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Isolation and identification of probiotic bacteria from Iranian traditional cheese produced by lamb or kid abomasum microorganisms

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ABSTRACT

Different strains of lactic acid bacteria are the most common micro-organisms known as probiotics. Two major kinds of probiotic bacteria are members of the genera Lactobacillus and Bifidobacterium, that are generally associated with the genus Lactobacillus which play a vital role in the body health, as well as the ability to produce antimicrobials and vitamins in the gastrointestinal tract. The purpose of the study was the isolation of probiotics in traditional cheeses that are produced from the natural flora of the newborn mammalian digestive tract. Bacteria from lamb and kid abomasum are added to milk. The curd is slaughtered from the abomasum of a lamb or kid that is suckling and has now no longer began out to devour forage, after which its belly is dried, that is referred to as curd. In this study, sixteen samples of cheeses local to Markazi province of Iran have been prepared. The isolated bacteria have been examined for morphological, biochemical, probiotic properties, and molecular identity. Out of sixteen traditional cheese samples, five kinds of Lactobacillus have been diagnosed through PCR. *L. casei* strain J026 strain was identified in the traditional cheese samples as the most genera. Using probiotic starters from local sources is a beneficial strategy for producing traditional cheese, which the native strains are more compatible with the humans' intestinal flora and therefore may also higher play their probiotic's characteristic.

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1. Introduction

Today, Lactobacilli play a vital role in the health of the gastrointestinal tract and have also been shown to produce antimicrobials and vitamins. Among probiotic microorganisms, lactic acid bacteria have been recognized as the most important group. Among them, *Lactobacillus genus* has been recognized and introduced as the most common organism in the production of probiotic products (1). Some lactic acid bacteria have also been identified as the natural flora of the gastrointestinal tract, mouth, and urogenital tract in humans and animals (2).

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Among fermented milk products, cheese is more popular and consumed per capita in many countries. Cheese is a product of milk that results from the coagulation of casein by the enzyme renin or similar enzymes in the presence of lactic acid produced by microorganisms (3). Today, in some regions between Asia and Europe, in Tibet, and some parts of Iran, milk is still fermented as in the old testament, and yogurt and cheese are made from it. From throwing the leaves of fig trees into milk to stop milking, humans later learned to obtain cheese from the stomachs of mammals, especially wild rabbits.

Cheese is made from an enzyme in the mammalian digestive tract. Traditional yeast from calves and lambs is added to milk. Cheese from the abomasum of a lamb or a cow that is suckling and has not started to eat fodder, its stomach is dried after slaughter, which is also called curd. To do this, the milk is boiled and cooled, and then to the curd that is added from the abomasum of lamb or lamb, and poured into a bag, and then the hardened milk is turned into cheese, and then fragrant ingredients are added to it. It is salted with alum or compressed salt and placed in the stomach or skin until it is used after 20 days or more. Depending on the type of cheese produced, fattening or adding natural fat to milk is done. For the preparation of all Iranian cheeses to preserve the taste and texture of the cheese, no fat is taken. Local cheese has a mild taste. Its water is completely absorbed but not squeezed, and in the preparation of this type of cheese, solid milk is usually washed after the operation to reduce its pH and the cheese is prepared with a sweet taste.

Cheeses produced by the industrial methods do not have the taste of traditional cheeses and are very weak in terms of some of their sensory properties, which can be related to the pasteurization of milk and the use of specific commercial primers in the manufacture of industrial cheeses (4).

Cheeses traditionally made from raw milk show a wider and more palpable taste range. The characteristics of these cheeses are apparently due to the diversity of local species and species and the native microbial flora of the milk that is used in the preparation of this type of cheese (5). Lactobacillus is the predominant species in cheeses made with raw milk, because these organisms can continue to grow in unfavorable conditions for other bacteria, and with their excellent proteolytic activity, play an essential role in creating the sensory properties of the product. they do. Therefore, knowing the composition of natural lactic flora in traditional cheeses, it is possible to prepare a primer to prepare a healthy and standard product while maintaining the basic characteristics of the product (6).

Markazi province, due to the diversity of the environment and the existence of different ethnicities in it, is witnessing the emergence of a variety of traditional foods, especially dairy products. This study aimed to identify Lactobacillus species isolated from traditional cheeses of Markazi province of Iran, which is the first step in developing a pure primer from the identified species.

2. Materials and Methods

2.1. Sampling Method

First, sixteen kinds of cheeses local to Markazi province, including Ashtian, Saveh, Tafresh, and Farahan, four samples of 200 g every have been prepared under sterile conditions. The cheese samples have been taken as 5 g and added to 45 mL of sterile 2% sodium citrate solution at 45°C after which homogenized for 1 min. The supernatant changed into used as a dilution of 0.1 to put together next dilutions. After 24 h of storage at room temperature, serial dilutions of 1×10^{-2}, 1×10^{-3}, 1×10^{-4}, 1×10^{-5}, and 1×10^{-7} have been organized from the surface liquid and have been collected at a rate of 20 µL and primary enriched in MRS broth medium, after which linearly cultured on pre-prepared MRS agar medium. The plates have been positioned in a CO_2 incubator at 37°C for 72 h (7).

2.2. Biochemical preliminary identity

2.2.1. Gram Stain test

The bacterial colony was first removed by loop and an extension was prepared on the slide. The slides have been then dried at room temperature. After the blades have been dried, the blades have been fixed on the flame after which stained. First, crystal and yolk paint have been poured on the slides for 1 min. The slides have been then washed with distilled water. Next, the color was added to the slides for 1 min. The slides have been washed once more after which, for 10 to 15 s, acetone alcohol was poured on the slides and have been quick washed with distilled water. Finally, the saffron dye was poured on the slides for 1 min and washed with distilled water. After drying the blades, the blades have been tested first with a 40 lens after whihc with a 100 light microscope lens (7).

2.2.2. Catalase test

Bacteria smash down in the event that they have the enzyme catalase. If a bubble forms, the bacteria will be catalase-positive, and if no bubbles shape, catalase will be negative. To carry out this experiment, a single bacterial colony was first removed with a sterile loop and positioned on a slide. Then a drop of hydrogen peroxide was poured on it and the result was observed (7).

2.2.3. Oxidase test

To carry out this test, a 1% solution of tetramethyl paraphenyl diamine dihydrochloride was first poured onto filter paper into a petri dish. Then a colony of the favored bacterium was removed and placed on filter paper and the result was observed (8).

2.3. Secondary identity of bacteria

2.3.1. Fermentation test of sugars

Fermentation of sugars was used to detect Lactobacillus. First, a sugar-based medium was made, using MRS broth compounds, but instead of glucose and meat extract, 2% sugars (arabinose, fructose, lactose, sorbitol, sucrose, galactose, rhamnose, ribose, mannitol) were added. First, the glucose-based medium, that is the MRS of glucose-free broth and meat extract, was prepared and divided into 9 equal parts. After autoclaving them, 2% of every sugar was added to each part by a 0.45 micron filter.

Lactobacilli isolated from cheese have been then inoculated. Results have been evaluated after 24, 48, and 72 h (9).

Fermentation of 9 kinds of sugars was used to identify the genus Lactobacillus. The major function of the carbohydrate fermentation test is to examine the ability of bacteria to ferment exceptional kinds of sugars. The indicator of this phenol test is the red broth.

2.3.2. Growth at different pH

To study the growth of lactobacilli at different pHs, an MRS broth medium was first made. Then, with NaOH and HCl, MRS broth media have been made with pHs of 2, 3, 4, 5, 6, 9, 10. It was then removed from the desired bacterium and inoculated in different media. After 24 h of storage at 37°C, the growth of bacteria at different pHs was observed by turbidimetric approach with a spectrophotometer at 600 nm (10,11).

2.3.3. Resistance to bile salts

To examine the bile tolerance test in lactobacilli isolated from cheese samples, first MRS agar medium containing 0.3% Oxgall was prepared. Oxgall was used as bile on this experiment. After autoclaving the medium, the bacteria have been inoculated and after 24 h of storage at 37°C, bacterial growth was observed. Probiotics should be able to show resistance to bile with inside the gastrointestinal tract. (10,11).

2.3.4. Motion test

The SIM semi-solid medium was used for this experiment. This medium was made according to the manufacturer's instructions and poured into test tubes, then autoclaved.

With a sterile needle ounce, a colony of bacteria was removed and inserted directly into the medium. The effects have been evaluated after 24 h of storage at 37°C (8).

2.3.5. Triple Sugar Iron (TSI) test

The TSI test is primarily based totally at the bacterium's ability to ferment the three sugars lactose, glucose, sucrose, and to supply carbon dioxide and hydrogen sulfide. This medium includes lactose, sucrose, and glucose as 1% of every sugar. It represents a phenol-red medium that turns yellow at acidic pH, bright red at neutral pH, and dark red at alkaline pH (8). This medium was made in keeping with the manufacturer's instructions and poured into test tubes, then autoclaved and positioned diagonally. With a sterile needle ounce, the bacterium was removed and cultured deep and superficially. The effects have been evaluated after storage for 24 h at 37°C. Catalase and oxidase tests were negative for all Lactobacillus. None of the isolated Lactobacillus are motile in the SIM environment. Lactobacillus turn the TSI environment yellow (acidic) after 48 h (8).

2.4. Molecular identity of the genus Lactobacillus

2.4.1. Detection of lactobacilli through molecular PCR

extraction was performed from isolated Lactobacillus isolates obtained by pure culture by PCR. To evaluate and detect the genus of Lactobacillus received in subculture approach with 16S rRNA sequence (12,13).

2.4.2. Preparation of genome extraction

Each of the isolated bacteria was first cultured in Luria broth (LB) medium and kept at 37°C for 18 h. DNA extraction begins when the turbidity of the tubes equals 2 McFarland. The sample was then transferred to 2 mL Eppendorf tubes and centrifuged for 2 min at 13×10^3 rpm at 25°C. The supernatant was then discarded to obtain sufficient sediment from the bacteria. To this bacterial precipitate, 20 µL of lysozyme was added and placed in a shaker at 100 rpm for 5 h (14).

2.4.3. Polymerase chain reaction (PCR)

2.4.3.1. Buffers used for PCR

A- TBE buffer

A 50-fold concentrated solution (50×TBE) was used as a buffer 1×TBE for this study, which was 10 mL of 50×TBE with 500 mL of deionized water.

B- Ethidium solution

One gram of ethidium bromide was dissolved in 100 mL of distilled water. This material was stored away from light.

C- DNA loading buffer

Contains bromophenol blue with acidic tris and glycerol and distilled water (14).

2.4.3.2. Polymerase chain reaction (PCR) requirements

A- DNA template

In this study, the DNA of the purified Lactobacillus genome was used as the template DNA. This molecule acts as a template for the function of the Taq enzyme.

B- Doxy-nucleotide triphosphate (dNTP)

10 mM dNTP was used. The final concentration of each nucleotide in PCR was 50 µM.

C- PCR buffer for DNA Taq polymerase enzyme

This buffer with concentration 10×contains tris acid and potassium chloride.

D- DNA Taq polymerase enzyme

DNA Taq polymerase is a polypeptide with DNA polymerase properties $5' \rightarrow 3'$. The optimum temperature for this enzyme is 70°C and its optimum pH is about 9. Metabion DNA Taq Polymerase was used in this study.

E- Primers

As it is shown in table 1, a universal primer (27F, 1492R) was used to perform the polymerase chain reaction.

Table 1. The primer sequence used to detect Lactobacillus genus

$5' \rightarrow 3'$ Sequence	Primer	Target organism
AGAGTTGATGTGTGGTCAG	27F	Genus Lactobacillus
GGTTACCTTGTTACGACTT	1492 R	

These primers have been used to detect and determine the highly specific region of the 16S rRNA gene (15).

2.4.4. PCR reaction

First, the extracted DNA samples have been taken out of the freezer and melted at room temperature. Then PCR microtubes have been numbered so as samples. For more accurate identity, a negative control was considered that had all of the substances required in a PCR reaction besides the pattern DNA.

The reason of using negative control is to investigate the presence or absence of PCR solution contamination with external DNA. If PCR solutions are not contaminated with foreign DNA, no banding have to beshped on the agarose gel in the negative control sample after PCR. First, a negative control was prepared and the door was immediately closed to prevent possible contamination.

To prevent contamination, prevent the loss of reagents and increase the accuracy of the operation, a PCR mixture was prepared for each sample daily according to the reaction volume of 25 µL per sample. PCR reaction was performed at a final volume of 25 µL (16 , 17). The material in Table 2 was added to the Eppendorf tubes for PCR.

Table 2. Amount required to perform PCR reaction

Mixing PCR materials	Volume (µl)
Water for PCR	18
PCR buffer	2.5
dNTPs	1
Forward Primer	1
Primer Reverse	1
Genomic DNA	1
Taq polymerase enzyme	0.5

After the primers have been added, a vortex was performed and then genomic DNA and Taq polymerase enzyme have been added and vortexed once more and immediately transferred to PCR.

2.4.5. Thermocycler device

The PCR reaction was performed using specific primers in keeping with the program, details of which are given in Table 3. PCR strings have been amplified in a Thermocycler Ferrotec, Japan. It has to be referred that the melting temperature (Tm) for each of the primers was calculated using the following formula and checked in Oligo analysis software program:

$$Tm=4\ (G+C)+2\ (A+T)$$

Table 3. Thermal and temporal characteristics of PCR

Time (seconds)	Temprature (°C)	Reaction steps
180	95	Pre – denaturation
30	95	Denaturation
30	61	Annealing
60	73	Extention
300	73	Final Extention

2.4.6. Preparation of electrophoresis buffer (Tris - Boric acid-EDTA) (10X) TBE

After weighing Tris base: 27.2475 g, Boric acid: 13.9125 g, EDTA: 1.86, g and distilled water was added to this set by 200 mL digital scale (increased to 200 volume) and after which through pH, the pH of the solution was set to 8, at the end of which 50 mL of distilled water was added to the solution (increased to 250 volumes). To prepare this buffer, 50 mL of TBE (10X) buffer was added to 950 mL of distilled water. TBE (10X) buffer was diluted at 1:20 with distilled water.

2.4.7. Preparation of gel electrophoresis

To prepare the agarose gel, first, 1 g of agarose powder was poured into an Erlenmeyer flask, and then a 1X TBE buffer was added to make a volume of 100 mL. Erlenmeyer was heated in the microwave to obtain a clear solution. The Erlenmeyer was cooled to about 40°C and gently poured into an electrophoresis mold containing a comb to solidify the gel. The mold was then placed in an electrophoresis tank containing an electrophoresis buffer and gently removed from the shoulder (18).

2.4.8. Detection of PCR products by electrophoresis

For electrophoresis 3 µL of each PCR product was mixed with 2 µL of loading buffer. PCR products were mixed in a ratio of 5 to 1 loading buffer made by Fermentas Company and transferred to wells. About 50 µL of DNA ladder was poured as a marker in the last well. The electrophoresis conditions have been set at 60 v for 1 h. After about 40 to 50 min, when the dye loading buffer was two-thirds the length of the gel, the power was turned off and the gel was injected into ethidium bromide for 10 to 20 min. After washing with distilled water, it was placed on an ultraviolet (Uvidoc) device (19,20). Then the image observed in the monitor of the device was adjusted and the gel image was prepared and the results have been checked.

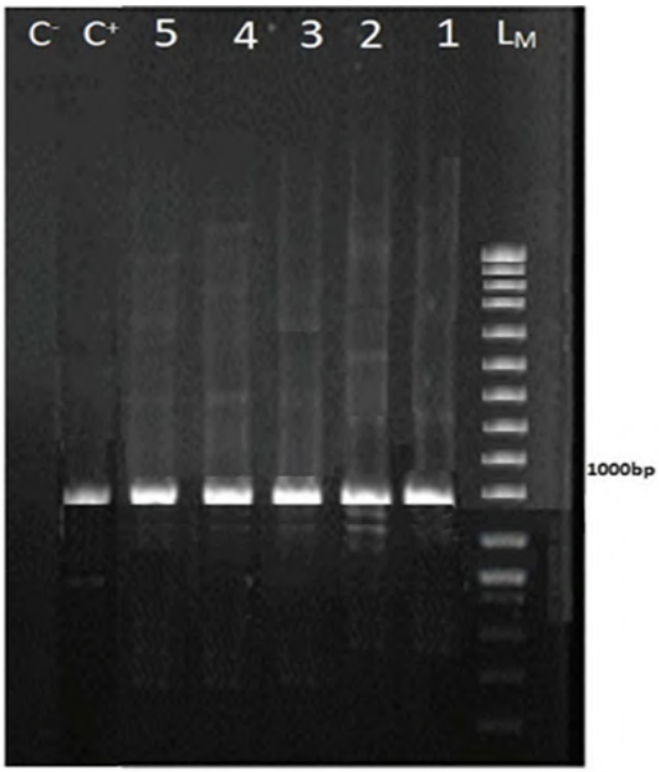

Figure 1. PCR result of samples with Lactobacillus 16S rRNA specific primers

2.4.9. DNA extraction from gel and Clean UP of PCR product for DNA sequencing

To extract DNA from the gel, a DNA extraction kit called (Biospin Gel Extraction Kit) containing Elution Buffer,Wash Buffer, Extraction Buffer, and Spin Column was used. First, a piece containing DNA was cut from agarose gel with a sharp, sterile razor blade and transferred to a 1.5 mL microtube.

Then the volume of this piece was added to the Extraction Buffer. In this study, 600 µL of this buffer was added to the microtube. In the next step, the microtube was placed inside the snake pan at 50°C until the gel was completely melted. At this stage, the solution was transferred to a purification column (Spin Column) and centrifuged for 6 min at 6×10^3 rpm. The bottom solution was then discarded. After discarding the bottom solution, 500 µL of the Extraction Buffer was added to the purification column and centrifuged for 30 s at 12×10^3 rpm. After the centrifugation, the bottom solution was discarded. At this stage, 750 µL of Wash Buffer was added to the purification column, and centrifugation was performed again for 30 s at 12×10^3 rpm, and finally, the bottom solution was discarded. At this stage, an additional centrifuge was performed again for 1 min at 12×10^3 rpm and the purification column was transferred to a sterile 1.5 microtube. After transferring the purification column to a 1.5 mL microtube, 30 µL of Elution Buffer was added to this column and kept at room temperature for 1 min. In the final stage, centrifugation was performed for 1 min at 12×10^3 rpm (The buffer in the microtube contained DNA).

3. Results

3.1. Isolation and preliminary identification of bacteria

From the grown bacteria, slides were prepared and stained by the hot method. Bacteria were observed in the form of elongated and narrow rods, single, double, or multiple, and sometimes chains. The bacteria lacked spores.

These bacteria are narrow and fully elongated during the initial separation, but after a while, they become short and thick (Figure 2). Sometimes, other bacteria were seen along with lactobacilli. To isolate and purify Lactobacillus, the grown bacteria were cultured twice in MRS medium for 72 h and stained by the hot method. This was repeated twice to obtain bacteria.

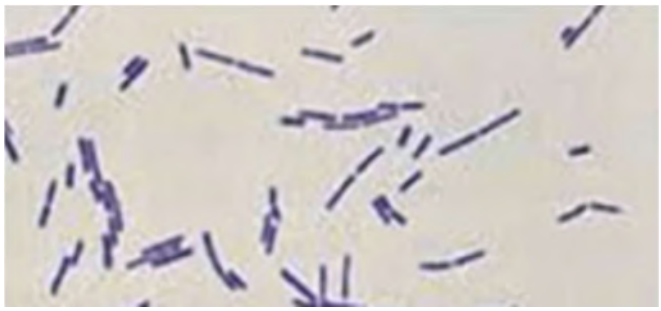

Figure 2. Gram coloring of Lactobacilli isolated from cheese samples

Catalase and oxidase tests were negative for all Lactobacillus. None of the isolated Lactobacillus are motile in the SIM environment. Lactobacilli turn the TSI medium yellow (acidic) after 48 h. The results of preliminary biochemical tests are given in Table 4.

Table 4. Results of biochemical tests for preliminary identification of Lactobacillus

Biochemical tests	I_1	I_2	I_3	I_4	I_5
Gram coloring	+	+	+	+	+
Catalase test	-	-	-	-	-
Oxidase test	-	-	-	-	-
TSI test	+	+	+	+	+
Motion test	-	-	-	-	-

The results of the sugar fermentation test in lactobacilli isolated from cheese are listed in Table 5. The results showed that all isolates were able to ferment lactose (100%), galactose (100%), fructose (100%), ribose (100%), and sucrose (100%), but none fermented rhamnose (0%). Arabinose (81.8%), mannitol (81.8%), and sorbitol (54.5%) were fermented by isolated strains.

Table 5. Sugar fermentation test in Lactobacillus isolated from cheese samples

Isolate / Sugar	I_1	I_2	I_3	I_4	I_5
Lactose	+	+	+	+	+
Sucrose	+	+	+	+	+
Galactose	+	+	+	+	+
Arabinose	+	-	+	+	+
Mannitol	+	-	+	-	+
Sorbitol	+	-	-	+	-
Fructose	+	+	+	+	+
Rhamnose	-	-	-	-	-
Ribose	+	+	+	+	+

The results of Lactobacillus growth at different pHs are shown that the lactobacilli isolated in this study tend to grow at pH = 6 and no growth was seen at pH = 2 and 3, 4, 7, 8, 9, and 10. Growth was seen very slowly. The growth curve of Lactobacillus isolated from strains 11-1 at pH (2-10) is shown in Figure 3.

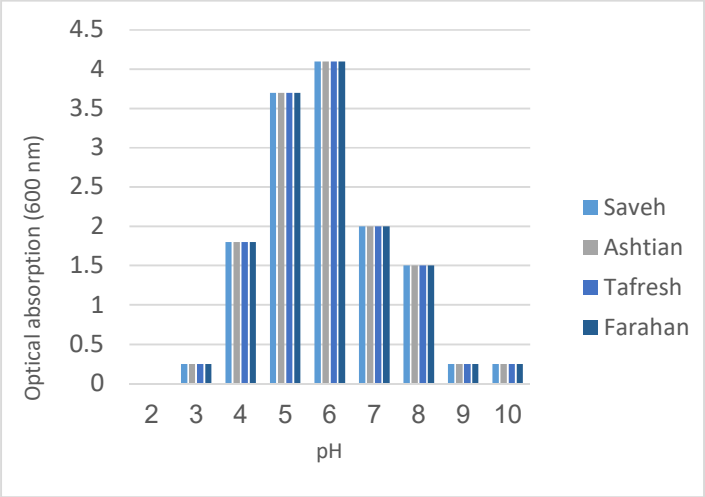

Figure 3. Growth diagram of isolated Lactobacillus at pH (2-10)

The results of the bile tolerance test of the isolated Lactobacillus from different cities impressed that 81.8% of them were resistance to 0.3% of bile salts.

3.2. Nucleotide Blast results

The results of PCR, sequencing at the NCBI site, and using BLAST were the basic tools for searching for local overlaps, and then Nucleotide Blast and with Chromas software the extracted sequences were analyzed in the EzTaxon database. The identified Lactobacillus are shown in Table 6, respectively. Observation of Lactobacillus band using 16S rRNA primer was observed by PCR and gel formation, which formed a band from 5 isolated samples of *Lactobacillus* obtained by pure culture. This means that these samples contain the genus Lactobacillus.

Table 6. Isolated strains of Lactobacillus from traditional cheeses of Markazi province of Iran

Isolated bacteria	Microorganisms with the closest 16S rRNA sequence	Accession No.	Similarity
1	Lactobacillus delbrueckii subsp strain: YIT 0080	AB008207.1	99
2	Lactobacillus fermentum strain SK152	CP016803.1	100
3	Lactobacillus plantarum strain MSD1-4	MH620395.1	100
4	Lactobacillus acidophilus strain LAG8011	CP054559.1	98
5	Lactobacillus casei strain J026	GQ395613.1	100

3.3.Results of 16S rRNA gene sequencing using Lacto primer (27F, 1492R)

Lactobacillus casei strain J026**>** GQ395613.1

TTCCAGAGATTGAGAGTTTGATCCTGGCTCAGGA
TGAACGCTGGCGGCGTGCCTAATACATGCAAGT
CGAACGAGTTCTTGTTGATGATCGGTGCTTGCAC
CAAGATTCAACATGGAACGAGTGGCGGACGGGT
GAGTAACACGTGGGTAACCTGCCCTTAAGTGGG
GGATAACATTTGGAAACAGATGCTAATACCGCA
TAGATCCAAGAACCGCATGGTTCTTGGCTGAAA
GATGGCGTAAGCTATCGCTTTTGGATGGACCCGC
GGCGTATTAGCTAGTTGGTGAGGTAATGGCTCA
CCAAGGCGATGATACGTAGCCGAACTGAGAGGT
TGATCGGCCACATTGGGACTGAGACACGGCCCA
AACTCCTACGGGAGGCAGCAGTAGGGAATCTTC
CACAATGGACGCAAGTCTGATGGAGCAACGCCG
CGTGAGTGAAGAAGGCTTTCGGGTCGTAAAACT
CTGTTGTTGGAGAAGAATGGTCGGCAGAGTAAC
TGTTGTCGGCGTGACGGTATCCAACCAGAAAGC
CACGGCTAACTACGTGCCAGCAGCCGCGGTAAT
ACGTAGGTGGCAAGCGTTATCCGGATTTATTGG
GCGTAAAGCGAGCGCAGGCGGTTTTTTAAGTCT
GATGTGAAAGCCCTCGGCTTAACCGAGGAAGCG
CATCGGAAACTGGGAAACTTGAGTGCAGAAGA
GGACAGTGGAACTCCATGTGTAGCGGTGAAATG
CGTAGATATATGGAAGAACACCAGTGGCGAAG
GCGGCTGTCTGGTCTGTAACTGACGCTGAGGCTC
GAAAGCATGGGTAGCGAACAGGATTAGATACC
CTGGTAGTCCATGCCGTAAACGATGAATGCTAG
GTGTTGGAGGGTTTCCGCCCTTCAGTGCCGCAGC
TAACGCATTAAGCATTCCGCCTGGGGAGTACGA
CCGCAAGGTTGAAACTCAAAGGAATTGACGGG
GGCCCGCACAAGCGGTGGAGCATGTGGTTTAAT
TCGAAGCAACGCGAAGAACCTTACCAGGTCTTG
ACATCTTTTGATCACCTGAGAGATCAGGTTTCCC
CTTCGGGGGCAAAATGACAGGTGGTGCATGGTT
GTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAG
TCCCGCAACGAGCGCAACCCTTATGACTAGTTG
CCAGCATTTAGTTGGGCACTCTAGTAAGACTGCC
GGTGACAAACCGGAGGAAGGTGGGGATGACGT
CAAATCATCATGCCCCTTATGACCTGGGCTACAC
ACGTGCTACAATGGATGGTACAACGAGTTGCGA

GACCGCGAGGTCAAGCTAATCTCTTAAAGCCAT
TCTCAGTTCGGACTGTAGGCTGCAACTCGCCTAC
ACGAAGTCGGAATCGCTAGTAATCGCGGATCAG
CACGCCGCGGTGAATACGTTCCCGGGCCTTGTA
CACACCGCCCGTCACACCATGAGAGTTTGTAAC
ACCCGAAGCCGGTGGCGTAACCCTTTTAGGGAG
CGAGCCGTCTAAGGTGGGACAAATGATTAGGGT
GAAGTCGTAACAAGGTA.

3.4. Drawing a phylogenetic tree by neighbor joining NJ method

In this study, the phylogenetic relationship between strain J026 *L. casei* and sequences recorded in the EZ Taxon database was investigated. After aligning the sequence of the 16S rRNA gene with the sequences of nearby species, the phylogenetic tree was plotted using the Neighboring_joining algorithm, which matched the taxonomic position of the strain in the drawn tree to the similarity of the 16S rRNA. The numbers in parentheses indicate the access number of the sequences in the EZ Txon database. The phylogenetic tree drawn by the neighbor-joining NJ method is shown in Figure 4.

3.5. Identification of the genus Lactobacillus

In this study, a total of 5 lactobacilli isolates were identified from 16 samples of local cheeses from central Farahan, Saveh, Tafresh, and Ashtian using 16S rRNA primer.

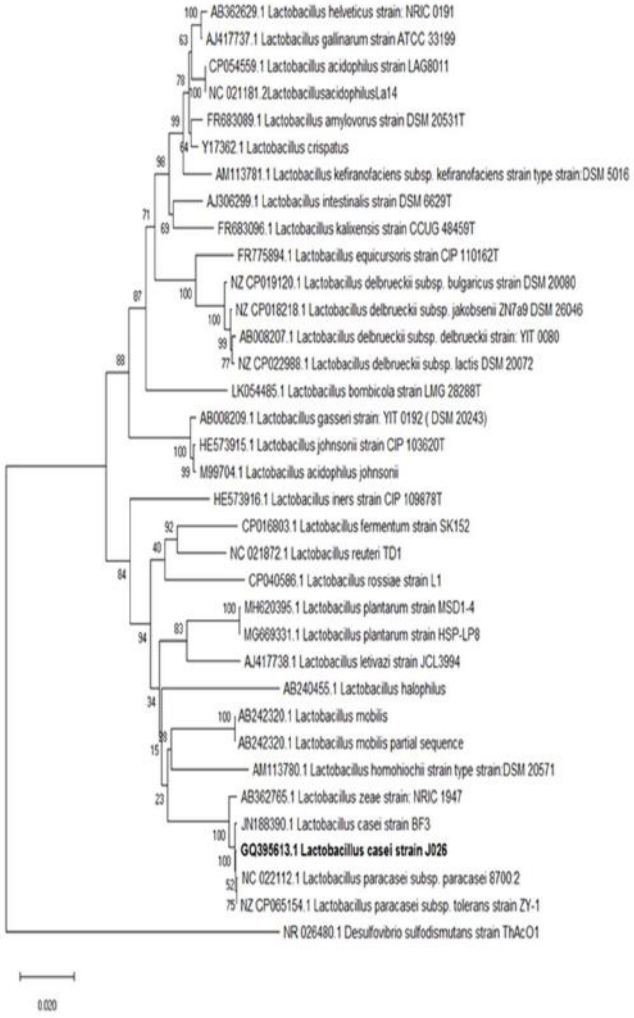

Figure 4. Phylogenetic tree drawn by neighbor-joining NJ method

4. Discussion

In a world where human health is important, the use of lactic acid bacteria as probiotics in food is very important. Probiotics are living microorganisms that have therapeutic and prophylactic effects on the host. Probiotics are usually consumed as part of fermented foods with live cultures specifically added to the food (21,22).

The gastrointestinal mucosa acts as a barrier that removes numerous antigens from the external environment. Probiotics introduce new microbes into the gastrointestinal tract that increase the gastrointestinal tract's defense mechanisms and Increase intestinal microbial balance (23).

Probiotics are used in both food and medicine. Probiotics, either endogenously or exogenously, can improve nutrition or improve host health. Probiotics are indigestible short-chain carbohydrates that increase the growth and activity of several intestinal bacteria and stimulate intestinal metabolism, which promotes host health. On Lactobacillus isolated from native cheeses of Markazi province of Iran can be an effective idea in the production of probiotic products using native strains. In this study, lactobacilli species were isolated from several samples of cheeses native to Markazi province of Iran, which is similar to research conducted by Afrikian E in 2012 to study lactic acid bacteria in traditional fermented dairy products in Armenia and Central Asia. The researchers performed their physiomorphological, biochemical, bacterial, and probiotic antimicroscopic studies, as well as the methods performed in this study, and achieved similar results in the above fields. They isolated different species of Lactobacillus from traditional Armenian products (24)

Foroutan et al. isolated the native Lactobacillus strain from traditional yogurts and performed biochemistry and molecular tests, known as *Lactobacillus casei*. Identified PM01.

Like the methods of this study, they separated Lactobacillus from traditional yogurts and Lactobacillus casei as the most microbial population in traditional yogurts and its probiotic properties along with proving the probiotics of *Lactobacillus acidophilus* ATCC 43556 and *L.ramenus* ATC strains. put. They examined resistance to bile acids and salts, as in this study, and assessed $CaCo_2$ cell adhesion and antibiotic resistance, which differed from this study. In this study, *L.casei* and *L.acidophilus* were isolated from traditional yogurts. Based on the results, all three strains can be considered as potential probiotics and are good choices for further evaluations (25).

5. Conclusion

In the present study, MRS agar, MRS broth, and PCR were used to identify and isolate the culture medium, and then by determining their gene sequence, the most lactobacilli *L. casei* strain J026 strain was identified in the traditional cheese samples of Markazi province of Iran. Increasing the use of industrial dairy products instead of traditional may increase the likelihood of elimination of probiotic bacteria. Based on the results obtained in this study, bacteria isolated from fermented products of local cheese samples of Markazi province of Iran as native probiotics in the economically viable industry. It has a strategy towards producing probiotic starters needed by the country. Of course, the native strains of Iran are more compatible with the intestinal flora of the people of our country and may be able to play their probiotic role better. Probiotic bacteria are among the dietary supplements that have beneficial effects on human health.

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Conflict of interest

The authors declare that there is no conflict of interest.

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