MOLECULAR CHARACTERIZATION OF ISOLATED LACTIC ACID BACTERIA FROM DIFFERENT TRADITIONAL DAIRY PRODUCTS OF TRIBES IN THE FARS PROVINCE, IRAN

Maryam Jafari¹, Nabi Shariatifar¹, Gholamreza Jahed Khaniki°, Mehran Sayadi°, Abbas Abdollahi²

Address(es): Gholamreza Jahed Khaniki, Mehran Sayadi
¹ Department of Food Safety and Hygiene, School of Public Health, Tehran University of Medical Sciences, Tehran, Iran.
² Department of Food Safety and Hygiene, Faculty of Health, Fasa University of Medical Sciences, Fasa, Iran

Abstract
Various traditional dairy products could be considered as an abundant source for isolation/collection of new lactic acid bacteria (LAB) with unique characteristics. The present research aimed to investigate the morphological, biochemical and genotypic characterization of dominant lactic acid bacteria that were isolated from traditional dairy products in Iranian tribes. A total of 75 samples of traditional yogurt, doogh from dairy units in Fars province were randomly collected. Isolation of lactic acid bacteria, biochemical and genotypic identification were conducted. Totally, 157 LAB isolates were selected. Cocci and rod shape LAB were 53.50% and 46.49%, respectively. Biochemical tests showed the occurrence of 40.6% of the strains were Streptococcus, 2.25% of the strains were enterococci and 20.3% of the strains were lactococci. Most isolated lactobacilli were related to Lactobacillus plantarum and Lactobacillus bulgaricus. Genetically the presence of the following species was verified: Lactococcus lactis subsp. cremoris, Lactobacillus plantarum, Streptococcus thermophilus, Lactobacillus fermentum, Lactococcus lactis subsp. lactis, Lactobacillus bulgaricus, Lactobacillus helveticus, and Lactobacillus casei. The current study showed that strains which were isolated from traditional dairy products were not only appropriate for use as starter adjuncts or cultures, but also they may provide a valuable gene pool for research and production of commercial starters with specific traits.

Keywords: Biochemical Identification; Genotypic Identification; Lactic acid bacteria; Strain selection; Traditional dairy products

INTRODUCTION
Fermentation is the most economical old methods for food preservation and storage that maintain quality for long period (Maslak, Sadogari, Mashak, & Niknafs, 2014). Fermentation of lactic acid is a known method for producing several dairy products, such as yogurt, doogh (savory yogurt-based beverage), kaskh (drained yogurt product), gharaghooroot (a nonfat dairy product), kashk (drained yogurt product), gharaghooroot (a nonfat dairy product), and yogurt), cheese, etc. (Abd El Gawad, Abd El Fattah, & Al Rubaayi, 2010; Azadnia & Khan Nazer, 2009). Traditionally, doogh is referred to a drinking product which is produced from yogurt dilution following a strong agitation stage in special waterproof sacs made from sheep or goat skin, called ‘Mashe’ (Kirdar, 2012; Noori, Keshavarzian, Mahmoudi, Yousefi, & Nateghi, 2013). Produced centuries ago in Bulgaria, yogurt is a semisolid fermented product which currently being consumed in different countries. Yogurt is a dairy product which is generated by Streptococcus salivarius subsp. thermophilus (S. thermophilus) and Lactobacillus delbrueckii subsp. bulgaricus (L. bulgaricus), two bacteria which convert lactose into lactic acid (Celik, 2007; Tumine & Marshall, 1997). The existence of living organisms in fermented food is well known and many scientific research were done regarding the traditional fermented products. The microorganisms present in food mostly specify the properties of the fermented food (flavor, texture, and acidity) and provide several nutritional benefits. These organisms exist as food natural microflora or result from adding starter cultures in food fermentation (Abdi, Sheikh-Zeinaldin, & Soleimanian-Zad, 2006). Dairy starter cultures are vital to the production of high quality and safe products in the modern dairy industry. Starter cultures are utilized as single strain, mixed strain, or multiple strains depending on the product type (El Soda, Ahmed, Omran, Osman, & Morsi, 2003). Lactic acid bacteria (LAB) are frequently known as dairy starter cultures which are used in various fermented milk products (Moulay et al., 2006). LABs widely exist as indigenous raw milk microflora in nature. These microorganisms are Gram-positive, catalase-negative and have an important part in food fermentation (Masood, Hasan, & Masud, 2013). The objective of the current research was to study the phenotypic and genotypic diversity of dominant LABs that were isolated from traditional dairy products in some tribes in Fars province, Iran.

MATERIALS AND METHODS
Sample collection
75 traditional doogh and yogurt samples were gleaned randomly from local areas of Fars in Iran and were sampled by transferring 10 ml into sterile plastic bags maintained at 4°C and analyzed within 24 h. After being transferred under refrigerated conditions (4°C) to the laboratory, the collected samples underwent microbiological analysis during the next 24 h.

Lactic Acid Bacteria Isolation
In completely sterile conditions, the collected samples were homogenized and subsequently diluted in Ringer’s solution and the aliquots (100 μl) of each dilution were spread-plated for the isolation of LAB. Plate Count Agar (PCA) (Merck, Germany) was employed to improve the isolation of LAB by incubating petri dishes at a temperature of 30°C for 72 h. De Man Rogosa Sharpe (MRS) agar (pH 5.7, Merck, Germany) was utilized to isolate lactic acid bacteria, while GM17 agar (pH 7.15, Himedia, Hindu) was used for the isolation of streptococci, lactococci and enterococci. The culture media were supplemented with 50 mg L-1 of natamycin to stop the mold and yeasts from growing (Botes, Todorov, Von Mollendorff, Botha, & Dicks, 2007). Through the use of the gas pack system, MRS plates were incubated in aerobic and anaerobic conditions (Merck Anaerocult Type A) at 37°C for 48-72 h, and GM17 plates were incubated under the same conditions at 30°C and 42°C for 24-48 h (Jokovic et al., 2008). Morphologically distinct colonies from PCA, GM17 and MRS agar plates were randomly-picked, sub-cultured and purified by streak plating via the same medium and were incubated at 37°C for 72 h. Subsequently, the strains were maintained at 4°C and refreshed by streaked every 2 weeks. Pure strains were further tested for catalase production, gram staining, spore formation, cell...
morphology, and oxidoase activity. Catalase negative and Gram-positive, non-
spore forming, oxidase negative, cocci or rods isolates were selected as
presumptive LAB and stored in growth (MRS or GM17 or Tryptic soy broth
(TSB)) medium containing 15% (v/v) glycerol as stocks frozen at -80°C.

Biochemical identification

Biochemical identification of the isolated/selected bacteria was carried out
following Bergey’s manual of determinative bacteriology.(Breed, Murray, &
Smith, 1957) For biochemical identification, every single isolate was activated in
5 ml GM17 or MRS or TSB broth medium for 24 h at 37°C prior to use. The tests
used for preliminary characterization of isolates included Gram staining, catalase
test (H2O2, 3%), oxidase test and endospore formation(spoore staining). Isolates
with Gram-positive, catalase, and oxidase-negative, non spore forming
characteristics were selected for further identification (Nikita & Hemangi,
2012).

Identification of cocci

Inoculated bacterial isolates in GM17 broth medium were incubated at 10°C,
40°C and 45°C. At different NaCl concentrations (2%, 4% and 6.5%), the growth
ability was evaluated in the modified GM17 broth media. The change of the color
as the evidence for cell growth (Nikita & Hemangi, 2012). Growth at pH 9.6,
was detected by cultivating of isolates in the GM17 broth media with pH 9.6 (by
pH adjustment with NaOH).

Arginine hydrolysis and gas production from citrate and Reduction of
Methylyene Blue

Inverted Durham tubes and Reddy broth were applied in this test. The color of
cultures usingarginasechanged from yellow to violet. To reduce Methylyene Blue,
night cultures were transferred into GM17 broth tubes with Methylyene Blue.

Carbohydrate fermentations

The properties of the isolates were further determined according to the profiles
related to their sugar fermentation. Sixteen different sugars were utilized. In each
test, the strains were inoculated in 3 ml of Phenol red base broth media
containing 150 μl of filter sterilized (0.22 μm, Merck Millipore) 10% sugar
solutions (0.5%). The acidification as a measure of fermentation ability is
reflected in color change from red to yellow.

Identification of lactobacilli

Inverted Durham tubes and MRS broths were used to identify lactobacilli. The accumulation of gas in Durham tubes indicated the production of CO₂ from
glucose. Lactobacilli isolates were screened to determine if they were able to ferment:D (-) Raffinos, D Mannitol, D (+) Galactose, D (-) Ribose, Lactose,
Maltose, glucose, L (+) Arabinos, D (-) Salicin, D (+) Xylose, D (+) Mannose,
Fructose sucrose, Sorbitol, Trehalose, and Rhamnose as described above.

Genotypic identification

DNA extraction

To extract the DNA, the cultures of isolates grown in MRS and GM17 broth for
18h were employed. Genomic DNA extraction was done through phenol-
chloroform extraction, which is a modified bacterial DNA extraction protocol
depicted by Federici et al., and the samples of DNA were maintained at -20°C
(Federici et al., 2014). A single colony of each isolate was inoculated into 10 ml
of the appropriate medium broth and incubated overnight at 37°C. Using
centrifugation at 7500-8000 rpm for 5 min, the cells were harvested, and genomic
DNA was isolated based on a modified genomic DNA isolation protocol
(Ashmaig, Hasan, & El Gaali, 2009). The collected bacterial pellet was
resuspended in 2.5 ml of TE buffer (1M Tris-HCl, 0.5 mM EDTA pH 8.0) and
washed two times with the same buffer. Once washed, the pellet was re-
suspended into 250 μl of lysis solution (1% (v/v) SDS, 0.2 mol NaOH). Through
gentle shaking, the cell suspension was incubated for 1 h in a water bath at 65°C.
The solution was gently emulsified by an equal amount of TE buffer saturated
phenolchloroform (1:1); it was then centrifuged for 5 min at 7500-8000 rpm, and
the aqueous phase was transferred into new tubes. Next, the aqueous solution
was washed two times with an equal amount of chloroform:isoamylic alcohol, 24:1,
and it was centrifuged for 5 min at 7500-8000 rpm. Once the aqueous phase was
centrifuged, it was transferred to Eppendorf tube (400 μl / tube) to which 1/10
volume of three molar sodium acetate (pH 2.5) was added. Via adding 2 volumes of
ice cooled absolute ethanol, the nucleic acids were precipitated. Subsequently,
the mixture underwent centrifugation at 14000 rpm for 10 min; after that, the
supernatant was discarded and the created pellet was washed two times by 70%
ethanol. The remaining ethanol was eliminated through drying the pellet at
room temperature. It was then dissolved in TE buffer and kept at -20°C.

Amplification of 16S rRNA genes and specific PCR conditions

The mixtures of the PCR reaction were prepared in 25 μl volumes containing 2.5
μl of 10X Taq buffer, 1.5 μl MgCl₂ (50 mM), 2.5 μl dNTPs (2 mM/μl), 2 μl random
primer (16S-FA 5’-AGAGTTTGATCCTGCGTGCA-3’ and 16S-RA 5’-
AGAGGTTGZ7CCAGCAG-3’), 0.5 μl Taq-DNA polymerase (5 U/μl). Through
adding sterilized distilled water, the mixture was diluted up to 25 μl . The amplifications were carried out via an Applied Biometra thermocycler programmed to repeat the thermal profile. The PCR program included 5 min at
96°C, 30 cycles of 96°C for 30 s, 55°C for 30 s and 72°C for 30 s; another
extension step at 72°C for 5 min was also performed in a mastercycler. Using
electrophoresis, the amplification products were assessed. Afterwards, the
standard marker and the DNA fragments were separated in 1.5% ethidium
bromide-stained agarose gels. Then, the separated fragments(1200-1500 bp)
and their patterns were visualized and photographed with gel documentation system
(Terzic-Vidojevic et al., 2014).

Multiple sequence alignment

Ultimately, the amplified genes were obtained from the gels by use of a QIA
quick gel extraction kit (Bioneer, USA) according to the manufacturer’s
instructions. Buffer 1 (gel binding buffer) was added to the cutted piece of gel,
incubated for 10 min at 60°C to completely dissolve the gel. The solution was
transferred to the DNA binding column tube, centrifuged for 1 min at 13000 rpm
and poured in the flow-through, re-assembling the DNA binding filter column
with 2.0 ml collection tube. 500 μl of buffer 2 (cell lysis buffer) was added to the
DNA binding column tube, centrifuged for 1 min at 13000 rpm., poured in
thetrough, re-assembling the DNA binding filter column with the 2.0 ml
collection tube and dried via another centrifugation for 1 min at 13000 rpm. DNA
was eluted from column by adding 30 μl buffer 3 (EB) to the center of the DNA
binding filter column and remaining for at least 1 min at room temperature and
centrifugation 1 min at 13000 rpm. The purified DNA fragments of amplified
genes for 16S RNA were finally subjected to sequencing service (Macrogen,
South Korea) for standard sequencing (Poormontaseri, Hosseinzadeh, &
Shekarforoush, 2014).

DNA sequence Analysis

DNA sequences were obtained by use of forward and reverse primers assembled
and edited through the use of BioEdit sequence alignment editor version 5.0.9.
Using BLAST, the homology was searched in the Genbank DNA database, and
the sequence similarity was estimated.

RESULTS AND DISCUSSION

Lactic Acid Bacteria Isolation

From the collected samples, approximately 280 colonies were picked at random
from selective media for each group of LAB.

Phenotypic Identification

Selected colonies from the fermented food samples showed comparable
differences in their colony morphology (size, shape, shine and color). Among the
280 Gram-positive isolates that were extracted from the dairy products, 117 (all
belonging to the LAB family) were negative for catalase and oxidase activity
and/or spore forming bacteria. A large number of isolates (60 isolates) had a red
shape, thereby related to the genus Lactobacillus; the rest were cocci (57
isolates).

Physiological and Biochemical Identification

The results of identification of cocoid shaped isolates are shown in Table 1. According to obtained results, the strains were Streptococcus (29 isolates),
enterococci (12 isolates) and lactococci (16 isolates). The ability of lactobacilli
isolates to ferment different types of carbohydrates is shown in Table 2.
different dairy samples considered as the predominant bacteria characterization. In addition, the results show that LAB isolates can be identified based on their phenotypic characteristics. Figure 1 shows identification results of the isolated LAB based on the phenotypic characterization.

**Table 1** Technological properties of cocci shaped of cocci LAB that were isolated from dairy products and the fermentation profile of sugars.

| Characteristics          | Streptococcus thermophilus | Enterococcus faecium | Enterococcus faecalis | Enterococcus durans | Lactococcus lactis subsp. lactis | Lactococcus lactis subsp. cremoris |
|--------------------------|----------------------------|----------------------|-----------------------|--------------------|----------------------------------|-----------------------------------|
| Gram staining            | +                          | +                    | +                     | +                  | +                                | +                                 |
| Catalase production      | -                          | -                    | -                     | -                  | -                                | -                                 |
| Oxidase activity         | -                          | -                    | -                     | -                  | -                                | -                                 |
| Spore formation          | Non-spore                  | Non-spore            | Non-spore             | Non-spore          | Non-spore                        | Non-spore                         |
| Growth at pH 9.6         | -                          | +                    | +                     | +                  | +                                | +                                 |
| Reduction Methylene Blue | -                          | -                    | -                     | -                  | -                                | -                                 |
| Growth at 10°C           | +                          | +                    | +                     | +                  | +                                | +                                 |
| Growth at 40°C           | +                          | +                    | +                     | +                  | +                                | -                                 |
| Growth at 45°C           | +                          | +                    | +                     | +                  | -                                | -                                 |
| Growth in 2% NaCl        | ±                          | +                    | +                     | +                  | +                                | +                                 |
| Growth in 4% NaCl        | -                          | +                    | +                     | +                  | -                                | -                                 |
| Growth in 6.5% NaCl      | -                          | +                    | +                     | +                  | -                                | -                                 |
| Hydrolysis of arginine   | -                          | +                    | +                     | +                  | +                                | -                                 |
| CO₂ from citrate         | -                          | +                    | -                     | -                  | -                                | -                                 |
| Acid formed from         |                           |                      |                       |                   |                                  |                                   |
| Mannitol                 | -                          | ±                    | +                     | -                  | ±                                | -                                 |
| Raffinose                | -                          | ±                    | -                     | -                  | -                                | -                                 |
| Ribose                   | -                          | +                    | +                     | +                  | +                                | +                                 |
| Galactose                | -                          | +                    | +                     | +                  | +                                | +                                 |
| Maltose                  | -                          | +                    | +                     | +                  | +                                | -                                 |
| Lactose                  | +                          | +                    | +                     | +                  | +                                | +                                 |

+: Growth / Fermentable carbohydrates - : Non-growth / Non-fermentable carbohydrates

**Table 2** Carbohydrates fermentation pattern of LAB isolated from traditional yogurt and doogh

| Lactobacillus species | Lb. plantarum | Lb. delbrueckii | Lb. heleviticus | Lb. brevis | Lb. casei subsp. tolerans | Lb. casei | Lb. acidophilus | Lb. bulgaricus | Lb. salivarius |
|-----------------------|---------------|-----------------|-----------------|------------|--------------------------|-----------|----------------|----------------|---------------|
| Arabinose             | +             | -               | -               | +          | -                        | -         | -              | -              | -             |
| Lactose               | +             | -               | +               | +          | +                        | +         | +              | +              | +             |
| Glucose               | +             | +               | +               | +          | +                        | +         | +              | +              | +             |
| Glucose (gas)         | -             | -               | -               | +          | -                        | -         | -              | -              | -             |
| Xylose                | +             | -               | -               | +          | -                        | -         | -              | -              | -             |
| Mannitol              | +             | -               | -               | -          | -                        | +         | -              | -              | -             |
| Rhamnose              | -             | -               | -               | -          | +                        | -         | -              | -              | -             |
| Salicin               | +             | -               | -               | -          | +                        | +         | -              | -              | -             |
| Mannose               | +             | +               | -               | +          | +                        | +         | -              | -              | +             |
| Ribose                | +             | -               | -               | +          | +                        | -         | -              | -              | -             |
| Galactose             | +             | -               | +               | +          | -                        | +         | +              | -              | +             |
| Sucrose               | +             | +               | -               | +          | +                        | +         | -              | +              | +             |
| Raffinose             | +             | -               | -               | -          | +                        | -         | +              | -              | +             |
| Fructose              | +             | +               | -               | +          | +                        | +         | +              | +              | +             |
| Sorbitol              | +             | -               | -               | -          | +                        | +         | -              | -              | -             |
| Trehalose             | +             | -               | +               | -          | -                        | -         | +              | -              | -             |

: Growth / Fermentable carbohydrates - : Non-growth / Non-fermentable carbohydrates

Fig 1 shows identification results of the isolated LAB based on the phenotypic characterization. In addition, the results show that LAB isolates can be considered as the predominant bacteria in collected dairy products. Nonetheless, different dairy samples had various predominant LAB.
In addition, we found that cocci especially those cultured by pouring plate, have completely different forms, however with the aid of a microscope, they were seen as spherical or oval, mostly forming short or long chain and in single or pairs. Bacilli were observed to be so differently from each other under a microscope; although, on the plate, they were almost similar in size and shape. Diversity of bacterial species can be related to different factors, such as raw milk composition and the breed of the animal, regional influences, the kind of the utilized culture medium, effect of reducing pH environment at different stages of growth, concentration of salt in the environment, and the existence of carbohydrate and other nutrients, including proteins.

In order to classify cocci shaped isolates, growth ability at 10°C, 40°C, and 45°C as well as growth at 2%, 4% and at the concentration of 6.5% NaCl were used. Streptococcus thermophilus was not able to show growth at 10°C, but it had a good growth at 40°C and 45°C; 47 isolates showed these characteristics. Enterococcus faecium was isolated at 10°C whereas some strains of E. coli were isolated at 40°C and 45°C. Some of these characteristics, unlike other species, Lactococcus species indicated growth at 10°C but had no growth at 45°C and only 26 isolates possessed such property. L. lactis subsp. lactis strain could grow poorly at 45°C, while L. lactis subsp. cremoris grew just at 10°C. S. thermophilus and L. lactis subsp. cremoris did not have the ability to grow at salt levels of 4 and 6.5%, while L. lactis subsp. lactis and Enterococcus species could grow at different levels of salt. Reduction methylene blue, growth at a pH of 9.6, arginine hydrolysis, and citrate utilization and carbohydrate fermentations also were examined. Only 11 isolates were able to reduce methylene blue and hydrolyze the arginine. At a pH of 9.6, very few (only 2 isolates) could grow and 5 of them were able to produce gas from arginine. On citrate utilization, only 21% of the isolates were capable of using citrate (mannitol, ribose, lactose, maltose, galactose) were also evaluated. S. thermophilus and L. lactis subsp. cremoris bacteria had less ability to ferment carbohydrates, while Enterococcus species and some species of Lactococcus are able to ferment majority of tested sugars. S. thermophilus and L. lactis subsp. cremoris bacteria did not participate in fermentation of galactose. S. thermophilus produce poorly its fermentable galactose because its enzyme beta-galactosidase is not able to ferment this sugar.

Similarly, most researchers agree on the fermentation of lactose by cocci bacteria (Begovic et al., 2011; Patil, Pal, Anand, & Ramana, 2010). On the contrary, atypical characteristic of some Streptococcus strains is their galactose fermentation. Almost none of LAB cocci were able to ferment raffinose, however, they are able to break the disaccharide lactose into glucose and galactose that can be used for energy.

For identification of bacilli shaped isolates, carbon dioxide from glucose and carbohydrate fermentation tests were performed by applying 13 different type of carbohydrates, bacterial strains were capable of oxidizing various sugars: mannanot, ribose, lactose, maltose, galactose were also evaluated. S. thermophilus and L. lactis subsp. cremoris bacteria had less ability to ferment carbohydrates, while Enterococcus species and some species of Lactococcus are able to ferment majority of tested sugars. S. thermophilus and L. lactis subsp. cremoris bacteria did not participate in fermentation of galactose. S. thermophilus produce poorly its fermentable galactose because its enzyme beta-galactosidase is not able to ferment this sugar. Moreover, some researchers have suggested that lactobacillus species can ferment the galactose. L. delbrueckii, L. bulgaricus and L. helveticus possess weak fermentation ability and are only able to ferment few sugars (Maqsoud et al., 2013). In a previously reported research by Kirdar (2012) in meaditerian area, three Lactobacillus species were noted. Unlike to other lactobacilli, L. casei subsp. pseudoplantarum is able to obtain energy and produce acid from most of the carbohydrates (Abdulrah & Osman, 2010). The ability to ferment galactose is considered as the main factor for distinction between L. helveticus species and galactose positive and L. bulgaricus known as galactose negative. L. lactis and L. acidophilus ferment sugars in the same way, however, the only distinction between them is in the fermentation of raffinose, in which L. lactis is not able to use the sugar for its energy supply. Roshanzade et al. reported the similar action of these two species in the fermentation of sugars (Roshanzadeh, Eskandari, Shemshaki, & Tajeddine, 2014). Enterococcus species can produce energy sources by producing different enzymes; however, using energy sources depends on the synthesis of specific enzymes by each bacteria (Abmaga et al., 2009). According to the literature, new bacterial species identified are discovered in almost the same studies carried out previously, pointing that the main reason for enterococcus species could grow at 10°C, 40°C and 45°C is their proteolytic activities were genetically identified and proved to play crucial role in cheese ripening (Absoreh, Ab Eli Ghani, Gomaa, & Fouad, 2016; Garcia-Cano et al., 2019).

Genotypic identification

Since 1990, some alternatives have emerged for the classical phenotypic and biochemical identification of LAB. These methods might not be sufficient to definitively attribute a strain to a certain species. To thoroughly identify and classify the species in bacterial systematics, polyphasic approaches (Coueurt, Dubernet, Bernardau, Gueguen, & Vernoux, 2003), such as phenotypic, chemotaxonomic, and genotyping methods are still recommended.

To identify the LAB species more accurately, PCR assays were used with primers that target 16s rRNA gene (Table 3). To confirm the belonging to certain species, the nucleotide sequences of the 16S rRNA gene were examined and specified using the BLAST program on NCBI. The results were categorized based on maximum identity and nonduplicated in terms of coverage. The sequence similarity with 90% or higher cut-off was regarded as significant. The optimum hit was considered as the sequence with the most maximum similarity to the query sequence.

Table 3 Genotypes of the selected isolated LAB as 16S rDNA gene sequence alignments submitted to the NCBI GeneBank database.

| Species                  | Similarities (%) | Source of Isolation   |
|-------------------------|------------------|-----------------------|
| Enterococcus faeae      | 99%              | Doogh                 |
| Streptococcus thermophilus | 99%             | Yogurt                |
| Lactobacillus subsp. lactis | 99%           | Doogh                 |
| Lactobacillus durans     | 100%             | Yogurt                |
| Lactococcus salivarius  | 100%             | Yogurt                |
| Lactobacillus helveticus | 98%             | Yogurt                |
| Lactobacillus delbrueckii subsp. Bulgaricus | 100% | Yogurt |
| Lactobacillus plantarum  | 97%              | Yogurt                |
| Lactobacillus casei      | 98%              | Yogurt                |
| Lactobacillus acidophilus | 95%            | Yogurt                |

With the current rapid growth in biotechnology, more and more molecular techniques are utilized in the genetic diversity studies on LAB. Typing methods based on major advantages because of their highly discriminatory power, being applied in a universal way and having independent culture. Many different molecular biology methods include PCR, DNA/RNA hybridization, ribotyping, polymerase chain reaction (PCR), real-time PCR hybridization and sequencing of rRNA are able to distinguish even the close related strains (Miljovic et al. 2019; Axelsson, 2004; Furet, Quenné, & Tailliez, 2004).

In category of LABs, a large number of studies have compared the foregoing methods. A highly comprehensive study in this regards still the collaborative work carried out by M. Staal, M.-L. Johansson and colleagues. They made use of a set of Lb. reuteri and Lb. plantarum strains. These strains were systematically characterized via phenotypic tests, DNA/DNA homology, REA, automated PCR sequencing of rRNA, ribotyping, and RAPD. Their work clearly elucidates that every method has its advantages and disadvantages and that one technique cannot be chosen to answer all problems, but rather different methods complement one another.
CONCLUSION
In the present study, in-vitro approaches were chosen to examine the diversity of the LAB in dairy products from Iran. Previous studies have been conducted on LAB isolates from the Iranian traditional cheeses and yogurts, with less attention to others traditional and industrial dairy products. This work showed that other fermented dairy products could be significant sources of LAB. Some studies reflected the fact that LAB strains detected from dairy products, produce more pleasant flavor and volatile compounds than industrial strains. Local LABs are highly potent as starters or adjuncts. To optimally utilize novel strains, the specific conditions of use have to be defined and more detailed research must be conducted on their technological features.

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