cells with fixed properties. The materials for this purpose need to be accepted, biocompatible, supplement penetrable, and metabolites [16]. After measuring the viability of LABs by the pour plate method, the results demonstrated that the number of free bacterial colonies after six weeks of storage at -80 °C is less than the microcapsule samples. In general, BCC10 and BCC7 isolates showed the best performance, and the BCC11 isolate had the lowest survival rate.

As shown in Figures 2 and 3, the viability of bacteria microencapsulated with sodium alginate/ chitosan is higher than that of bacteria microencapsulated with sodium alginate alone. Compared to sodium alginate/chitosan, the lactic acid bacteria microencapsulated with sodium alginate had higher porosity, so they were less resistant to stresses such as cold and humidity. Chitosan can be used in conjugation with alginate to extend the physical steadiness and devotion of alginate microcapsules in the large intestine. The highest survival rate of microencapsulated bacteria with sodium alginate/chitosan was related to the BCC10 strain and, the lowest survival rate was related to the BCC11 strain. Table 3 revealed that the BCC7 and BCC10 isolates, when microencapsulated with sodium alginate/chitosan, exhibited significantly enhanced survival rates compared to their non-encapsulated counterparts. Specifically, the survival rate of BCC7 and BCC10 isolates microencapsulated with sodium alginate/chitosan was observed to be increased by 23.50% and 20.78%, respectively, comparing their free counterparts when subjected to storage conditions of -80 °C for a duration of six weeks. It can be concluded that the survival of encapsulated cells was higher than free cells. This may be due to the fact that LAB cells enclosed in protective carriers stabilize cellular structures and thus reduce environmental stress by restricting molecular motion.

Figures 4A and 4B show scanning electron microscopy of the best isolates (BCC10 and BCC7 isolates) microencapsulated with sodium alginate chitosan. As shown in these figures, the morphology of microencapsulated bacteria was almost spherical. The outer surface of the beads is covered with a group of tiny cracks and fissures. The chitosan coating tainted the morphology of the microcapsules and altered the external wall of alginate particles. Tiny capsules holding strains have a lot of benefits compared to free strains. They can protect the strains from harsh conditions and make them work more efficiently [12].

Molecular Identification of LABs Using 16srDNA Gene Amplification: Since the two species, BCC7 and BCC10 showed the best performance after several experiments, their gender and species were identified by PCR and sequencing. Identification and alignment of the genus and species of unknown lactic acid bacteria with other existing sequences were made through BLASTN software in the National Center of Biotechnology Information (NCBI). The results indicate that the BCC7 isolate is 99.03% similar to the *Enterococcus faecium* SKL-4 strain, and the BCC10 isolate is 99.89% identical to the *Enterococcus fascium* 276-18 strain.

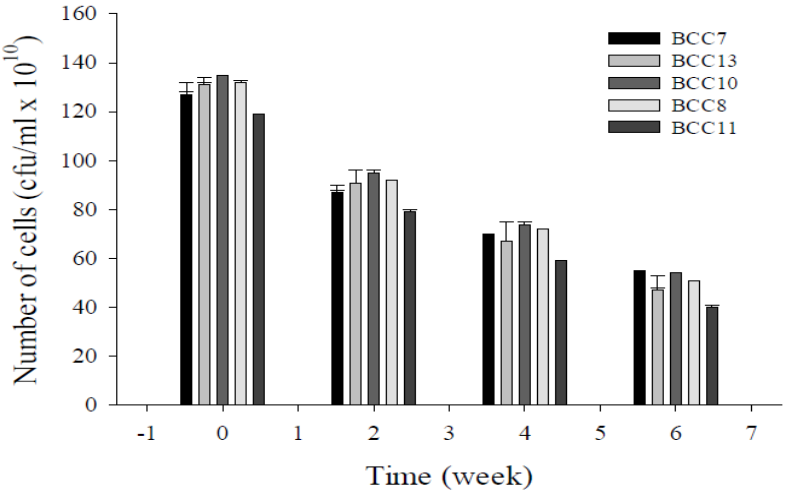

Fig 2-The Survival Rate of Bacteria Trapped with Sodium Alginate, after Storage at -80 °C for Six Weeks

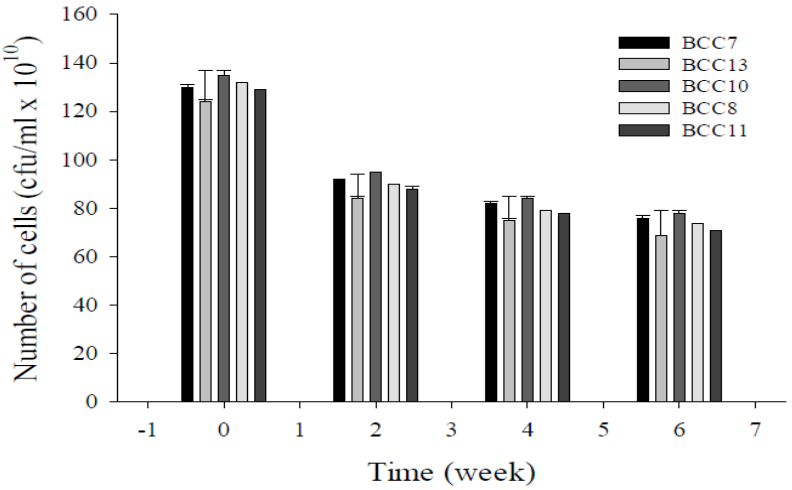

Fig 3- The Survival Rate of Bacteria Trapped with Sodium Alginate Microcapsules with Chitosan Coating after Storage at -80 °C for Six Weeks

Table 3- The Survival Percentage of Free Lactic Acid Bacteria Microencapsulated with Sodium Alginate and Sodium Alginate/Chitosan After Six Weeks of Storage at -80 °C

LABs	%Survival of microencapsulated bacteria with sodium alginate/ chitosan	%Survival of microencapsulated bacteria with sodium alginate	%Survival of free cells
BCC7	58.50%	43.31%	35%
BCC13	55.65%	35.88%	30%
BCC10	57.78%	40%	37%
BCC8	56.06%	38.64%	35%
BCC11	55.04%	33.61%	31%

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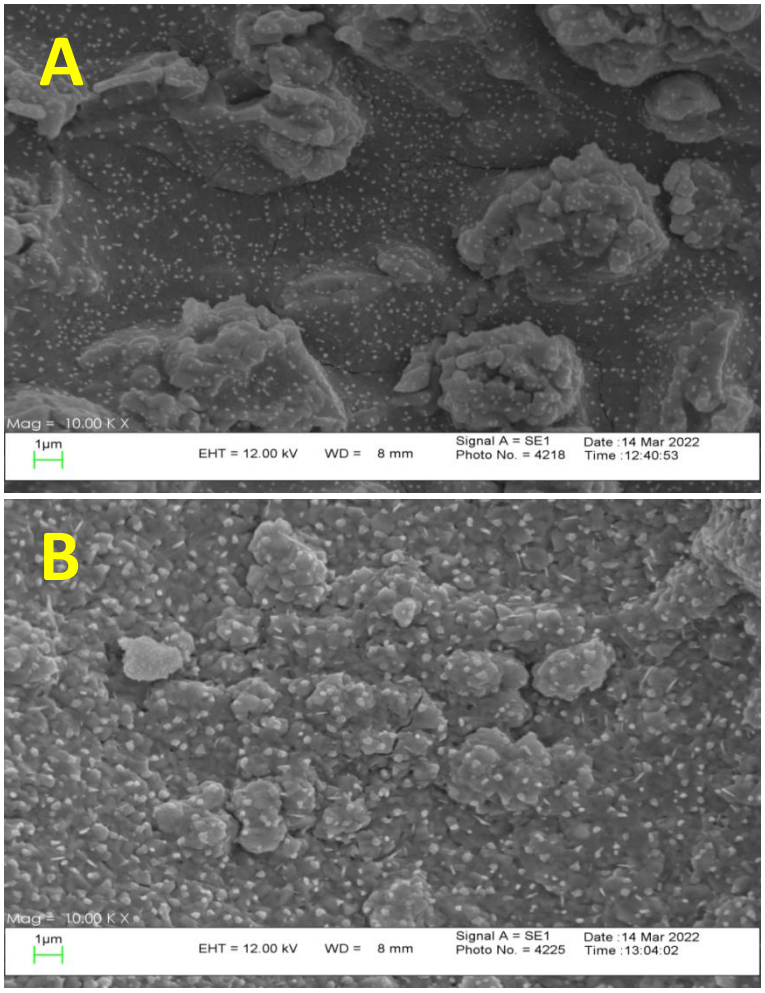

Fig 4- Scanning Electron Microscopy of Sodium Alginate Beads Coated with Chitosan Containing (A) BCC10 and (B) BCC7 isolates

Discussion and Conclusion

The survival of lactic acid bacteria in the gastrointestinal tract is one of the first evaluation features for consumers. Human digestive enzymes are highly complex and contain a mixture of proteases, amylases, and lipases in different isoforms. A study by Faye et al. was done on the survival of 9 strains of lactic acid bacteria under conditions of gastric acidity. *Lactobacillus* and *Enterococcus hirai* strains had much higher acid tolerance than *lactococci*. In the first hour of gastrointestinal simulation, the number of bacteria decreased. Still, during the rest hours, lactic acid bacteria were able to resume their growth, and the number of cells increased [41]. *Lactobacillus bulgaricus kefri 673*, when encapsulated with high molecular weight alginate particles and chitosan coatings, has shown more remarkable survival in similar gastrointestinal conditions and more excellent stability during storage at 4-22 °C. It has also played an essential role in the delivery of lactic acid bacteria to the intestines and their survival during storage in the refrigerator [42-43]. In addition, the morphological and biochemical properties of lactic acid bacteria are better preserved in lower temperatures [44-45]. As this study showed, the survival of lactic acid microencapsulated bacteria with sodium alginate and chitosan is increased. Different studies have proven that encapsulated microorganisms' lives improved at excessive temperatures than uncoated

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bacteria exposed to numerous deadly conditions. In another research, coating alginate microcapsules with chitosan was detailed to lead to an extension of the shelf life of *Lactobacillus plantarum*, which was consistent [36]. Nualkaekul et al. mentioned that alginate-coated chitosan microencapsulation enhanced the survival rate of *Lactobacillus plantarum* by approximately 0.5-1 within the logarithmic phases compared with alginate-coated. Also, coating alginate with chitosan compared to uncoated capsules has increased the shelf life of *Lactobacillus plantarum* by 0-0.5 in the logarithmic phases in the virtual gastric condition [46]. Trabelsi et al. organized that sodium alginate-chitosan coating forms a thick protective membrane that preserves the survival of most bacteria at refrigerator temperatures for 60 days [47]. In formulated products, the viability of probiotics finely coated with chitosan using sodium alginate granules has been improved compared to free cells. To maintain stability in the refrigerator temperatures, their survival has been quadrupled [20]. Kanmani et al. also reported in a study that lactic acid bacteria of *Enterococcus faecium MC13*, encapsulated by alginate with microcytosine coating, have led to more prolonged survival in gastrointestinal conditions. Alginate and chitosan microcapsules are much more stable in gastric conditions. When applied to the intestine, the capsules are broken and the cells released from the capsule are quickly released directly into the intestinal system [48] Studies were done by Krasaekoopt et al. on strains of *Lactobacillus acidophilus* and *Lactobacillus casei* encapsulated in alginate grains and chitosan coatings showed that the survival of encapsulated cells was higher in simulated gastrointestinal conditions than in non-encapsulated cells [49-50].

Production of high-quality cheeses, the most widely consumed dairy product in the world, extensively depends on the starters. The desirable starter cultures have characteristics such as the ability to produce high acidity, create appropriate taste and odor using the desired dose and composition, low proteolytic activity to prevent rapid maturation and bitterness, high antagonistic activity to inhibit pathogens, resistance against antibiotics, phage and specific salt concentration as well as growth at cheese production temperature. The dairy industries must facilitate the addition of agents during cheese processing, especially the addition of lactic acid strains, and focus on their viability. In the present study, the strains from traditional cheeses of Iran were isolated for cheese production. Various properties of LABs such as their acidity and storage stability were investigated. The results confirmed that the microencapsulation of LABs had a responsibility for maintaining and increasing their survival under simulated gastric conditions. The BCC7 and BCC10 LABs strains had higher growth rates and good resistance to the simulated conditions than others. Consequently, it is possible to develop and introduce the new and improved cheese starter to industrial factories; however, wider experiments and the development of new technologies are required to achieve this benefit.

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Declaration of Interest

The authors declare no competing interest.

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