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Comparison of lactic acid bacteria diversity during the fermentation of Tarhana produced at home and on a commercial scale

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Abstract In this study, lactic acid bacteria diversity during the fermentation of homemade and commercially prepared Tarhana, a traditional fermented cereal food from Anatolia, was determined and compared. The isolates collected from Tarhana dough were differentiated according to their $(GTG)_5$ profiles and then identified using 16S rDNA and *phe*S gene sequences. The variation of lactic acid bacteria during fermentation was also screened using PCR-DGGE. Commercially prepared Tarhana dough was fermented with higher *Lactobacillus* spp. diversity than homemade Tarhana dough. *Lactobacillus casei, L. alimentarius, L. fabifermentas*, and *L. paralimentarius* were identified differently from the fermentation of commercially prepared Tarhana dough. PCR-DGGE analysis revealed that *L. plantarum* was the main strain for homemade Tarhana, whereas *L. brevis* and *L. alimentarius* were observed in commercially prepared Tarhana dough fermentation. In conclusion, *L. plantarum, L. brevis* and *L. alimentarius* can be useful as a potential starter culture for the industrial production of Tarhana.

Keywords: Tarhana, lactic acid bacteria, diversity, industrial production

Introduction

Traditional fermented foods are an inexpensive, practical, and convenient source of nutrients for the modern world, particularly in areas that are prone to famine. Fermentation is a natural way of enhancing the nutritional value of food through the synthesis of essential amino acids and vitamins during the fermentation process. Furthermore, fermentation also helps preserve foods and prevent spoilage (1). Tarhana is a traditional Anatolian fermented food, produced by mixing wheat flour, yogurt, sourdough, and different vegetables and spices (also includes tomato, red pepper, onion, mint, and salt), which is dried and ground following fermentation at room temperature for one week. Accordingly, the fermentation of Tarhana is important for the development of the product's flavor and aroma (2,3).

Although they function together with yeasts, lactic acid bacteria (LAB) play an important role during the fermentation of Tarhana (4,5). The metabolites produced by LAB yield the characteristic taste and flavor of Tarhana and extend its shelf life by reducing the pH. Therefore, studies on LAB identification and their alteration during fermentation are required to understand the complexity of the fermentation process. In one study (6), Tarhana fermentation was reported to include 27% *Pedicoccus acidilactici*, 19% *Streptococcus thermophilus*, 19% *Lactobacillus fermentum*, 12% *Entericoccus faecium*, 7% *P. pentosaceus*, 5% *Leuconostoc pseudomesenteroides*, 4% *Weissella cibaria*, 2% *L. plantarum*, 2% *L. bulgaricus*, 2% *Leu. citreum*, 1% *L. paraplantarum*, and 0.5% *L. casei*. In another study, *P. acidilactici*, *L. brevis*, and *L. plantarum* were identified from 2 different Tarhana doughs, which were fermented at 30 and 40°C. *Pediococcus* strains were predominant at 40°C, whereas mesophilic *Lactobacillus* spp. were predominant at 30°C (7).

In recent years, due to the busy daily life of consumers, the production of Tarhana on a domestic scale has decreased, whereas the production of commercial Tarhana on an industrial scale has substantially increased. Therefore, the present study identified LAB diversity during the fermentation of both homemade and commercially prepared Tarhana dough, which may facilitate the development of potential Tarhana starter cultures, by a combination of techniques such as using enriched media for isolation, genomic identification, and culture-independent PCR-based methods.

Materials and Methods

Tarhana dough samples, referred to as homemade and commercially prepared Tarhana (Table 1) in the study, were obtained from four

homes (coded as A, B, C, and D) and 4 plants (coded as E, F, G, and H). The Tarhana dough samples were obtained from each producer at 5 fermentation time points (day 0, 1, 3, 5, 10, and 15) under aseptic conditions. Acidity analysis of the dough samples before and after fermentation was performed according to standard (TS 2282) authorized by the Turkish Standards Institution. Ethanol (67%, 50 mL) was added to 10 g of the sample and transferred to a flask (250 mL). Following stirring and filtration, titration was performed with 0.1 N NaOH until a permanent pink color was obtained. The acidity values were expressed after multiplying the amount of spent NaOH with a dilution factor of 5.

Bacterial cultures isolated during the study were stored in a growth medium containing 30% sterile glycerol at 70°C.

Isolation and identification of LAB LAB were anaerobically isolated from 0.01% cycloheximide-containing MRS-5C agar (8) plates incubated at 30°C for 48 h. Tarhana samples (10 g) were mixed with 90 mL sterile physiological saline (0.85% w/v, NaCl) to prepare the initial dilution. Serial dilutions were made for each sample and then 1 mL of the appropriate dilution was plated on MRS-5C agar with cycloheximide to enumerate the total LAB using the pour plate method. The prepared plates were incubated anaerobically at 30°C for 48 h. Approximately 50 colonies were collected from each sample and then the catalase test and Gram staining were done for all isolates. Gram-positive, catalase-negative, and non-motile micro-organisms were preserved.

The isolates were differentiated using $(GTG)_5$ profiles generated by PCR (Techne, Cambridge, UK) using a primer (5'-GTGGTGGTGGTGGTG-3') and genomes of LAB as templates (9). The PCR mixture consisted of 4 μL master mix (5*FIREPol Master Mix/SOLIS Bio Dyne, Tartu Estonia), 0.75 μL primers (50 mM), 2 μL DNA, and the final volume was adjusted to 20 μL with dH_2O. PCR (Techne) was conducted with an initial denaturation at 95°C for 3 min, 30 cycles of 95°C for 1 min, 45°C for 30 s, 72°C for 5 min, and final extension at 72°C for 10 min.

The isolates were identified by sequencing the V3 region (1,533 bp) of the 16S rDNA, which was amplified using a primer pair: pA (5'-AGAGTTTGATCCTGGCTCAG-3') and pH (5'-AAGGAGGTGATCCAGCC GCA-3') (9). PCR mixture was prepared using 5 μL buffer, 2 μL (400 μM each) dNTP mixture (Thermo Fisher Scientific, Waltham, MA, USA), 1 μL (10 mM each) pA and pH primers, 1 μL (5 U) Platinum Hi-Fi Taq DNA polymerase (Thermo Fisher Scientific), and 5 μL genomic DNA. The total volume was adjusted to 50 μL with sterile ultrapure dH_2O. The PCR protocol was as follows: denaturation at 95°C for 5 min, followed by 30 cycles at 95°C for 30 s, 53°C for 30 s, 72°C for 1 min, and a final extension at 72°C for 10 min (9).

The 500 bp of phenylalanyl-tRNA synthetase alpha subunit (*phe*S) gene was also amplified by a primer pair pheS21-F and pheS22-R (5'-CAYCCNGCHCGYGAYATGC-3' and 5'-CCWARVCCRAARGCAAARCC-3') and subsequently sequenced for identification (10). The PCR mixture comprised 8 μL master mix (5*FIREPol Master Mix/SOLIS Bio Dyne), 1 μL primer, and 2 μL genomic DNA, and the final volume was completed to 40 μL using sterile ultrapure water. A touch-down PCR protocol (denaturation at 95°C for 5 min, 10 cycle at 95°C for 1 min, first: 52°C for 45 s, last: 42°C for 45 s, 72°C for 1 min, 20 cycles of 95°C for 30 s, 46°C for 45 s, 72°C for 1 min, and final extension at 72°C for 10 min) was applied (10).

Culture-independent analysis of Tarhana fermentation The LAB diversity during Tarhana fermentation was determined using a culture-independent approach using the Polymerase Chain Reaction Denaturating Gradient Gel Electrophoresis (PCR-DGGE) method using a D-Code Universal Mutation Detection System (BioRad, Hercules, CA, USA) (11). Bacterial genomic DNA was isolated from the Tarhana dough sample as previously described with few modifications of the food samples (12). The Tarhana sample (10 g) was homogenized in 90 mL of peptone physiological water and then 50 mL of this homogenate was centrifuged at 1,000×*g* for 5 min. The supernatant was transferred and centrifuged at 5,000×*g* for another 15 min. Lysozyme (50 mg mL^{-1}, Sigma-Aldrich, St. Louis, MO, USA) was used to disrupt the LAB cell wall and finally genomic DNA was extracted and purified using a DNA isolation kit (Invitrogen, Carlsbad, CA, USA).

In the PCR-DGGE analysis, bacterial genomic DNA samples were used to amplify the V3 region of 16S rDNA with F338 primer (5'-ACTCCTACGGGAGGCAGCAG-3') with a GC clamp together with the R518 primer (5'-ATTACCGCGGCTGCTGG-3') (12). The PCR mixture comprised 8 μL master mix (5*FIREPol Master Mix/SOLIS Bio Dyne), 1 μL primer, and 2 μL genomic DNA. The amplification program was 95°C for 5 min initial denaturation, 30 cycles of 95°C for 30 s, 55°C for 45 s, 72°C for 1 min, and a final extension step at 72°C for 10 min. The amplified fragments were loaded on 8% polyacrylamide gel containing 25–50% denaturants (7 M urea and 40% formamide) at 50 V for 15 min at 60°C. Gels were stained with ethidium bromide and monitored under UV illumination.

The amplified 16S rDNA V3 regions of isolated and identified LAB strains were separated on 8% polyacrylamide gel containing 25–50% denaturant before loading the fragments amplified from Tarhana samples to correlate each band that appeared with the corresponding LAB species. Three reference markers (M1, M2, and M3) were prepared according to the migration of the amplified 16S rDNA V3 regions of different LAB strains. The LAB profiles of Tarhana dough samples for each day were determined by using these reference markers.

Sequencing Amplified fragments from the 16S rDNA and *phe*S genes were purified using a PCR purification kit (Thermo Fisher Scientific) according to the supplier's instructions. In addition, the unidentified DGGE bands were excised from the gels, re-amplified with primers (F338 without a GC clamp and R518), and then purified as described above. The DNA sequence was determined by the dideoxy chain termination method using the relevant primers. The search for DNA similarity was undertaken through the National Center for Biotechnology Information Gene Bank.

Results and Discussion

The LAB diversity of homemade and commercially prepared Tarhana dough The acidity values of homemade and commercially prepared dough prior to fermentation were 4.51 and 5.10, respectively, whereas after fermentation, the acidity values increased to 18.45 and 17.25, respectively. According to the TS2282 standard requirements, which should be between 10 and 35 after extraction by 67% ethanol, all the dough samples had sufficient acidity values at the end of the fermentation period.

The 2,000 LAB isolates that were collected during the Tarhana dough fermentation period were divided into 37 groups according to the similarities in the $(GTG)_5$ profiles. Each strain in these groups had three to six bands. The dough samples coded C, E, G, and H included the highest number LAB isolates showing different $(GTG)_5$ profiles. When the total number of LAB isolates showing different $(GTG)_5$ profiles at each dough sample were compared, commercially prepared Tarhana dough showed a higher level of different LAB isolates than that of the homemade Tarhana dough, indicating that these dough samples could have high LAB diversity (Table 1).

One representative sample of the LAB isolates was selected from each of the 37 groups for identification. According to the 16S rDNA sequences, LAB isolates obtained from the homemade Tarhana dough samples were identified as *L. plantarum* (BL3, BL4, BL5, CL4, CL6, DL2, and DL3), *Lactococcus lactis* (CL3, CL5, and DL1), *L. namurensis* (AL2 and AL3), *L. brevis* (CL2 and BL1), *L. farciminis* (BL2), *Leu. mesenteroides* (CL1), and *P. acidilactici* (AL1), whereas *L. plantarum* (EL3, EL5, FL1, FL2, FL3, FL4, GL4, GL5, and HL4), *L. brevis* (EL1, FL5, GL3, and HL6), *L. casei* (GL1), *L. fabifermentas* (HL1), *L. mindensis* (HL2), *L. paralimentarius* (HL3), *L. namurensis* (HL5), and *L. farciminis* (EL4) were obtained from the commercially prepared Tarhana dough samples with more than 97% homology. Only *L. alimentarius* (GL2) was identified as having an insufficient identification ratio (85%) (Table 2).

To support the identification results of the isolates considering the 16S rDNA sequences, the partial *phe*S gene of isolates was also sequenced. When the identification results based on the sequences of both 16S rDNA and *phe*S were compared, all strains except AL2 and BL2 had results consistent with the 16S rDNA. Accordingly, these strains were identified as *L. arizonensis* (AL2) and *L. vini* (BL2). However, the homology values of strains AL2 and BL2, which were identified differently using the *phe*S DNA sequence, were lower than the threshold value (97%) (Table 2).

The ultimate identification was achieved by comparing the sequences of 16S rDNA and *phe*S gene to determine the LAB diversity in the Tarhana dough samples. The isolates BL3, BL4, BL5, CL4, CL6, DL2, DL3, EL3, EL5, FL1, FL2, FL3, FL4, GL4, GL5, and HL4 were identified as *L. plantarum* wherein BL1, CL2, EL1, FL5, GL3, and HL6 were identified as *L. brevis*; CL3, CL5, and DL1 were identified as *Lc. lactis*; AL2, AL3, and HL5 were identified as *L. namurensis*; BL2 and EL4 were identified as *L. farciminis*; CL1 was identified as *Leu. mesenteroides*; AL1 was identified as *P. acidilactici*; HL1 was identified as *L. fabifermentas*; GL2 was identified as *L. alimentarius*; GL1 was identified as *L. casei*; HL2 was identified as *L. mindensis*; and HL3 was identified as *L. paralimentarius*.

L. plantarum and *L. brevis* were prevalent and persistent in the tested Tarhana dough samples. Similarly, *L. plantarum* and *L. brevis* were the most frequently isolated strains from the Tarhana fermentation incubated at 30°C (5,7). In contrast, *P. acidilactici* and *S. thermophilus* were predominant in the Tarhana dough samples collected from a different region (6). This is most likely related to the fermentation temperature used during the production. In fact, when the fermentation temperature of Tarhana dough was increased to 40°C, the resulting LAB biota changed to a *P. acidilacitici* dominance (7).

In this study, *L. plantarum*, *L. alimentarius*, *L. brevis*, *Lc. lactis*, *L. mindensis*, *L. pentosus*, and *Leu. mesenteroides* were isolated from the fermented Tarhana dough, indicating a similar LAB diversity characteristic as sourdough (13-15). Essentially, sourdough is one of the ingredients of Tarhana production (1-3) as the main component is flour. However, interestingly, *L. fabifermentas*, which has not been previously isolated from sourdough, was isolated from several Tarhana dough samples. This species was first isolated from cocoa bean heap fermentations (16); furthermore, it has been reported as one of the dominant species in grape marc, and its genome has been sequenced (17).

The Tarhana dough samples actually had a higher LAB diversity when compared with the previous findings (6,15). This might be due

Table 1. The Tarhana dough characteristics, fermentation conditions, and number of different $(GTG)_5$ profiles determined from homemade (A, B, C, and D) and commercially prepared Tarhana (E, F, G, and H) dough samples

Dough code	Characteristics and fermentation conditions	Different $(GTG)_5$ profiles
A	Traditional homemade Tarhana dough was produced in 10 kg batches at home-scale. Wheat flour, yogurt, tomato puree, chopped red pepper, onion, mint, salt, and sourdough were used as ingredients. The homemade Tarhana dough was fermented for 15 days and mixed every day.	3
B		5
C		6
D		3
E	Commercially prepared Tarhana dough was produced on an industrial scale in 100 kg batches. The ingredients used for Tarhana doughs were 40% wheat flour, 20% chopped red pepper, and 12% onion, 16% yogurt, 10% tomato puree, 1% salt, 0.5% mint, and 0.5% sourdough. The fermentation temperature of the commercially prepared Tarhana dough was maintained at 25°C for 15 days and the dough was mixed periodically.	4
F		5
G		5
H		6

Table 2. Identification of LAB isolates with partial sequences of the 16S rDNA and *phe*S genes

Isolate Code	Identification with 16S rDNA sequence	Homology %	Identification with *phe*S gene sequence	Homology %	Genebank[2)] Accession Number
Homemade Tarhana doughs					
AL1	*P. acidilactici*	98	*P. acidilactici*	97	KT285575
AL2	*L. namurensis*	99	*L. arizonensis*	82	KT285576
AL3	*L. namurensis*	99	*NI*[1)]	-	KT285577
BL1	*L. brevis*	99	*L. brevis*	100	KT285578
BL2	*L. farciminis*	98	*L. vini*	77	KT285579
BL3	*L. plantarum*	99	*L. plantarum*	99	KT285580
BL4	*L. plantarum*	100	*L. plantarum*	100	KT285581
BL5	*L. plantarum*	99	*L. plantarum*	100	KT285582
CL1	*Leu. mesenteroides*	97	*Leu. mesenteroides*	97	KT285583
CL2	*L. brevis*	97	*L. brevis*	97	KT285584
CL3	*Lc. lactis*	99	*NI*	-	KT285585
CL4	*L. plantarum*	99	*L. plantarum*	99	KT285586
CL5	*Lc. lactis*	99	*Lc. lactis*	99	KT285587
CL6	*L. plantarum*	99	*L. plantarum*	100	KT285588
DL1	*Lc. lactis*	99	*NI*	-	KT285589
DL2	*L. plantarum*	99	*L. plantarum*	100	KT285590
DL3	*L. plantarum*	99	*L. plantarum*	99	KT285591
Commercially prepared Tarhana doughs					
EL1	*L. brevis*	99	*L. brevis*	99	KT285592
EL3	*L. plantarum*	99	*L. plantarum*	99	KT285593
EL4	*L. farciminis*	99	*L. farciminis*	100	KT285594
EL5	*L. plantarum*	99	*L. plantarum*	99	KT285595
FL1	*L. plantarum*	99	*L. plantarum*	99	KT285596
FL2	*L. plantarum*	99	*L. plantarum*	99	KT285597
FL3	*L. plantarum*	100	*L. plantarum*	99	KT285598
FL4	*L. plantarum*	99	*L. plantarum*	99	KT285599
FL5	*L. brevis*	99	*L. brevis*	100	KT285600
GL1	*L. casei*	99	*L. casei*	100	KT285601
GL2	*L. alimentarius*	85	*L. alimentarius*	97	KT285602
GL3	*L. brevis*	100	*L. brevis*	99	KT285603
GL4	*L. plantarum*	98	*L. plantarum*	99	KT285604
GL5	*L. plantarum*	98	*L. plantarum*	96	KT285605
HL1	*L. fabifermentas*	99	*L. fabifermentas*	99	KT285606
HL2	*L. mindensis*	99	*L. mindensis*	99	KT285607
HL3	*L. paralimentarius*	99	*L. paralimentarius*	99	KT285608
HL4	*L. plantarum*	99	*L. plantarum*	100	KT285609
HL5	*L. namurensis*	99	*NI*	-	KT285610
HL6	*L. brevis*	99	*L. brevis*	99	KT285611

[1)]NI, Could not be identified.
[2)]The highest nucleotide homology recorded at the Genebank.

to the use of a modified MRS growth medium (supplemented with different sugar and vitamins) for isolation. Evidently, the use of modified MRS increases the isolation diversity when performing isolations from sourdough (8).

The alteration of LAB strains in homemade and commercially prepared Tarhana dough samples during fermentation The LAB strains existing in each dough and the fermentation time point are shown in Fig. 1. Moreover, the homemade and commercially prepared Tarhana dough samples were compared according to the occurrence of each LAB strain during fermentation, as shown in Table 3. In dough A, *L. namurensis*, *P. acidilactici*, *L. mindensis*, and *S. thermophilus* appeared from day 1 to 10; however, in the same sample, the presence of *L. plantarum* was detected throughout the fermentation process (Fig. 1A). In dough B, *L. crispatus* and *S. thermophilus* were present in all fermentation periods, whereas *L. crispatus* was unique for this sample (Fig. 1A). In dough C, *Lc. lactis, Leu. mesenteroides*, and *L. bulgaricus* were present throughout the fermentation process and *L. plantarum, L. alimentarius, L. brevis,* and *L. sanfranciscensis* were also present after day 3 (Fig. 1B). In dough D, *L. plantarum*, *Lc. Lactis*, and *L. casei* were present throughout the fermentation process; however, *L. namurensis, L. bulgaricus*, and *P. acidilactici*

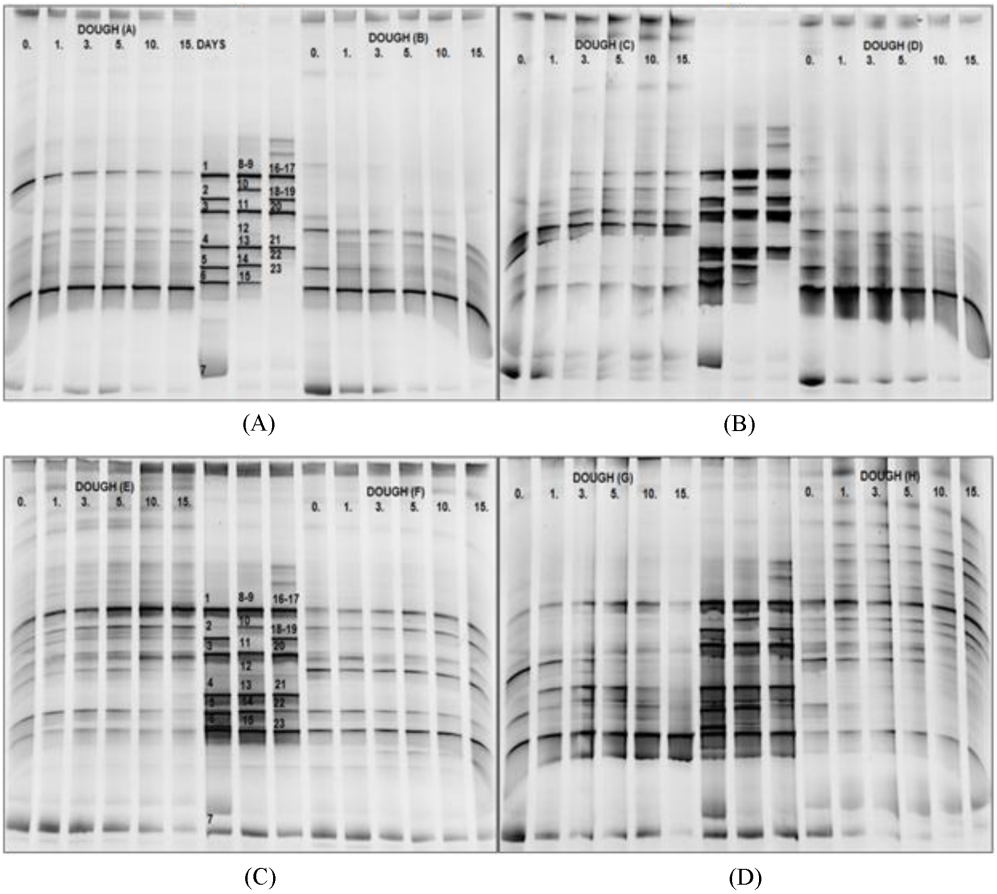

Fig. 1. PCR-DGGE profiles of homemade (A and B) and commercially prepared Tarhana (C and D) dough samples obtained at fermentation day 0, 1, 3, 5, 10, and 15. Reference strains: 1. *L. plantarum*, 2. *Leu. mesenteriodes*, 3. *L. brevis*, 4. *L. farciminis*, 5. *P. acidilactici*, 6. *L. namurensis*, 7. *L. casei*, 8. *L. plantarum*, 9. *Leu. citreum*, 10. *L. alimentarius*, 11. *Lc. lactis*, 12. *L. mindensis*, 13. *L. farciminis,* 14. *L. namurensis*, 15. *Lc. lactis*, 16. *L. fabifermentas*, 17. *L. plantarum*, 18. *L. brevis,* 19. *L. brevis*, 20. *Leu. mesenteriodes*, 21. *L. paralimentarius*, 22. *Leu. pseumesenteriodes*, and 23. *L. farciminis*.

disappeared by day 5 (Fig. 1B).

The DGGE profile of dough E showed that *L. plantarum*, *L. brevis*, *L. mesenteroides, L. casei*, *L. alimentarius*, and *L. pentosus* were detected at all fermentation periods (Fig. 1C). In dough samples F and G, *L. plantarum* and *L. brevis* were present until the end of fermentation. In addition to these species, *L. alimentarius and L. mindensis* were found in dough F and G (Fig. 1C), whereas *L. namurensis* was present in dough G (Fig. 1D); however, among these strains in dough G, *L. mindensis* disappeared on day 3, whereas *L. namurensis* disappeared on day 10. In dough H, *L. plantarum, L. farciminis*, and *L. brevis* existed until the end of fermentation but *L. bulgaricus* disappeared toward the end of fermentation (Fig. 1D).

In the PCR-DGGE analysis, *L. sanfranciscensis, L. bulgaricus*, and *S. thermophilus* were detected in some of the homemade and commercially prepared Tarhana dough samples during the initial fermentation period but were not detected during the end of fermentation. It can be noted that these strains could not be identified using culture-dependent methods. In the present study, *S. thermophilus* and *L. bulgaricus* in both homemade and commercially prepared Tarhana dough were only detected using the PCR-DGGE method. This result differs from previous reports (6), indicating that although yogurt is the main ingredient of Tarhana dough, the relevant cultures *S. thermophilus* and *L. bulgaricus* could not grow during the fermentation probably due to the low fermentation temperature (25°C).

The PCR-DGGE analysis supported the results of culture-dependent analysis. In fact, *L. plantarum* was observed constantly in both the homemade and commercially prepared Tarhana dough samples wherein *L. brevis* was present in the commercially prepared Tarhana dough samples. *L. alimentarius* increased with fermentation in the commercially prepared Tarhana dough samples but the persistence of *L. bulgaricus* and *S. thermophilus* gradually decreased with fermentation (Table 3). As a result, the DGGE analysis also revealed that the commercially prepared Tarhana dough showed greater LAB diversity during fermentation than the homemade Tarhana dough.

In conclusion, Tarhana production from home to the industrial scale enriched the LAB diversity existing in the Tarhana fermentation. Commercially prepared Tarhana dough is fermented with a large diversity of LAB, consisting mainly of sourdough-associated *Lactobacillus* strains. In this study, *L. plantarum* and *L. brevis,* as well as *L.*

Table 3. Occurrence of LAB strains in homemade (A, B, C, and D) and commercially prepared Tarhana (E, F, G, and H) dough samples during fermentation

LAB strains	Fermentation days					
	0	1	3	5	10	15
L. plantarum	A,D E,F,G,H	A,D E,F,G,H	A,B,C,D E,F,G,H	A,B,C,D E,F,G,H	A,B,C,D E,F,G,H	A,B,C,D E,F,G,H
L. namurensis	A,D G	A,D G	A,D G	A,D G	A G	- G
P. acidilactici	A,D -	A,D -	A,D -	A,D -	A -	- -
L. mindensis	A F	A F	A F	A F	A F	A F
L. crispatus	B -	B -	B -	B -	B -	B -
L. bulgaricus	B,C,D F,G,H	C,D F,G,H	C,D G,H	C,D G,H	C G	C -
S. thermophilus	A,B,C,D G	A,B,C,D G	A,B,C,D G	A,B,C G	A,B G	A,B G
L. farciminis	- H	B H	B H	B H	B H	B H
Lc. lactis	C,D -	C,D -	C,D -	C,D -	C,D -	C,D -
Leu. mesenteroides	C E,H	C E,H	C E,H	C E,H	C E,H	C E,H
L. casei	C,D E,F	C,D E,F	D E,F	D E,F	D E,F	D E,F
L. sanfranciscensis	- -	H -	C, H	C, H	C, H	C, H
L. alimentarius	- E,F	- E,F,G	C E,F,G	C E,F,G	C E,F,G	C E,F,G,H
L. brevis	- E,F,G,H	- E,F,G,H	C E,F,G,H	C E,F,G,H	C E,F,G,H	C E,F,G,H
L. pentosus	- E	- E	- E	- E	- E	- E
L. fabifermentas	- G	- G	- G	- G	- G	- G
Leu. citreum	- H	- H	- H	- H	- H	- H
P. pentosaceus	- H	- H	- H	- H	- H	- H

alimentarius, were monitored constantly in commercially prepared Tarhana, which could be useful as potential starter cultures at the industrial production of Tarhana.

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