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Retracted Article: Isolation of *Lactobacillus* Species from Domestic Dairy Products of Mahabad City

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Abstract

Background: *Lactobacillus* is the most important group of lactic acid bacteria that produce acid lactic through fermentation of glucose, which is an important process in dairy production. Due to the natural microbial ecosystems of milk, the traditional dairy products provide a suitable culture for growth and proliferation of *Lactobacillus* strains.
Objectives: The aim of this study was to investigate *Lactobacillus* species from domestic dairy products of Mahabad city.
Methods: In this study, 90 samples of cheese, yogurt, and dough were collected from the nearby villages of Mahabad. For the isolation of *Lactobacillus*, samples were cultured in MRS agar and grown colonies were tested gram stain, catalase test, and morphology. Catalase-negative, gram positive, and without spores bacillus identification and purification. Then, oxidase tests, reduce nitrate, and oxidation-reduction was performed on the SIM medium. To identify species of *Lactobacillus*, growth in 15°C - 45°C, gas production from glucose with Durham tube to detect homo or hetero Fermentative, and fermentation Fructose, Maltose, Lactose, Galactose, Mannose, Ribose, Arabinose, Xylose, Cellobiose, Inositol, Mannitol, Melezitose, Melibiose, Raffinose, Rhamnose, Trehalose, Salicin, Glycerol and Gluconate and esculin hydrolysis were studied.
Results: In 3 products, *Lactobacillus plantarum* strain was identified, which was in cheese (46%) in comparison with yogurt and dough (respectively 13% and 36%).
Conclusions: Based on findings in this study, Cheese provides better culture for growth and proliferation of *Lactobacillus* when compared with yogurt and dough and the *Lactobacillus plantarum* strain was the most of milk microflora and traditional dairy products of Mahabad town.

Keywords: Traditional Dairy Products, Mahabad, *Lactobacillus*

1. Background

A *lactobacillus bacterium* is gram-positive, non-sporulation, motionless, and catalase-negative. These are normal flora of the human body and have a great resistance against salt and antibiotics (1). Probiotic being is the most important of the *Lactobacillus* characteristics; probiotics are the microorganisms where a sufficient number of them were used as a food supplement and provides beneficial effects to the host (2)

Lactobacillus is the normal flora in traditional dairy products and containing probiotic potential such as resistance to gastric acid and bile salts. Production of organic acids and decrease of pH, secretion of antimicrobial compounds, and inhibitors such as bacteriocins, hydrogen peroxide, and compete with pathogens for adhering to the intestine wall, were the *Lactobacillus* effects (3). They are effective in disease improvements such as cancer, peptic ulcer, intestinal inflammation, high blood pressure and cholesterol, diarrhea, allergies, weakened immune systems, as well as lactose intolerance (4, 5). They can be used as natural preservatives in foods with secretion of bacteriocins, which has an inhibitory effect on the growth of spoilage microorganisms (3).

Another important effect of *Lactobacillus*, is anti-aflatoxin activity in dairy products (6). This group of bacteria contains the beta-galactosidase enzyme and can hydrolysis lactose in milk and produce lactic acid by this en-

zyme during the fermentation process. Lactic acid is very effective in creation of dairy products flavor (1). *Lactobacillus* also contains proteinase, lipase, and esterase enzyme. Activity of these enzymes in dairy products, leading to create new compounds such as short-chain fatty acids, digest peptides, and production of new amino acids (7).

One of the traditional dairy products that was evaluated in this study is Kurdish dairy cheese that is known as Coupe cheeses. The cheese is prepared by crushing and putting it into a pottery or tin and placed underground for 2 to 3 months. These conditions will provide the perfect environment for growth the microaerophilic bacteria. Coupe cheese is prepared using rennet cheese and without adding any starters. The Coupe cheese structure is granular and dry (8). Traditional yogurt is prepared using yogurt as the starter (9). Natural enzymes of milk such as protease and lipase are the important factors in the development of favorable cheese taste, which were destroyed in the pasteurization process, however the enzymes were not destroyed in traditional cheese (10). The aim of this study is isolation of the *Lactobacillus* strains from traditional dairy products (cheese, yogurt, dough) of Mahabad city.

2. Methods

From villages in and around Mahabad city, 90 samples of traditional dairy products were collected, which included 30 samples of yogurt, 30 samples of dough, and 30 samples of cheese, which were prepared from 2 types of cattle and sheep milk. These samples transported to the microbiology laboratory. At the beginning 10gr from each samples were added into the erlens which was containing of 90 mL sterilized physiologic serum. After homogenization, samples were diluted up to 5^{0} and were cultured in MRS agar (Merck, Germany). The following cultured samples were placed in aerobic jar and then incubated at 30°C for 48 - 72 hours (11). After incubation, the obtained colonies were performed by gram stain, catalase, and morphology test. Gram-positive, catalase-negative, and without spore bacillus were identified and cultured in the new plates and were incubated in the same condition.

For detection of *Lactobacillus* genus, other tests such as oxidase, reduce of nitrate, oxidation-reduction, and movement in the SIM medium were performed. To identify, the *Lactobacillus* species examined their growth in 15°C and 45°C temperature and fermentation of Fructose, Maltose, Lactose, Galactose, Mannose, Ribose, Arabinose, Xylose, Cellobiose, Inositol, Mannitol, Melezitose, Melibiose, Raffinose, Rhamnose, Trehalose, Salicin, glycerol and Gluconate, and hydrolysis of Esculin.

Furthermore, gas production from glucose was performed with Durham tube for identification of homo or hetero fermentative (12). Finally statistical analysis were performed by Ver21 SPSS software and using t-Test.

3. Results

Among the 90 studied samples, there were *Lactobacillus* in 4 cases of yogurt, 11 cases of dough, and 14 cases of cheese. All *Lactobacillus* were diagnosed as *Lactobacillus plantarum*, due to inability to gas production from glucose, inability to growth at 45°C temperatures, and inability to fermentation of Rhamnose, Glycerol, Inositol, and Trehalose. The *Lactobacillus plantarum* frequency is 46%, 36%, and 13% in cheese, dough, and yogurt, respectively.

According to that may be pH, salt and age of samples were effective in *Lactobacillus* isolation, the samples with *Lactobacillus* were compared with samples without *Lactobacillus*. There were no significant differences in cheese samples ($P > 0.05$). Additionally, there were no observed significant differences in yogurt and dough in terms of age and salt ($P > 0.05$), however, pH differences were statistically significant ($P < 0.05$).

4. Discussion

According to our results, the isolation of *Lactobacillus* from cheese was more compared to the other products (yogurt and dough). The isolated *Lactobacillus* from cheese were about 46% and all of them were *Lactobacillus plantarum*. Cheese has special advantages in comparison to other dairy products for probiotic bacteria. Cheese increased viability of probiotics, due to high pH, solid, and pressed structure (that probiotics protect against unsuitable environmental factors), high buffering capacity (due to high levels of protein), fat percent, and particular storage conditions (10). Cheese has a high pH (4/8 - 5/6) in comparison to other products (3/7 - 4/3), which provides more favorable conditions for the growth of probiotics. Cheese contains micronutrient such as decomposed peptides, decomposed fatty acids (from the process of lipolysis), lactose and organic acids (produced by glycolysis), which considered food source of probiotics in the cheese (10).

Hashemi et al., (2013) reported that there were increased numbers of mesophilic *Lactobacillus* at the end of the reaching process in comparison to the beginning of reaching process, in Kurdish cheese. Slowly metabolism of *Lactobacillus* and adaptability with adverse environmental conditions (low pH, low water activity and high NaCl) in comparison to other lactic acid bacteria, were the cause of existence of this bacteria in large amounts at the end of reaching period (13).

Milani (2012), in a study on biological diversity of traditional Kurdish cheese, reported that in the beginning of

Table 1. Mean of the pH, Age and Salt and Standard Deviation in Cheese, Yogurt and Dough Traditional

Dairy Type	pH	Age (Day)	Salt
Cheese	5.45 ± 0.29	56.83 ± 8.8	4.13 ± 1.77
Yogurt	3.59 ± 0.79	4.13 ± 0.81	
Dough	3.63 ± 0.11	7.86 ± 1.33	

Table 2. Number, Source and Gathering Location of Samples with *Lactobacillus*

	Cheese		Yogurt		Dough	
Samples	**Cattle**	**Sheep**	**Cattle**	**Sheep**	**Cattle**	**Sheep**
Lactobacillus	n (8)	n (6)	n (1)	n (3)	n (9)	n (2)
Sampling location	South (8)	South (5)	South	South (2)	South (3)	South (2)
	North (3)	North (1)		North (1)	North (6)	

Table 3. pH, Age and Salt Difference in Cheese Samples in Terms of *Lactobacillus* Presence[a]

	pH	Age	Salt
Cheese			
Group 1	5.45 ± 0.299	59. ± 7.68	3.89 ± 1.86
Group 2	5.46 ± 0.292	5.3 ± 6.3	4.33 ± 1.72
P value	0.713	0.563	0.911

[a] Group 1: *Lactobacillus* (+); Group 2: *Lactobacillus* (-).

Table 4. pH, Age and Salt Difference in Yogurt and Dough Samples in Terms of *Lactobacillus* Presence[a]

	pH	Age
Yogurt		
Group 1	3.64 ± 0.153	4 ± 0.816
Group 2	3.58 ± 0.646	4.15 ± 0.833
P value	0.044	0.352
Dough		
Group 1	3.71 ± 0.006	6.63 ± 0.809
Group 2	3.67 ± 0.08	6.77 ± 1.33
P value	0.006	0.097

[a] Group 1: *Lactobacillus* (+); Group 2: *Lactobacillus* (-).

the ripening period, *Enterobacter* and lactic acid bacteria were the most important bacterial groups in terms of frequency. Contrary to yeast and mildew, the speed of reduce the number of coliforms and *E.coli* were very high in during ripening period. The results showed that the absence of positive coagulase coliforms, *Salmonella*, and *Staphylococcus* in all cases at the end of the 60 days of the ripening period, which seems has been due to reduced water activity, pH decrease and increase the population of useful microflora of lactic acid bacteria. Furthermore, in the end, the ripening period increased the free fatty acids and amino acids, which were enhances the positive characteristics in terms of the flavor (14).

Lactobacillus is the dominant species in cheeses that is prepared from raw milk, due to the fact that these bacteria can grow in difficult conditions (15). According to our results, 4 and 11 *Lactobacillus plantarum* strains from yogurt and dough were isolated, respectively. Several factors decreased or had a loss of probiotic growth in the yogurt, such as high acidity, low pH, presence of hydrogen peroxide, bacteria-inhibiting production, dissolved oxygen, and high concentrations of soluble substances. The rapid decline of pH in yogurt fermentation causes the growth of probiotics to stop before enough duplication and increase

the other bacteria population in yogurt; the result reduced the number of probiotics bacterial after fermentation and during of storage (10). Lactic acid bacteria constitute an important part of the yogurt microbial population, which with the increase in yogurts age during storage, causes an increase in acidity by lactic acid bacteria and reduces the number of probiotic bacteria (16).

A study was performed by Jokovic et al., (2008) on Kajmak (fermented fatty milk) traditional dairy products and isolated 3 species such as *Lactobacillus kefir*, *Lactobacillus paracasei*, and *Lactobacillus plantarum* (17). During the examination of lactic acid bacteria in Armada traditional cheese (typical Spanish cheese which production from goat's milk) by Herreros et al., (2003) *Lactobacillus plantarum*, *Lactobacilluscasei*, and *Lactobacillusbrevis* species were identified (18). The Fiore Sardo cheese was studied by Munnu et al., (2000) (traditional Italian cheese which production from sheep's raw milk) and showed that hetero fermentative *Lactobacillus* such as *Lactobacillus plantarum* and *Lactobacillus casei* were dominant species during reach (11).

Abdi et al., (2006) studied Lighvan cheese and isolated *Lactobacilluscasei* as well as *Lactobacillusplantarum*, which were dominant species (19). Navidghasemizad et al., (2009) reported that *Lactobacillusplantarum* was the dominant strain in traditional Lighvan cheese (20), as well as the Abdi study (19). Durlu-Ozkaya et al., (2001) studied the activity of lactic acid bacteria in Beyaz cheese and reported that 14 isolates of *Lactobacillus* plantarum and 3 isolates of *Lactobacillus paracasei* were found. They showed that the *Lactobacillus* plantarum was the dominant strain in the Beyaz cheese (21). A study performed by Edalatian et al., (2012) on the Koozeh cheese isolated *Lactobacillusplantarum*, *Lactobacillusbrevis*, *Lactobacillus hlavaty*, *Lactobacillus delbrueckii*, *Lactobacillus fermentum*, and *Lactobacillus ferment* from this cheese and showed that the *Lactobacillus plantarum* was the dominant strain (8). Hasanzad Azar and Ehsani (2013) reported that the *plantarum* strain was the dominant strain in the Coupe local cheese (22). Manolopoulou et al. (2003) in assessing the microbial population of Feta cheese (traditional cheese in southern of Greece), showed that the *plantarum* strains was the dominant strain in this cheese (23). All of these studies are similar to the present study. Results of above studies showed that *Lactobacillus plantarum* was identified as one of the most common species in the traditional dairy products, however, several studies were unlike our study.

Akabanda et al., (2013) examined fermented milk that was called Nuno (prepared in Ghana and west Africa) in terms of presence of *Lactobacillus* and was clear that *Lactobacillus fermentum* was dominant during the fermentation process and played an important role in the first 6 - 8 hours of fermentation time (24). Mathara et al., (2008) isolated 23 species of *Lactobacillus* from traditional fermented milk (Kulenato) in Kenya, and *Lactobacillus acidophilus*, *Lactobacillus rhamnosus*, *Lactobacillus fermentum*, and *Lactobacillus casei* were most of the isolated strains (25). Yu et al., (2011) isolated 7 strains of *Lactobacillus plantarum* from Mongolian traditional dairy products, while *Lactobacillus delbrueckii*, *Lactobacillus hlavaty*, and *Lactobacillus fermentum* were identified as dominant species (26). The research done by Torres-Lanez et al., (2006) on the properties of natural microflora of Mexican Fresco cheese, showed that *Lactobacillus casei* was the dominant strains (15). In the Rossetti et al., (2008) research on the Grana padano traditional cheese, *Lactobacillus hlavaty* and *Lactobacillus delbrueckii* strain were the dominant strain (27).

Generally, in all of research that has been performed in identifying the species of lactic acid bacteria in traditional dairy products, the difference in bacterial populations and species was observed. One of the reasons for these differences is the difference in the compounds of cow, sheep, and buffalo milk, which were used in production of traditional dairy products. Furthermore diversity of the *Lactobacillus* genus and strains in the traditional dairy products depends on the native milk flora, methods of production, and geographical areas, which all influence the diversity of *Lactobacillus* population and other lactic acid bacteria in traditional dairy products (26).

Characteristics of traditional cheeses are a result of genus diversity, local species, and milk especial native flora (28). Therefore, it is possible that *Lactobacillus* species population are different in each region in traditional dairy products. Based on these results, we can concluded that *Lactobacillus plantarum* is the dominant native micro flora in traditional products (yogurt, dough, and cheese) in Mahabad city. Since natural microbial flora of milk were effective on the flavor, it is possible that *Lactobacillus plantarum* play an important role in the taste and aroma in this product.

Footnotes

Authors' Contribution: Saman Mahdavi designed the study concepts, analyzed the data and prepared the manuscript; Alireza Isazadeh, Saba Haj Azimian, Fariba Shekar, and Ali Asgharian conducted experimental studies and drafted the manuscript; Nazila Moghtaran Bonab was involved in drafting the manuscript and final approval the manuscript.

Conflicts of Interest: The authors declared that they had no conflicts of interest.

References

1. Gomes AMP, Malcata FX. Bifidobacterium spp. and Lactobacillus acidophilus: biological, biochemical, technological and therapeutical properties relevant for use as probiotics. *Trends Food Sci Technol*. 1999;**10**(4-5):139–57. doi: 10.1016/s0924-2244(99)00033-3.
2. Soccol CR, Vandenberghe L, Spier MR, Medeiros ABP, Yamaguishi CT, Lindner JDD. The potential of probiotics: a review. *Food Technol Biotechnol*. 2010;**48**(4):413–34.
3. Kailasapathy K, Chin J. Survival and therapeutic potential of probiotic organisms with reference to Lactobacillus acidophilus and Bifidobacterium spp. *Immunol Cell Biol*. 2000;**78**(1):80–8. doi: 10.1046/j.1440-1711.2000.00886.x. [PubMed: 10651933].
4. Parvez S, Malik KA, Ah Kang S, Kim HY. Probiotics and their fermented food products are beneficial for health. *J Appl Microbiol*. 2006;**100**(6):1171–85. doi: 10.1111/j.1365-2672.2006.02963.x. [PubMed: 16696665].
5. Ceapa C, Wopereis H, Rezaiki L, Kleerebezem M, Knol J, Oozeer R. Influence of fermented milk products, prebiotics and probiotics on microbiota composition and health. *Best Pract Res Clin Gastroenterol*. 2013;**27**(1):139–55. doi: 10.1016/j.bpg.2013.04.004. [PubMed: 23768559].
6. Tropcheva R, Nikolova D, Evstatieva Y, Danova S. Antifungal activity and identification of Lactobacilli, isolated from traditional dairy product "katak". *Anaerobe*. 2014;**28**:78–84. doi: 10.1016/j.anaerobe.2014.05.010. [PubMed: 24887637].
7. Khalid NM, Marth EH. Lactobacilli-their enzymes and role in ripening and spoilage of cheese: A review. *J Dairy Sci*. 1990;**73**(10):2669–84.
8. Edalatian MR, Najafi MBH, Mortazavi SA, Alegria A, Nassiri MR, Bassami MR. Microbial diversity of the traditional Iranian cheeses Lighvan and Koozeh, as revealed by polyphasic culturing and culture-independent approaches. *Dairy Sci Technol*. 2012;**92**(1):75–90.
9. Iranmanesh M, Ezzatpanah H, Mojgani N, Torshizi MAK, Aminafshar M, Maohamadi M. Isolation of lactic acid bacteria from ewe milk traditional yoghurt and sour buttermilk in Iran. *Euro J Food Res R*. 2012;**2**(3):79–92.
10. Najafi MZDH, Karimian A, Abedinia Nejad MH. Microbiological Changes of Pousti Cheese During Ripening. *Food Technology & Nutrition*. 2011;**8**(2):85–92.
11. Mannu L, Comunian R, Francesca Scintu M. Mesophilic lactobacilli in Fiore Sardo cheese: PCR-identification and evolution during cheese ripening. *Int Dairy J*. 2000;**10**(5-6):38[illegible]9. d[illegible] 10.1016/s0958-6946(00)00074-1.
12. Barrow GI, Feltham RKA. *Cowan and Steel's manual for the identification of medical bacteria*. Cam-bridge United Kingdom: Cambridge University Press; 1993. doi: 10.1017/cbo9[illegible]51152[illegible]4.
13. Hashemi MT, Yavarmanesh M, Mila[illegible] E, Pa[illegible]an A. Effect of rennet type, container and ripe[illegible]ng p[illegible]iod [illegible] physicochemical and microbial properties of lo[illegible]l K[illegible]dish cheese. *Iran J Food Sci Technol*. 2013;**9**(37):135[illegible].
14. Milani E. *Evaluation of synbi[illegible]Kordish cheese production using isolated nati[illegible]strains [illegible]ditional Kordish cheese & other pure probiotic strains*. Mas[illegible] University of Ferdowsi of Mashhad; 2012.
15. Torres-Lianez M, Vallejo-Cordoba B, Diaz-Cinco M, Mazorra-Manzano M, Gonzalez-Cordova A. Characterization of the natural microflora of artisanal Mexican Fresco cheese. *Food control*. 2006;**17**(9):683–90.
16. Lourens-Hattingh A, Viljoen BC. Yogurt as probiotic carrier food. *Int Dairy J*. 2001;**11**(1):1–17.
17. Jokovic N, Nikolic M, Begovic J, Jovcic B, Savic D, Topisirovic L. A survey of the lactic acid bacteria isolated from Serbian artisanal dairy product kajmak. *Int J Food Microbiol*. 2008;**127**(3):305–11. doi: 10.1016/j.ijfoodmicro.2008.07.026. [PubMed: 18775578].
18. Herreros M, Fresno J, Prieto MG, Tornadijo M. Technological characterization of lactic acid bacteria isolated from Armada cheese (a Spanish goats' milk cheese). *Int Dairy J*. 2003;**13**(6):469–79.
19. Abdi R, Sheikh-Zeinoddin M, Soleimanian-Zad S. Identification of lactic acid bacteria isolated from traditional Iranian [illegible]ghv[illegible] cheese. *Pakistan J Biologic Sci*. 2006;**9**(1):99–103. doi: [illegible]0.3923[illegible]jbs[illegible]006[illegible]9.103.
20. Navidghasemizad S, Hesari J, Saris P, Na[illegible]ei M. [illegible]solati[illegible]n of lactic acid bacteria from Lighvan cheese, a semihar[illegible]chees[illegible]ade from raw sheep milk in Iran. *Int J Dairy Techno[illegible]* 2009;**62**(2)[illegible]60–4.
21. Durlu-Ozkaya F, Xanthopo[illegible]os V, [illegible]nail N, [illegible]topoulou-Tzanetaki E. Technologically important pr[illegible]erti[illegible]of lact[illegible] acid bacteria isolates from Beyaz cheese ma[illegible]from[illegible]w ewes' milk. *J Appl Microbiol*. 2001;**91**(5):861–70. [PubMed: [illegible]72266].
22. Hassanzadazar H, Eb[illegible]i A. Phe[illegible]otypic characterization of lactic acid bacteria isolated from [illegible]raditional koopeh cheese. *Global Veterinaria*. 2013;**10**(2)[illegible]
23. Manolopo[illegible]ou [illegible]rantinopoulos P, Zoidou E, Aktypis A, Moschopou[illegible]Kandarakis IG, et al. Evolution of microbial populations during [illegible]ditional Feta cheese manufacture and ripening. *Int J [illegible]ood Microbiol*. [illegible]003;**82**(2):153–61. [PubMed: 12568755].
24. [illegible]banda F, Owusu-Kwarteng J, Tano-Debrah K, Glover RL, Nielsen DS, Je[illegible]ersen L. Taxonomic and molecular characterization of lactic acid b[illegible]eria and yeasts in nunu, a Ghanaian fermented milk product. *Food Microbiol*. 2013;**34**(2):277–83. doi: 10.1016/j.fm.2012.09.025. [PubMed: 23541194].
25. Mathara JM, Schillinger U, Guigas C, Franz C, Kutima PM, Mbugua SK, et al. Functional characteristics of Lactobacillus spp. from traditional Maasai fermented milk products in Kenya. *Int J Food Microbiol*. 2008;**126**(1-2):57–64. doi: 10.1016/j.ijfoodmicro.2008.04.027. [PubMed: 18539351].
26. Yu J, Wang WH, Menghe BL, Jiri MT, Wang HM, Liu WJ, et al. Diversity of lactic acid bacteria associated with traditional fermented dairy products in Mongolia. *J Dairy Sci*. 2011;**94**(7):3229–41. doi: 10.3168/jds.2010-3727. [PubMed: 21700007].
27. Rossetti L, Fornasari ME, Gatti M, Lazzi C, Neviani E, Giraffa G. Grana Padano cheese whey starters: microbial composition and strain distribution. *Int J Food Microbiol*. 2008;**127**(1-2):168–71. doi: 10.1016/j.ijfoodmicro.2008.06.005. [PubMed: 18620769].
28. Garabal JI, Rodriguez-Alonso P, Centeno JA. Characterization of lactic acid bacteria isolated from raw cows' milk cheeses currently produced in Galicia (NW Spain). *Food Sci Technol*. 2008;**41**(8):1452–8. doi: 10.1016/j.lwt.2007.09.004.