# Application of Repetitive Extragenic Palindromic Elements Based on PCR in Detection of Genetic Relationship of Lactic Acid Bacteria Species Isolated from Traditional Fermented Food Products

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## ABSTRACT

Tarkhineh is one of the traditional foods in Iran and a rich source of probiotic bacteria. The objective of this study was to identify the probiotic bacteria isolated from Tarkhineh using 16S rRNA gene sequencing and molecular typing with repetitive extragenic palindromic–PCR (REP-PCR). In total, 20 different bacteria were isolated from traditional dairy products and Tarkhineh. Molecular identification of the isolates was carried out by 16S rRNA gene sequencing, and DNA sequences of isolates deposited in GenBank. The REP-PCR reaction by REP1R-I, REP2-I and REP1R-I+REP2-I markers was performed for fingerprinting and characterization of the isolates. Unweighted pair group method with arithmetic mean (UPGMA) clustering methods were performed based on Dice similarity. The REP1R-I primer grouped isolates into three, and REP2-I and REP1R-I+REP2-I grouped all isolates into four main clusters in dendrograms. In all analyses, isolates of *Lactobacillus casei*, *Lactobacillus brevis*, *Lactobacillus plantarum*, and *Entrococcus facium* formed separate clusters. The results of sequencing corresponded to clustering in the dendrogram. According to the results, REP-PCR is an accurate technique for determining the genetic diversity of lactic acid bacteria species.

Keywords: 16S rDNA sequencing, REP-PCR fingerprinting.

## INTRODUCTION

Tarkhineh is one of the traditional foods that are consumed in the west of Iran, which also has medical and pharmaceutical applications. Studies have indicated that Tarkhineh and Iran's traditional dairy products are two important sources of lactic acid bacteria that have probiotic properties [31, 32]. Recent studies have investigated the microbial communities in Tarkhineh to isolate and identify lactic acid bacteria (LAB). The isolates were identified as *L. nagelii* (67%), *L. bifermentans* (21.3%), *Leu. cermoris* (6%), *L. fructosus* (1.45%), *L. fermentum* (1%), *L. intestinalis* (0.9%), *L. agilis* (0.9%) *L. acidipiscis* (0.9%), and approximately %1 of isolated samples remained unknown. The naturally occurring lactic acid bacteria load was found to vary between $1.97 \times 10^5$ and $4.3 \times 10^5$ cfu gr$^{-1}$ [29].

LAB play a key role in the production of many types of fermented food [8]. Since the application of conventional biochemical methods is often not sufficient to discriminate closely related lactic acid bacteria species, use of accurate and reliable methods for the detection and evaluation of genetic variability in LAB at the strain level is very important. For a food microbiologist,

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it is important to have a correct and accurate genomic profile of LAB, both at the species level and to determine bacterium strains in fermented foods. The probiotic characteristic of each bacterium is unique and specific, thus, creating the taste, smell, preserving food texture, and other health-promoting properties of these food sources [1, 22, 34]. In previous years, molecular techniques have been used as effective methods for detecting the dominant flora of complex microbial populations, such as fermented foods [5]. In particular, PCR-based molecular techniques including; 16S rDNA sequencing [15, 26], hybridization with specific DNA probes [23], ribo-typing [24], random amplified polymorphic DNA (RAPD) [6, 25, 30], and repetitive extragenomic palindromic elements-polymerase chain reaction (REP-PCR), are used as reliable techniques compared to the highly inaccurate and time-consuming conventional phenotypic and biochemical methods [10]. Some methods such as amplified fragment length polymorphism (AFLP) and pulsed field gel electrophoresis (PFGE) are reliable and accurate, but they are time-consuming and expensive, and cannot be used as a routine test in all research laboratories. In addition, they also require specialized equipment [2, 9]. RAPD-PCR is a sensitive method that requires careful control of the test conditions to achieve reliable results. However, amplification of repetitive regions of bacterial genomes using the REP-PCR technique is considered a reliable method for taxonomy, molecular genotyping, and determination of phylogenetic relationships between closely related species and even in distinct bacterial strains [13, 11, 21, 19]. This method has the benefits of ease of use, fast, high differentiation power and it is practicable in all laboratories. REP-PCR is feasible with markers such as (GTG)5, REP1R-I, and REP2R-I that have been used successfully for the identification and detection of lactobacilli and bifidobacterium species [10, 14].

This study aimed to identify bacteria isolated from locally sourced Tarkhineh and traditional dairy products based on 16S rRNA sequence, and then, to investigate the genetic variety and genomic profile of the isolates detected with a REP-PCR molecular method based on REP1R-I, and REP2-I and combination of REP1R-I and REP2-I primers.

## MATERIALS AND METHODS

### Bacterial Strains and Culture Conditions

The LAB isolates used throughout this study were previously isolated from traditional Tarkhineh food, Tarkhineh drink and dairy products, by Tajabadi Ebrahimi (Table 1). All of the isolates exhibited probiotic potential activities, including acid and bile resistance and cholesterol removal [31, 32, 33].

Isolates were inoculated in selective media De Man-Rogosa-Sharpe (MRS) broth and incubated under anaerobic conditions (8% $CO_2$) at $37^\circ$C for 24 hours.

**Table 1.** Samples used in this study.

| Isolates | Origin |
|----------|--------|
| T2 | Traditional Tarkhineh food |
| T3 | Traditional Tarkhineh food |
| T4 | Traditional Tarkhineh food |
| T5 | Traditional Tarkhineh food |
| T9 | Traditional Tarkhineh food |
| T14 | Traditional Tarkhineh food |
| T15 | Traditional Tarkhineh food |
| T16 | Traditional Tarkhineh food |
| T20 | Traditional Tarkhineh food |
| T22 | Traditional Tarkhineh food |
| TD3 | Tarkhinh drink |
| TD4 | Tarkhinh drink |
| TD5 | Tarkhinh drink |
| TD10 | Tarkhinh drink |
| C6M3 (L2) | Traditional cheese |
| C6I4 | Traditional cheese |
| Y1L4 (L8) | Traditional Yogurt |
| Y2P3 (L16) | Traditional Yogurt |
| Y2B4 | Traditional Yogurt |
| 14 (L14) | Traditional Yogurt |

## DNA Extraction

Genomic DNA from the MRS broth cultures was extracted by a DNA extraction kit (MBST, Iran, Tehran) in accordance with the manufacturer's instructions. The first 2 mL samples from overnight cultures at 37°C were used for total genomic DNA extraction. The cells were harvested by centrifugation at 3,000×g for 5 minutes. The pellets were washed twice with TEN buffer (100 mM EDTA, 150 mM NaCl, 100 Mm Tris HCl, pH= 8) then re-centrifuged and resuspended in 200 μL of TEN buffer containing 4 mg mL$^{-1}$ lysozyme. The suspension was incubated at 37°C for 24 hours. In the lysis reaction, 180 μL lysis buffer (LB) was added to the solution and immediately mixed thoroughly by vortexing and incubated for 10 minutes at 55°C. Then, 20 μL proteinase K (10 mg ml$^{-1}$) was added to the sample and incubated at 55°C for 30 minutes. After adding 360 μL binding buffer and incubation for 10 minutes at 70°C, 270 μL ethanol (96-100%) was added to the solution. Then, it was vortexed and the complete volume was transferred to the MBST-column. The MBST-column was first centrifuged at 8,000×g and then washed twice with 500 μL washing-buffer at 8,000×g. Next, the columns were centrifuged with 12,000×g to remove the rest of the ethanol from the carrier. After that, the DNA was eluted from the carrier with 100 μL elution buffer. Finally, the quality of DNA was determined by electrophoresis on 0.7% agarose gel.

## 16S rDNA Amplification and Sequencing

The extracted DNA was used as a template for amplification of the 16S rRNA gene. PCR-mediated amplification was carried out in a Gradient Master Thermocycler (Bio Rad, USA). The amplification conditions were as follows: 1 μL genomic DNA, 10 μL 10X reaction buffer (Cinagen Company, Iran), 200 μM each of the four deoxynucleotides (Cinagen Company, Iran), 1 U Taq polymerase (Cinagen Company, Iran), 20 pmol of each primer (Takapouzist Company, Iran) (616V, 5`-AGAGTTTGATYMTGGCTCAG-3`; 630R, 5`-CAKAAAGGAGGTGATCC-3`), dH2O to a final volume of 25 μL. The PCR conditions were: (94°C 2 min$^{-1}$) 1X, (94°C 45 s$^{-1}$, 58°C 1 min$^{-1}$, 72°C 30 s$^{-1}$) 30X, (94°C 1 min$^{-1}$, 72°C 4 min$^{-1}$) 1X [7]. The PCR products were checked for correct amplification by electrophoresis on 1.5% agarose gel under UV illumination.

PCR products were purified and sequenced for both strands with the same primers used in a PCR (Bioneer Company, Korea). The sequences were examined for possible chimeric artifacts using Vector NTI 11 software.

Identification and similarity search of the 16S rDNA sequences was carried out using the BLAST (N) program at the NCBI database (http.www.ncbi.nlm.nih.gov) for identification of bacterial isolates at species levels.

## Rep-PCR Genomic Fingerprinting of Isolates

The primers used in this study were REP1R-I (5´-NNNNCGNCGNCATCIGGC-3') and REP2-I (5'-NCGNCTTATCNGGCCTAC-3') with some modifications (we used N instead of I in the primer sequence) for REP-PCR [10].

The PCR reaction mixture consisted of 1μL template DNA, 1X PCR buffer (10 mM Tris-HCL pH 8.8, 250 mM KCL), 200 μM dNTPs, 20 pmol of each primer and 3 units of Taq polymerase, in a total volume of 25 μL. All of the reagents were prepared from the Cinagen Company. Primers were synthesized by the Takapouzist Company. DNA amplification was performed on a Gradient Master Thermocycler (Bio Rad) with an initial denaturation step (92°C, 7 minutes), followed by 30 cycles of denaturation (92°C, 30 seconds), annealing (variable temperature, 1 minute) and

extension (72°C, 2 minutes), and a final extension step (72°C, 10 minutes). The annealing temperature was 55.9, 42, and 50°C, for primers; REP1R-I, REP2-I and REP1R-I+REP2-I, respectively. Each PCR experiment contained a negative control. The PCR amplified products were separated by electrophoresis on a 1.5% agarose gel using 0.5X TBE buffer (44.5 mM Tris/Borate, 0.5 mM EDTA, pH 8.0). The gels were stained with Rima Sight DNA stain and visualized under UV light. A 100 bp DNA ladder (GeneRuler, Fermentas, Germany) was used as the molecular standard in order to confirm the appropriate REP markers. These markers were named by primer origin, followed by the primer number and the size of the amplified products in base pairs. REP-PCR bands were treated as binary characters and coded accordingly (Presence= 1, Absence= 0). Dice similarity was determined among the isolates studied and used for grouping of the genotypes and the un-weighted pair group method with arithmetic mean (UPGMA) method was used for clustering analyses by NTSYS software version 2.02 (1998). The correlation between similarity and cophenetic matrices, as a measure of goodness of fit, was computed for each marker by NTSYS software version 2.02.

## RESULTS

### Species-specific Identification of Isolated Bacteria

Optimized PCR reaction for specific identification of species with 616V and 630R primers in bacteria amplified 1,500 bp amplicons in the previously mentioned isolates (Figure 1). After purification of the PCR products, bilateral sequencing of the segments (both sense and antisense strands), was performed using specific primers.

The 16S rDNA sequence alignment was performed using BLAST in the NCBI database and the following similarities were identified: 99% similarity with *Lactobacillus casei* for C6M3 (L2), Y1L4 (L8), Y2P3 (L16), T2, T3, T4, T14, T16, T20, T22, TD4, TD10, and Y2B4 isolates, 99% similarity with *Lactobacillus brevis* for 14 (L14), T9, T15, TD3, and TD5 isolates, 97% similarity with *Lactobacillus plantarum* for T5, and 97% similarity with *Enterococcus facium* for C614. Based on the similarity found in BLAST, the studied isolates belonged to the *Lactobacillus casei, L. brevis, L. plantarum,* and *Enterococcus facium* groups.

The 16S rDNA gene sequences from all the isolates were deposited in Genbank with the following accession numbers: *L. casei* isolate L2 *(*JQ412725*), L. casei* isolate L8 *(*JQ412726*), L. casei* isolate L16 *(*JQ412728*), L. casei* isolate T2 *(*JQ301797*), L. casei* isolate T3 *(*JQ771071*), L. casei* isolate T4 *(*JQ301798*), L. casei* isolate T14 *(*JQ771072*), L. casei* isolate T16 *(*JQ771073*), L. casei* isolate T20 *(*JQ412730*), L. casei* isolate T22 *(*JQ412731*), L. casei* isolate TD4 *(*JQ412732*), L. casei* isolate TD10 *(*JQ412734*), L. casei* isolate Y2B4 *(*JQ412735*), L. brevis* isolate L14 *(*JQ412727*), L. brevis* isolate T9 *(*JQ301799*), L. brevis* isolate T15 *(*JQ412729*), L. brevis* isolate TD3 *(*JQ771074*), L. brevis* isolate TD5 *(*JQ412733*), L. plantarum* strain T5 *(*JQ301796*),* and *Enterococcus facium* isolate C6I4 *(*JQ771070 *).
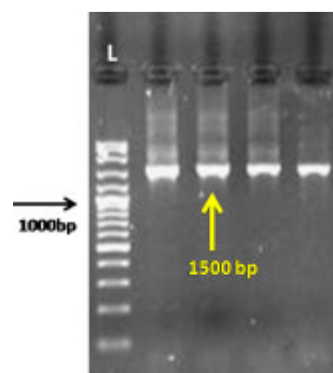


**Figure 1.** 16S rRNA Gene amplification of isolates on 0.7% agarose gel. L: DNA Ladder, 1: T15, 2: TD5, 3: T9, 4: TD3.

Investigation of Genomic Fingerprinting Based on REP-PCR with REPIR-I Primer

The genetic diversity of the isolates was investigated on 1.5% agarose gel. All clear and reproducible bands in the range of 250-2,500 bp were scored (Figure 2). A total of 13 polymorph, 2 unique and no monomorph bands were found. The 900 bp band proliferated in all isolates except T9 (*L. brevis*), and 550 bp band only in T5 (*L. plantarum*), while T14 isolates (*L. brevis*) were not amplified. Band 600 bp only in T5 isolate (*L. plantarum*), and band 650 bp only in T4 (*L. casei*) were observed. Band 1,200 bp were present only in amplified *L. brevis* isolates, and band 350 bp only in T2 isolate from *Lactobacillus casei*, and it was not observed in the remaining *L. casei* isolates.

Bands 200, 600, 1,200, 1,500 and 2,000 bp were not amplified in any of the *L. casei* isolates. In total, 112 bands were obtained. Most bands related to TD5 and T15 *L. brevis* isolates with 11 bands, and the least related to the T14 *L. casei* isolate (Table 2).

Bacteria were classified in three main clusters in the dendrogram (Figure 3). Bacteria C6M3, Y2P3, T22, TD4, TD10, T20, Y1L4, T14, T16, T2, T4, Y2B4, and T3 (*L. casei* isolates) and bacteria C6I4 (*Enterococcus facium*) were grouped in cluster I, bacteria T15, TD5, TD3, T9, and T14 (*L. brevis* isolates) in cluster II, and T5 isolates (*L. plantarum*) in cluster III.

**DNA Fingerprinting by REP2-I Primer**

A 1.5% agarose gel was used to detect DNA fingerprinting of the isolates. All reproducible bands in the range of 350-3,000 bp were scored (Figure 4). A total of 13 polymorphs, 1 monomorph and 1 unique band were found (Table 2). The 1,700 bp was reported as a monomorph band and the 600 bp unique band only amplified in the C6M3 and Y1L4 isolates. The 700 bp band was reported in all isolates except in two *L. casei* strains, namely, T22 and TD4. The 1,400 bp band was observed in all but the T9 and C6I4 isolates. In total, 165 bands
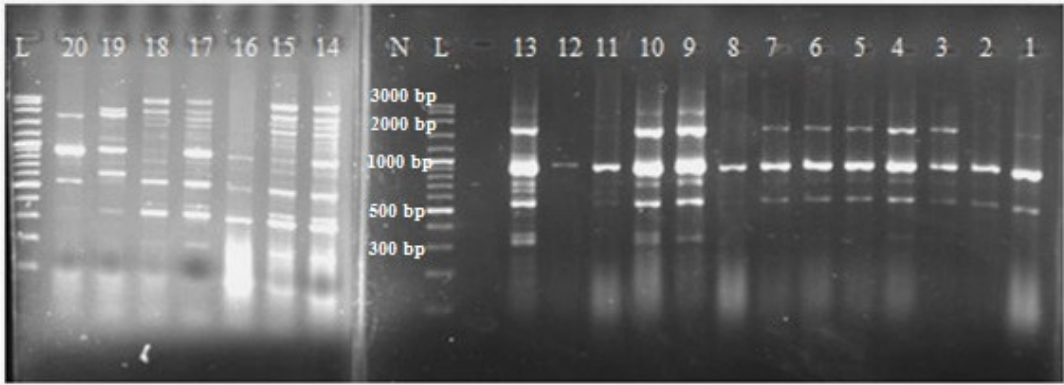


**Figure 2.** REP-PCR profile of isolates studied based on REPlR-I primer on 1.5% agarose gel. 1: C6M3, 2: Y1L4, 3: Y2P3, 4:T20, 5:T22, 6: TD4, 7: TD10, 8: Y2B4, 9: T16, 10: T2, 11: T3, 12: T14, 13: T4, L:100 bp DNA ladder, N: Negative control, 14: TD5, 15: T15, 16: 14, 17: TD3, 18: T9, 19: T5, 20: C6I4, L: 100 bp DNA ladder.

**Table 2.** Number and range of amplified bands by REP-PCR primers.

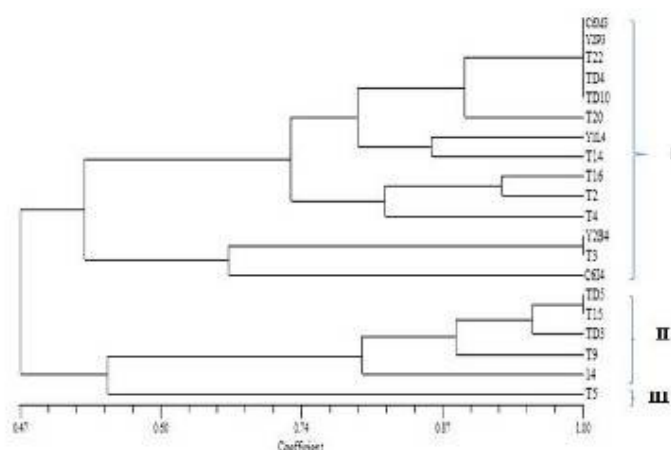| Primer | Total band in isolates | Polymorph band | Monomorph band | Unique band | Range of band |
|---|---|---|---|---|---|
| REPlR-I | 112 | 13 | 0 | 2 | 200-2500 bp |
| REP2-I | 165 | 13 | 1 | 1 | 350-3000 bp |
| REPlR-I+ REP2-I | 257 | 26 | 0 | 3 | 200-3000 bp |

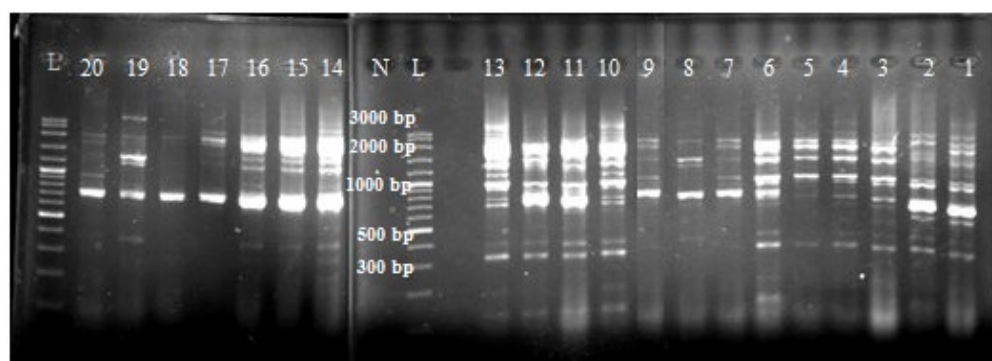**Figure 3.** UPGMA dendrogram of isolates based on REPlR-I marker by NTSYS software.



**Figure 4.** REP-PCR profile of isolates studied based on REP2-I primer on 1.5% agarose gel. 1: C6M3, 2: Y1L4, 3: T20, 4: T22, 5:TD4, 6: TD10, 7: Y2B4, 8: T3, 9: T14, 10: T16, 11: T2, 12: T4, 13: Y2P3, L: 100 bp DNA ladder, N: Negative control, 14: TD5, 15: T15, 16: 14, 17: TD3, 18: T9, 19: T5, 20: C6I4, L: 100 bp DNA ladder.

amplified, mostly in the C6M3, T16, and Y2P3 isolates (*L. casei*) with 13 bands, and the lowest number were observed in the C6I4 isolate with 4 bands. Bacteria C6M3, Y2P3, T22, TD4, TD10, T20, Y1L4, T14, T16, T2, T4, Y2B4, and T3 were grouped in cluster I (*L. casei* isolates), bacteria T15, TD5, TD3, T9, and T14 (*L. brevis* isolates) in cluster II, bacteria C6I4 (*Enterococcus facium*) in cluster III, and the T5 isolate (*L. plantarum*) in cluster IV (Figure 5).

markers was more complex, with 16 polymorph bands and 3 unique bands observed. Four main clusters were formed in the dendrogram. Bacteria C6M3, Y2P3, T22, TD4, TD10, T20, Y1L4, T14, T16, T2, T4, Y2B4, and T3 were classified in cluster I (*L. casei* isolates), bacteria C6I4 (*Enterococcus facium*) in cluster II, T15, TD5, TD3, T9, and T14 (*L. brevis* isolates) in cluster III, and T5 isolates (*L. plantarum*) in cluster IV (Figure 6).

### Detection of Genetic Diversity Based on Combination of REPIR-I and REP2-I Primers

The PCR profile of isolates based on combination of REPIR-I and REP2-I

### DISCUSSION

Local dairy products and traditional fermented foods are rich in probiotics, and owing to these bacteria, they have useful, health promoting properties. It has been
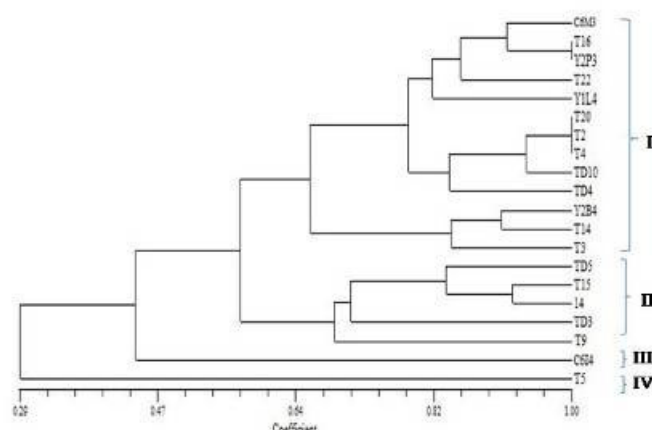
**Figure 5.** UPGMA dendrogram of isolates based on REP2-I marker by NTSYS software.
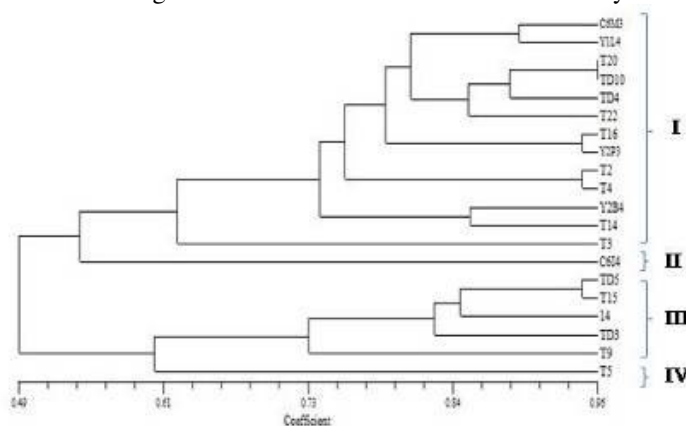


**Figure 6.** UPGMA dendrogram of lactobacilli isolates based on combination of REPIR-I and REP2-I primers by NTSYS software.

some years since the isolation and identification of probiotic bacteria in various traditional dairy products of the world, including Iran, have attracted researchers' interest. The application of biochemical methods, such as sugar fermentation and gas production, are considered to be common methods for identifying bacteria. However, these techniques are limited in terms of time and expense, as well as in the ambiguity of test results [3, 16]. Hence, applying various molecular techniques has resolved these problems. In the isolation process, a vast population of bacteria are always isolated and purified, and some of these bacteria are the same strains of a species. Therefore, choosing an accurate and reliable molecular

method is necessary for optimizing time and expenditure. One accurate molecular identification method is bacterial ribosomal RNA sequencing. This technique was utilized in the present study in the 16S rRNA region. This is a variable and protected region of the genome, in which strain differences result from differences in variable regions. To proliferate and identify bacteria in this region, researchers have designed different primers for sequencing the desired segment [27, 4, 35, 37]. In this research, using sequencing methods, isolated bacteria from various traditional dairy and local Tarkhineh food sources were identified and registered in the GenBank database: *L. casei, L. brevis, L. plantarum,* and

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*Enterococcus facium.* In the *L. casei* group, 7 bacteria were isolated from Tarkhineh, 2 from Tarkhineh yogurt drink, 3 from yogurt, and 1 from cheese. In the *L. brevis* group, 2 bacteria were isolated from Tarkhineh, 2 from Tarkhineh yogurt drink, and 1 from yogurt. Finally, in the *L. plantarum* group, 1 bacterium was isolated from Tarkhineh. Another typing method used in this study was a REP-PCR fingerprinting technique using REP1R-I and REP2-I primers. This method was first used by Versalovic in 1991 for typing gram-negative bacteria [36]. Later, other researchers used this method for gram-positive bacteria genotyping, and experiments showed that this technique had a high differentiating power for distinguishing bacteria. This technique has also been used in identifying and typing various bacteria such as lactic acid bacteria, lactobacilli, geobacilli, and enterococci [12, 20, 28].

Three main clusters are seen in the dendrogram plotted using an UPGMA method based on REP1R-I primer (Figure 3). In cluster I, *L. casei* isolates formed different clusters according to genetic similarities and differences, and they were grouped together. C6I4 isolate (*Enterococcus facium*) was also grouped with *L. casei*. According to DNA fingerprinting with a REP1R-I primer, C6M3, Y2P3, T22, TD4, and TD10 isolates showed the most genetic similarity with one another and were grouped together. As a result, REP1R-I primer was not able to differentiate these isolates. *L. brevis* isolates formed cluster II, and the greatest genetic similarity was found between TD5 and T15. The T5 isolate (*L. plantarum*) formed the separate cluster III, and maintaining genetic distance, it was placed near *L. brevis* isolates.

In the dendrogram based on REP2-I, four main clusters were formed (Figure 5). *L. casei* was placed in cluster I and *L. brevis* in cluster II. The C6I4 and T5 isolates formed separate clusters of III and IV, respectively. The C6I4 isolate was grouped near the *L. brevis* isolates, and T5 keeping genetic distance was also placed close to the *L. brevis* isolates. In cluster II, the greatest similarity was observed between the T15 and T14 isolates.

The combined REP1R-I and REP2-I primers dendrogram showed four main clusters (Figure 6). The *L. casei* isolates were placed in cluster I, and the most similarity was observed between T20 and TD10 isolates. The C6I4 isolate formed a separate cluster II, close to the *L. casei* isolates. *L. brevis* isolates grouped together in cluster III, and the T5 isolate (*L. plantarum*) was grouped separately in cluster IV.

The resulting profiles from amplification of REP1R-I and REP2-I and REP1R-I+REP2-I primers are indicative of the genetic loci of 13, 13 and 26, respectively, for each primer. Moreover, the creation of distinct polymorphic bands showed a high genetic variation in these isolates, and proved the ability of REP markers in distinguishing genetic variations between isolates.

The correlation between similarity matrixes of marker data was determined using a Mantel test. It was assessed to be; very good for REP1R-I (r> 0.9), good for REP2-I (0.9> r> 0.8), and good for the combined REP1R-I and REP2-I (0.9> r> 0.8) (Table 3).

The interesting point in all of the dendrograms is the distinction of different *Lactobacillus* isolates including *L. casei, L. brevis,* and *L. plantarum*, with each isolate grouped and clustered separately in its own group and cluster. This was precisely in line

**Table 3.** Mantel test results.

|  | REPlR-I | REP2-I | REPlR-I+ REP2-I |
| --- | --- | --- | --- |
| Correlation between similarity and Cophenetic matrices for each marker | r= 0.91 | r= 0.82 | r= 0.88 |

with sequencing results and exhibits the high distinction power of the REP markers used in investigating the genetic variety of isolates. It seems that the C6I4 isolate is genetically heterogeneous since in dendrograms plotted according to REP1R-I and REP1R-I+REP2-I primers, it was located near *L. casei* isolates, but in a dendrogram based on the REP2-I marker, it was grouped close to *L. brevis* isolates. Another point observed in the dendrograms is the different groupings of the *L. casei* and *L. brevis* isolates. Some isolates were grouped together with one primer, but changed groups with another primer. It should be noted that the results of all groupings from *Lactobacillus* species with all markers used are not totally confirmed, because different primers target different genetic regions and show genetic variations in a variety of forms. Different grouping of isolates extracted from honey with various REP markers were found by Lo´pez and Alippi in 2009 [17] and by Marco and Ralf in 2002 on *L. johnsonii* [18].

REP-PCR fingerprinting analysis can be considered as a complementary technique for 16S rRNA sequencing. REP-PCR was a useful tool for differentiating LAB isolated from Iranian Tarkhineh and dairy products in this study. This technique has some advantages of being easy, fast, repeatable, low-cost, and powerful for genomic typing of isolates.

## CONCLUSIONS

The present study revealed the efficacy of REP-PCR genome fingerprinting with modified REP1R-I and REP2-I primers in investigating phylogenetic relationships and genetic variation of gram-positive lactic acid bacteria isolates. As sequencing is considered an accurate and reliable technique, the genotyping performed with REP-PCR fully confirmed sequencing as a complementary method. It is hoped to provide an accurate data bank with the profiles obtained, for initial screening and

eventually, typing of isolated bacteria at lower cost and higher speed.

This is the first report on REP-PCR application and 16S rDNA sequencing for the identification and evaluation of genetic diversity of probiotic bacteria isolated from Tarkhineh food and dairy products in Iran.

## ACKNOWLEDGEMENTS

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## بکارگیری عناصر پالیندرومی تکراری بر پایه PCR برای شناسایی ارتباط ژنتیکی گونه های باکتری های اسید لاکتیک جدا شده از محصولات غذایی سنتی تخمیری

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### چکیده

ترخینه یکی از غذاهای سنتی ایران بوده و غنی از باکتری های پروبیوتیک می باشد. هدف از این تحقیق، شناسایی باکتری های جدا شده از ترخینه بر اساس توالی یابی 16S rRNA و تایپینگ مولکولی بر اساس توالی های تکراری پالیندرومی (REP-PCR) می باشد. در کل، ۲۰ باکتری مختلف از ترخینه و محصولات لبنی سنتی جدا شدند. شناسایی مولکولی ایزوله ها براساس توالی یابی

ناحیه 16S rRNA انجام شد و توالی ها در GeneBank ثبت شدند. واکنش REP-PCRبا
مارکرهای REP1R-I، REP2-I، REP1R-I+REP2-I جهت شناسایی و انگشت نگاری ایزوله
ها انجام گرفت. از روش خوشه بندی UPGMA بر اساس ضریب تشابه Dice برای رسم دندروگرام
استفاده شد. بر اساس پرایمر REP1R-I، ایزوله ها در سه کلاستر اصلی و بر اساس پرایمرهای
REP2-I و REP1R-I+REP2-I در چهار کلاستر اصلی در دندروگرام گروهبندی شدند. در
تمامی آنالیزها، ایزوله های *Lactobacillus casei, Lactobacillus brevis,*
*Lactobacillus plantarum, Entrococcus facium* کلاستر مجزایی تشکیل دادند. نتایج
حاصل از توالی یابی با خوشه بندی ایزوله ها بر اساس مارکرهای REP در دندروگرام مطابقت کامل
نشان داد. بر اساس نتایج حاصله، روش REP-PCR یک روش دقیق جهت تعیین تنوع ژنتیکی گونه
های باکتری های اسید لاکتیک می باشد.