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Isolation and Identification of Lactobacilli Found in Nomads Traditional Yogurt in the City of Jahrom Using PCR Method and, the Study of Their Interactional Effects on Streptococcus mutans as Cause of Tooth Decay Using Disc and Auger Hole Methods

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ABSTRACT

Background: The majority of the people are faced with tooth decay. Streptococcus mutans has been identified as the main cause of tooth decay. Drug resistance is common and is increasing among the bacteria cause of tooth decay. With increasing antibiotic resistance, to eliminate them, the new method of using probiotics instead of antibiotics can be used. Methods: In this study, the traditional yogurt is used as a source of probiotic bacteria such as Lactobacillus. Thus, first Lactobacillus have been isolated and purified, then PCR techniques have been used for identification of Streptococcus mutans and then their impact was investigated using disk and auger hole methods. In the first method (disk), disks impregnated with 24 and 48 -hour cultures of Lactobacillus solutions were used , and in the second method (auger hole) impregnated with 24, 48 , 72 and 96 cultures of Lactobacillus solutions used to inject in hole. In both methods, Streptococcus mutans cultivations of half McFarland turbid were used. Findings: In this study, 16 samples of pure lactobacillus, manly the four species of L.casei, L.zeae, L.plantarum and L.rhamnosus were identified using PCR techniques. Then, the impact of these four species on Streptococcus mutans was evaluated, and the result achieved on the disk method was not acceptable. The auger hole method show that Lactobacillus casei with an average diameter of 17.7 mm and Lactobacillus rhamnosus with a average diameter of 13.3 mm have significant inhibitory effects on the growth of Streptococcus mutans, but Lactobacillus zeae, Lactobacillus plantarum did not have significant inhibitory effects on Streptococcus mutans without apparent lack of growth halo. Conclusion According to this study and other investigations, each species of probiotic bacteria has different inhibitory effect on Streptococcus mutans compared to the others. Therefore, it can be recommended as a method of clinical research.

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INTRODUCTION

The term probiotic is derived from the Greek word βιωτικός meaning Life that is in contrast with the word antibiotics meaning antilife. The World Health Organization refers the term to live organisms that show health effects for their hosts if consumed effectively [1].

Probiotic bacteria administered in adequate amounts in different parts of the body (principally enteric), and mainly through maintaining a healthy balance of intestinal flora confer a health benefit on the host [2,3]. Recently, several clinical studies have shown the effects of specific probiotics in the treatment of systemic diseases.

Different species of bacteria are referred to as probiotics as the most common is Lactobacillus and Bifid bacterium [4]. Lactobacillus natural living environment is the human enteric and vagina. This bacteria is a genus of Gram-positive facultative anaerobic and is classified as lactic acid bacteria (LAB). According to the definition provided by International Dairy Federation (IDF) and the International Standards Organization (ISO) lactic acid bacteria are Gram-positive , non -spore-forming, catalase-negative, nitrate reduction negative and cytochrome oxidase negative that are not capable of melting the gelatin and indole production. All lactic acid bacteria have fermentative metabolism and are highly saccharolytic which is resulting final product of

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carbohydrate intake of lactic acid. Main lactic acid bacteria in the dairy industry belong to Leuconostoc ، Lactococcus ،Enterococcus ،Streptococcus ،Pediococcus and Lactobacillus genus [5].

Lactobacillus genus includes various species. Lactobacillus bacteria are 1-10 microns length and 0.5 -1.3 microns width, smooth and uniform with rounded ends. This bacteria shapes short chains of short bacilli, and or long bacilli, it is slim and elegant, sometimes like Cocobacilli , more often Coccus as short chains; so that it may be confused with Streptococcus [3,6]. Lactobacillus bacteria are gram-positive, but in successive cultures may be seen as gram-negative [7].

Lactobacillus species, which are considered as probiotics , including Lactobacillus Gasry, Lactobacillus rhamnosus, Lactobacillus casei, Lactobacillus reuteri, Lactobacillus bulgaricus, Lactobacillus plantarum, Lactobacillus acidophilus Lactobacillus Johnson [8,4]. Lactobacilli are widespread in the environment, especially in animal and plant food products, on crops, milk, dairy products and decaying organic materials. Also, they exist on enteric part of the birds and mammals as well as mammals vagina, and are rarely pathogens [3].

Animal studies showed that Lactobacillus lactis, Lactobacillus casei, Lactobacillus plantarum and Lactobacillus Helvetius are able to induce systemic and mucosal immune responses against Streptococcus pneumonia and tetanus toxin anti genes [9]. Today's competitive properties in bacteria are used in the treatment of infections caused by bacterial pathogens, Lactobacillus or lactic acid-producing bacteria are among the most important ones. The bacteria bind to mutagenic compounds inhibits their absorption and spread in the body [10] and is capable of degrading N-nitrosamine. Lactobacilli and their soluble compounds inhibit the growth of tumor cells directly or by activating cellular immune responses. These bacteria and their fermentation products have anti mutagenic and antioxidant activity against a wide range of mutagenic and carcinogenic [11].

Conventional phenotypic identification of lactic acid bacteria is very tedious and time consuming and not always reliable. In addition, there are certain species that are not identified based on phenotypic traits. Phenotypic responses can be influenced by environmental conditions. In order to design more stable and reliable detection methods, genetic testing should be used. One method is the use of nucleic acid markers [12]. Moreover, oral infections are most common and expensive form of human infections.

A new method, including the use of probiotics is a promising way to fight infections for removal of pathogenic bacteria in the oral cavity; here the harmful (beneficial) bacteria can be replaced with the pathogenic microorganisms [13]. Streptococcus mutans is a major factor in human dental decay.

These bacteria are gram-positive coccus-shaped of viridians streptococci group. Since the viridians group streptococcus is normal mouth and throat flora, this group Streptococcus all is found in mouth environment, among them streptococcus mutans and Streptococcus sobrinus is significant contributor to tooth decay [14]. Studies have been conducted on the microorganisms used in dairy products, with the potential for caries prevention. Lactic acid-producing bacteria due to the ability to ferment sugars and creates a low pH can be anticariogenic. Streptococcus mutans and Lactobacillus are associated with dental caries in particular [15]. Also, calcium and other substances protect the tooth surface and prevent attaching tooth pathogens. Probiotics prevent the growth of harmful micro flora in mouth and show beneficial effects. Given the above, the overall objective of this study is to isolate and identify the molecule Lactobacillus in nomadic traditional yogurt in the city of Jahrom to study their antimicrobial efficacy that specifically examine the antibacterial effect of Streptococcus mutans cause of tooth decay.

Methods:

47 yogurt samples have been prepared at different times in different parts of the city areas (Khafr, Kurdian, Symkan and mainly Chatiz) and were transferred in a cool box with ice in less than 4 hours to Islamic Azad University Microbiology Research Laboratories, Jahrom unit.

Sterilized Ringer's solution (NaCl $gr/_L$ 9) that was prepared 24 hours beforehand was used for the dilution of samples, gradual dilution up to 10^{-5} for each sample was performed [17,16].

MRS agar (Man Rogosa Sharpe agar) made of Himedia Samples was used as specific culture medium for Lactobacillus [16,17]. After 48 h of incubation, tiny colonies on plates containing concentrations with 10^{-4} and 10^{-5} were observed. The pure culture was attempted using loops, a single colony is removed and transferred to a new medium, this was done in a few steps and several pure cultures were obtained. After several purifications some steps of sub-culture were performed to ensure that bacterial culture is pure.

Studies on Lactobacillus conducted in Iran mainly aimed at applications in the food and improving the quality of dairy products and molecular analysis to identify or determine the biochemical properties has received less attention. Therefore, identification of Lactobacillus isolated from traditional yogurt in this study was performed by molecular methods or PCR. All of this is part of the study was performed at Medical University of Imam Jafar Sadiq (AS) Community Center. To perform PCR primers and the optimum temperature on Table 1 were used.

Table 1: primers and optimum temperature for PCR

Bacteria	Primer	denature	Annealing	Extension
L.casei	F-CAGACTGAAAGTCTGACGG R-GCGATGCGAATTTCTTTTTC	95 ℃	55 ℃	72 ℃
L.rhamnosus	F-CAGACTGAAAGTCTGACGG R-GCGATGCGAATTTCTATTATT	95 ℃	58 ℃	72 ℃
L.zeae	F-TGTTTAGTTTTGAGGGGACG R-ATGCGATGCGAATTTCTAAATT	95 ℃	58 ℃	72 ℃
L.plantarum	F-GCCGCCTAAGGTGGGACAGAT R-TTACCTAACGGTAAATGCGA	95 ℃	55 ℃	72 ℃

Effect of identified Lactobacilli on Streptococcus mutans

Disk method:

Streptococcus mutans with standard PTCC1683 code purchased from the Iran Center for Microbial Collection and cultured in nutrient broth made by Himedia Company and then incubated and reached to half McFarland turbidity. Lactobacillus bacteria were cultured in 50 cc MRS broth environment and after 24 and 48 hours, each time 10cc was removed and were centrifuged with 3000 RPM (rpm) for 10 minutes and supernatant were used for the subsequent steps.

Then the filter paper pre-punched into discs and were sterilized were located into supernatant for a period of 4-5 minutes [18]. Similarly, Streptococcus mutans were culture plated on mitis salivarius agar made of Quelab using a sterilized swab, discs containing Lactobacillus supernatant were placed in the center of plate. The plates were incubated for 48 hours at a temperature ℃ 37. In this method, first 100 µl suspensions of Streptococcus mutans were added to micro titer agars and 30 minutes later 100 µl Lactobacillus suspensions was added to it.

Agar hole method:

Streptococcus mutans have been grown in the nutrient broth culture to reach to half McFarland turbidity. The Lactobacillus bacteria were cultured in 50 cc MRS broth environment and after 24, 48, 72 and 96 hours, each time 10cc was removed and were centrifuged with 3000 RPM (rpm) for 10 minutes and supernatant were used for the subsequent steps. Simultaneously, Streptococcus mutans were culture plated on mitis salivarius agar using a sterilized swab, then 6 mm holes were prepared at the center of plates using Pasteur pipette. 100 λ of Lactobacillus supernatant were cast in each hole.

Plates were left to supernatant be absorbed medium for 30- 60minutes. Then, the plates were incubated for 48 hours at a temperature ℃ 37 [18]. In this way, 200 µl of the mixture of two above environments (100 µl Streptococcus mutans and Lactobacillus100 µl) were transferred to micro titer plate's holes. Finally, statistical analysis using was done at 5% level using SPSS software and Duncan test; and the significance of the results was studied.

Results:

After PCR processes completed and according to the images obtained by electrophoresis and previous studies a L.zeae sample (sample 17) with a bond between 750 bp; four samples L.rhamnosus (samples 2, 12, 14 and 18) with a bond between 200 bp; five L.plantarum (samples 3, 7, 13, 15 and 16) with a bond between 600 bp and six L.casei (samples 1, 4, 5, 6, 8 and 11) with a bond between 400 bp that have been arranged apart from nomadic traditional yogurt of Jahrom city area(figure 1).

Bands related to Lactobacillus rhamnosus (band 200 bp), Lactobacillus casei (band 400 bp), Lactobacillus plantarum (band 600 bp) and Lactobacillus zeai (band 750 bp) is quite clear and obvious Figure (1)

Effects of detected Lactobacilli by polymerase chain reaction from Streptococcus mutans

Disk method:

Using the disk method obtained from supernatant of 24 and 48 hours cultures did not result in acceptable results and lack of growth halo was not shown.

Auger hole method:

According to the results, supernatant obtained from 72 hours cultures of species Lactobacillus casei and Lactobacillus rhamnosus demonstrated high inhibition on the growth of Streptococcus mutans (Figure 2), but the species Lactobacillus plantarum and Lactobacillus zeai did not show any lack of growth halo at any time. Lack of growth halo diameter of Lactobacillus casei (mean 17.7 mm) and Lactobacillus rhamnosus (mean 13.3 mm) on culture media (Table 2) are listed.

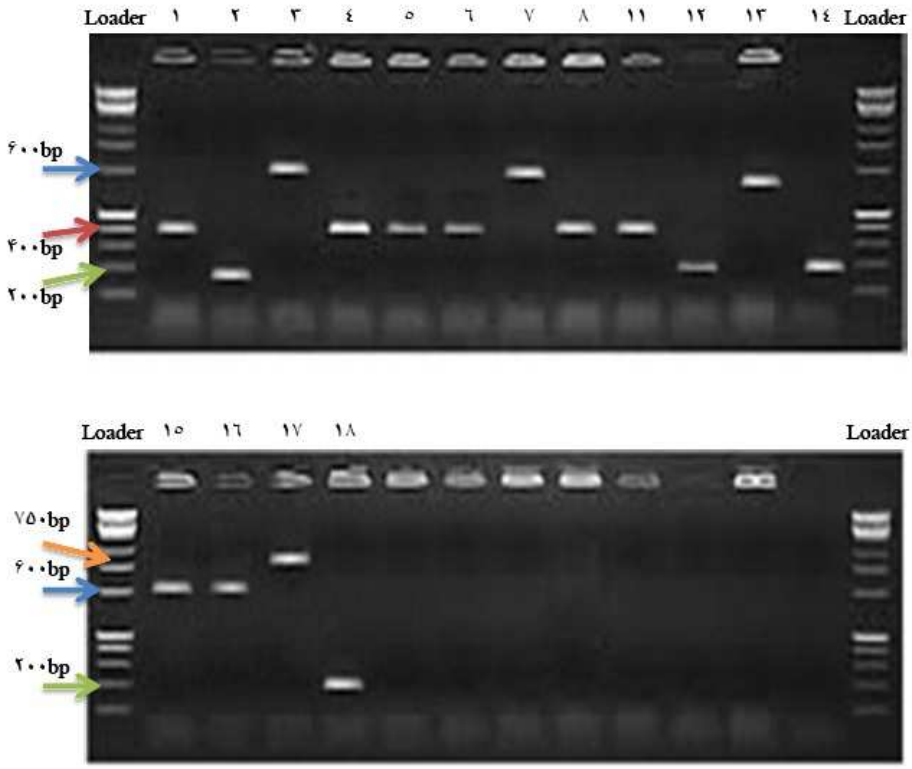

Fig. 1: PCR results in gel electrophoresis

Table 2: lack of growth halo of Lactobacillus casei and Lactobacillus rhamnosus diameter

Lactobacillus species	Halo diameter (mm)
L. casei 6	17
L. casei 8	14
L.casei 11	22
L.rhamnosus 12	11
L. rhamnosus 14	13
L.rhamnosus 18	16

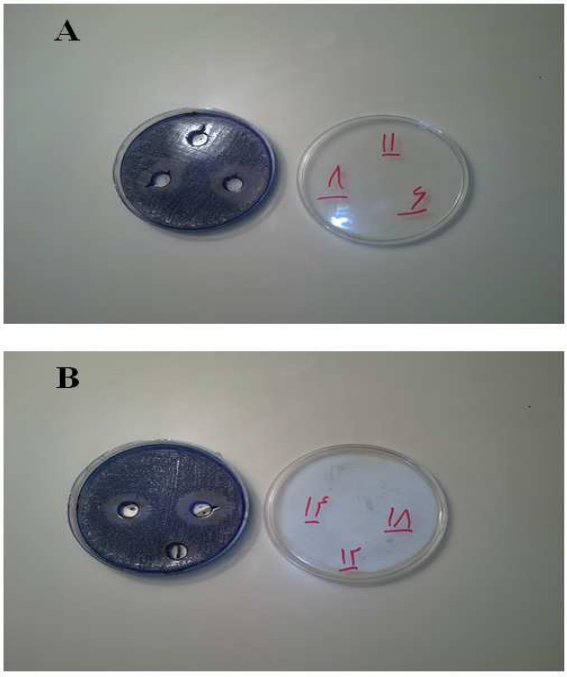

Fig. 2: lack of growth halo

A: lack of growth halo of Lactobacillus casei (mean diameter 17.7 mm)
B: lack of growth halo of Lactobacillus rhamnosus (mean diameter 13.3 mm)

Discussion and conclusions:

Isolation and Identification of Lactobacillus

In this study ,Using biochemical tests, 16 lactobacillus samples of the nomadic Jahrom was purified and using molecular identification methods, it was found that it is related to the four species of Lactobacillus casei, Lactobacillus ziae, Lactobacillus rhamnosus and Lactobacillus plantarum, which confirms previous researches, as well. For example Beukes et al could isolate and identify Lactobacillus delbruecki and Lactobacillus plantarum from samples of a traditional dairy products called amasi in South Africa and Namibia [19]. Mathara et al could identify Lactobacillus plantarum, Lactobacillus fermentum, Lactobacillus acidophilus, Lactobacillus paracasei in Kenya in from samples of traditional dairy products called kule naoto with a pH of less than 4.5.

Ayhan et al (2005) could isolate Lactobacillus bulgaricus subspecies for bulgarious from traditional yogurt samples, with special features for business [21]. Kostinek et al (2007) could isolate three Lactobacillus species (fermentum, pentosus and plantarum) in South Africa from traditional dairy called cassava [22]. Jokovic et al could isolate three species of Lactobacillus kefir, Lactobacillus plantarum and Lactobacillus paracasei from traditional dairy products that are also produced synthetically (called kajmak) in 2008 in Serbia [23]. Nita et al (2012) could identify Lactobacillus fermentum bacteria with a high potential of bacteriocins production in whey using sequencing measurements method [24].

Antibacterial properties of Lactobacillus:

In this study, effect of Lactobacillus strains isolated from traditional yogurt on streptococcus mutans on the human as a pathogen, especially in the formation of dental caries was investigated. Effect of Lactobacillus on pathogen has a strong background itself, forexample, Murry et al demonstrated the inhibitory effects of Lactobacillus salivarius and Lactobacillus plantarum on the growth Escherichia coli, Salmonella typhimurium and Clostridium perfringens [25]. Rajerm et al showed that L. lactis strains isolated from the aquatic environment has strong antimicrobial activity against food putrefactive bacteria and is usable as natural food preservative [26].

It seems likely that a probiotic mechanism in mouth is similar to that of in gastrointestinal, but the information regarding this fact is insufficient and it is not approved that probiotic species are able to improve and regulate oral cavity condition. Moreover, it must be kept in mind that there are differences between the oral cavities and gastrointestinal in the immune response, mucosal epithelial structure, and chemical composition of the resident flora of the digestive discharge tube, which varies with the saliva in the mouth. Therefore, the results of the impact of probiotics on gastrointestinal diseases can not directly be applied in the oral cavity and dentistry and further studies on the effect of probiotics in the oral cavity is required [4]. Studies have demonstrated that Lactobacillus a part of the oral flora may play an important role in micro ecological balancing in the oral cavity with probiotic properties. Although less information is available about the Bifid bacterium but so far good results have been seen in some studies [27,28], and further studies are required. Despite the reduction in caries bacteria in saliva after ingestion of probiotic bacteria, it should be noted that the decrease in saliva pathogen bacteria for a short or a long time does not necessarily mean better oral hygiene dental caries or cavities. However, it is better to postpone the use of probiotics in children who have untreated open cavities, and decay, to temporary restoration of teeth until further studies done in children [4]. Studies do not show that permanent establishment of probiotics in oral cavity after temporary use of probiotic products containing. It seems that probiotics to have significant residual effects after discontinuation. Therefore, a daily intake of probiotics seems necessary to reach its potential. It should be noted that the approving the effectiveness of a probiotic strain does not necessarily mean that other species have a similar effect. Also, using a combination of probiotics may have synergistic cumulative effects or the opposite [4,30,29]. It is likely the differences in study design, dose, and mode of administration, ages and the bacterial species in various studies are the reasons for conflicting results on the efficacy of probiotics in the oral cavity. Various studies have shown that Lactobacillus inhibits Streptococcus pathogen and Candida in oral cavity and is beneficial. Mokim indicated that Lactobacillus rhamnosus and Lactobacillus rotary are able to prevent decay by reducing Streptococcus mutans bacteria as probiotics. Probiotics interaction with pathogenic bacteria is one of the effective mechanisms that have been reported [31]. Nice et al have reported the beneficial effects of long-term consumption of probiotic milk on dental caries in children 3 to 4 years. Furthermore, they reported that the addition of Lactobacillus rhamnosus probiotic strain can reduce a strain of Streptococcus mutans bacteria Streptococcus Sorbinus [32].

Ahoula et al studied the effect of Lactobacillus rhamnosus GG and Lactobacillus rhamnosus LC705 on Streptococcus mutans, and a significant decrease of Streptococcus mutans was observed in those who initially had high levels of bacteria [33].

According to the studies, it seems that the reduced the level of Streptococcus mutans after treatment is not related to the prescriptions including milk, cheese, yogurt, Lozenge and etc [29,4]. Studying the effect of different probiotic strains on oral health can lead to the selection of the most effective one [4].

In recent decades ,Lactobacillus is highly regarded in dental researches and modern molecular methods have confirmed the hypothesis that L. is more associated with decayed dentin and caries lesions in progress spot and played no role in the advent of the process of decay. Although, there is evidence that Lactobacilli are associated with the dentin caries , the increased number of them in the oral cavity following probiotic products consumption of individuals without active cavities do not predict increase in the decay for several reasons:

- ✓ First, lactobacilli are generally rarely triggering the formation of cavities .
- ✓ Second, not all species of Lactobacillus-induced decay .
- ✓ Third, when the lactobacillus carrier is a dairy, the carrier can neutralize the effects of acid produced by the bacteria [4].
- ✓ Fourth, several studies have shown that the amount of Streptococcus mutans in saliva significantly reduced immediately after the daily consumption of a variety of probiotic Lactobacillus [33-35]

Conclusion:

According to this study and other investigations, each species of probiotic bacteria has different inhibitory effect on Streptococcus mutans compared to the others. Therefore, it can be recommended as a method of clinical research.

REFERENCES

[1] Yakarim, M., 1390. Effect of Aloe Vera on increasing the growth rate of bacteria in milk and yogurt probiotic Lactobacillus acidophilus and Bifidibacterium bifidum. Master's thesis. Islamic Azad University of Jahrom Branch.
[2] Izadi, M., M.H. Fouladi, G.h.R. Sharifi Sirchi, J. Amini, 1389. Isolation of Lactobacillus acidophilus yogurt samples of Shahrbabak city and its molecular identification. Journal of Agricultural Biotechnology, 2(2): 11-1.
[3] Holt, G.J., R.N. Krieg, H.A. Sneat P., T. Staley J., T .S. Williams, 1994. Bergeys Manual of Determinative Bacteriology. 9. USA: Williams and Wilkins, 787.
[4] Meurman, J.H., I. Stamatova, 2007. Probiotics: contributions to oral health. Oral Diseases., 13(5): 443-51.
[5] IDF149, I. 2008. Milk products -Acidifying starter cultures- Standard of identify.
[6] Jo Baron, E., M. Finegold S., 1990. Bailey and Scotts Diagnostic Microbiology. USA: The C.V. Company, 861.
[7] Banwart, G.J., 1989. Basic Food Microbiology. 2. NewYork: Van Nostrand Reinhold, p: 773.
[8] Nase, L., K. Hatakka, E. Savilahti, M. Saxelin, A. Ponka, T. Poussa, et al. 2001. Effect of long-term consumption of a probiotic bacterium, Lactobacillus rhamnosus GG, in milk on dental caries and caries risk in children. Caries Research, 35(6): 412-20.
[9] Wu, H.Y., H.H. Nguyen, M.W. Russell, 1997. Nasal lymphoid tissue (NALT) as a mucosal immune inductive site. Scandinavian Journal of Immunology, 46(5): 506-13.
[10] Bolognani, F., C.J. Rumney, I.R. Rowland, 2000. Influence of carcinogen binding by Lactic acid producing bacteria on tissue distribution and in vitro mutagenecity of dietary carcinogens. Food and Chemical Toxicology, 35: 535-545.
[11] LeBlung, A.M., G. Perdigon, 2004. Yogurt feeding inhibits promotion and progression of experimental colorectal cancer Med Sci monit. Medical Science Monitor, 10(4): BR96-104.
[12] Schleifer, K.H., M. Ehrmann, C. Beimfohr, E. Brockmann, W. Ludwig, R. Amann, 1995. Application of molecular methods for the Classification and identification of Lactic Acid Bacteria. International Dairy Journal, 5: 1081-1094.
[13] Caglar, E., S.K. Cildir, S. Ergeneli, N. Sandalli, S. Twetman, 2006. Salivary mutans streptococci and lactobacilli levels after ingestion of the probiotic bacterium Lactobacillus reuteri ATCC 55730 by straws or tablets. Acta Odontologica Scandinavica, 64(5): 314-8.
[14] Molana, Z., M. Ghasempour, F. Asgharpour, M. Mitra Alami, P. Bagheban Shaker, 1392. Comparison of Streptococcus mutans and lactobacilli frequency in children 5-3 years old with tooth decay and tooth decay. Journal of Laboratory Sciences, 7(1): 34-29.
[15] Tanzer, J.M., A. Tanzer, C. Lang, B. Cooper, L. Hareng, A. Gamer, et al. 2010. Caries inhibition by and safety of Lactobacillus paracasei DSMZ16671. Journal of Dental Research, 89(9): 921-6.
[16] Ghobadi Dana, M., A.H. Salmanian, B. Yakhchali, 1387. Study of folic acid production by lactobacilli isolated from Iranian native products. XVIII National Congress of Food Science and Technology, Mashhad, Iran, 25-22: 5-1.

[17] Ghobadi Dana, M., A.H. Salmanian, B. Yakhchali, Isolation and molecular identification of lactobacilli found in some indigenous dairy products . Journal of Food Science and Technology Research and Innovation, 1(2): 116-99.
[18] Dorri, K.A., V. Hemayatkhah Jahromi, M. Namdar, H. Karegar Jahromi, 1389. Antibacterial effect of Lactobacillus strains isolated from the feces of children on the growth of pathogenic bacteria in the stomach and intestines. Microbial World Magazine, 3(4): 237-229.
[19] Beukes, E.M., B.H. Bester, J.F. Mostert, 2001. The microbiology of South African traditional fermented milks. International Journal of Food Microbiology, 63: 189-197.
[20] Mathara, J.M., U. Schillinger, P.M. Kutima, S.K. Mbugua, W.H. Holzapfel, 2004. Isolation, identification and characterisation of the dominant microorganisms of kule naoto: The Maasai traditional fermented milk in Kenya. International Journal of Food Microbiology, 94: 269-278.
[21] Ayhan, K., F. Durlu-ozkaya, N. Tunail, 2005. Commercially important characteristics of Turkish origin domestic strains of Streptococcus thermophilus and Lactobacillus delbrueckii ssp. bulgaricus. International Journal of Dairy Technology, 58: 150-157.
[22] Kostinek, M., I. Specht, V.A. Edward, C. Pinto, M. Egounlety, C. Sossa, S. Mbugua, C. Dortu, P. Thonart, L. Taljaard, M. Mengu, CMAP. Franz, W.H. Holzapfel, 2007. Characterization and biochemical properties of predominant lactic acid bacteria from fermenting cassava for selection as starter cultures. International Journal of Food Microbiology, 114(3): 342-351.
[23] Jokovic, N., M. Nikolic, J. Begovic, B. Jovcic, D. Savic, L. Topisirovic, 2008. A survey of the lactic acid bacteria isolated from Serbian artisanal dairy product kajmak. International Journal of Food Microbiology, 127: 305-311.
[24] Nithya, k., S. Duraisamy, S. Balakrishnan, U. Narayanapillai, G. Ramasamy, 2012. Characterization of bacteriocin producing lactic acid bacteria and its application as a food preservative. African journal of microbiology research, 6(6): 1138-1146.
[25] Murry, Jr A.C., A. Hinton Jr, H. Morrison, 2004. Inhibition of growth of Escherichia coli, Salmonella typhimurium, and Clostridia perfringenes on chicken feed media by Lactobacillus salivarius and Lactobacillus plantarum. International Journal of Poultry Sciences, 3(9): 603-607.
[26] Rajaram, G., P. Manivasagan, B. Thilagavathi, A. Saravanakumar, 2010. Purification and characterization of a bacteriocin produced by Lactobacillus lactis isolated from marine environment. Advance Journal of Food Science and Technology, 2(2): 138-144.
[27] Masco, L., K. Van Hoorde, E. De Brandt, J. Swings, G. Huys, 2006. Antimicrobial susceptibility of Bifidobacterium strains from humans, animals and probiotic products. Journal of Antimicrobial Chemotherapy, 58(1): 85-94.
[28] Caglar, E., O.O. Kuscu, K.S. Selvi, C.S. Kavaloglu, N. Sandalli, S. Twetman, 2008. Short-term effect of ice-cream containing Bifidobacterium lactis Bb-12 on the number of salivary mutans streptococci and lactobacilli. Acta Odontologica Scandinavica, 66(3): 154-8.
[29] Anderson, M.H., W. Shi, 2006. A probiotic approach to caries management. American Academy of Pediatric Dentistry, 28(2): 151-3.
[30] Twetman, S., C. Stecksen-Blicks, 2008. Probiotics and oral health effects in children. International Journal of Paediatric Dentistry, 18(1): 3-10.
[31] Mokeem, S.A., 2007. The synergism of probiotics in dentistry. Saudi Dental Journal., 19(3): 1-3.
[32] Abbasi, M.A., 1388. Study thyme impact on the growth of Lactobacillus acidophilus bacteria and Bifidibacterium bifidum. PhD thesis of veterinary professionals. Islamic Azad University of Kazeroun Branch.
[33] Ahola, A.J., H. Yli-Knuuttila, T. Suomalainen, T. Poussa, A. Ahlstrom, J.H. Meurman, et al. 2002. Short-term consumption of probiotic-containing cheese and its effect on dental caries risk factors. Archives of Oral Biology, 47(11): 799-804.
[34] Busscher, H.J., A.F. Mulder, H.C. van der Mei, 1999. In vitro adhesion to enamel and in vivo colonization of tooth surfaces by Lactobacilli from a bio-yoghurt. Caries Research, 33(5): 403-4.
[35] Hasslof, P., M. Hedberg, S. Twetman, C. Stecksen-Blicks, 2010. Growth inhibition of oral mutans streptococci and candida by commercial probiotic lactobacilli--an in vitro study. BMC Oral Health, 10: 18.