The Effect of Iranian Customary Used Probiotic Yogurt on the Children’s Salivary Cariogenic Microflora

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ABSTRACT
Statement of the Problem: Dental caries is the most common disease of childhood. Using probiotics has recently been introduced to reduce the incidence of dental caries. It consists of live microbial food supplements that beneficially affect the host, and hence are considered an alternative way to eradicate the infections.

Purpose: The aim of this study is to evaluate the effect of consumption of probiotic yogurt on the children’s salivary cariogenic microflora.

Materials and Method: A double-blind randomized study was performed recruiting 2 parallel groups; 24 healthy children in the case and 25 children in the control group. All healthy children were followed-up over 4 periods. Periods 1 and 3 were wash-out periods with duration of 1 and 2 weeks, respectively. During periods 2 and 4 (2 weeks duration each), the case group consumed 200g yogurt containing Bifidobacterium lactis (1×10^6 per gram) once daily and the control group consumed normal yogurt. Salivary Streptococci mutans and Lactobacilli were enumerated before and after the yogurt consumption periods. Pre- and post-treatment values within and between regimens were compared using the t-test and paired samples.

Results: There was a reduction in Streptococcus mutans and Lactobacilli counts in the control group, but for Streptococcus mutans, the count reduction between phases 1 and 4 was statistically significant (p= 0.009). In the case group, neither the Streptococcus mutans count nor the Lactobacilli count was significantly reduced.

Conclusion: Based on the findings of this study, short-term daily consumption of probiotic yogurt containing Bifidobacterium lactis could not reduce the levels of salivary Streptococcus mutans and Lactobacilli in 6 to 12 year-old children, while normal yogurt could reduce the Streptococcus mutans counts significantly.

Introduction
Dental caries is the most common chronic disease affecting the majority of adults and 60-90% of school children. [1] Caries is a multifactorial disease with bacterial origin that can demineralize tooth enamel. [2] In dental caries, there is an increase in acidogenic and acid-tolerating species such as mutans Streptococci and Lactobacilli, although other bacteria can be found. Changes in the homeostasis of the oral cavity would enhance proliferation of bacterial biofilm, notably mutans, from the streptococci group. [3]
rtant role in the initiation of caries and Lactobacillus to have a part in the progression of tooth decay. [4] One of the methods that have recently been considered effective on the reduction of dental caries and decrement of cariogenic flora is the use of probiotic products. [5] Probiotics can promote oral health and also prevent oral diseases like caries, gingivitis, periodontitis and halitosis. The use of health promoting bacteria is one of the novel approaches in oral health. [5] The consumption of probiotics may have a favorable effect on the reduction of dental caries. [6-7]

Several studies have shown that using the probiotic products could replace and ex-change the cariogenic bacteria with non-cariogenic ones. [8] These studies have employed different approaches. One approach is making use of the ability of probiotic bacteria to colonize on teeth and influence the supragingival plaque. [8] Probiotics must be able to attach to the surface of teeth and integrate into the bacterial population, making the dental biofilm. [5]

The term probiotic, introduced by Gibson and Roberfroid, is a live non-digestible microbial food whose efficacy on human health has been established. [6] A number of probiotic products that can play a critical role in human health have been proposed to have the potential to improve intestinal balance and influence the immune system through molecular mechanisms. [9-11]

Other studies have investigated the application of probiotics in treating cardiovascular diseases, urogenital infections and cancer [12-15] but little attention is paid to its possible effect on oral health and risk factors of caries. The present study has focused on the effect of probiotics on oral microorganisms.

Nase et al. (2001) investigated the effect of probiotic Lactobacillus rhamnosus on caries risk and showed less caries and lower Streptococcus mutans levels in probiotic-milk consuming group. [16] Caglar et al. (2007) estimated the efficacy of Xylitol and probiotic chewing gums on the count of Streptococcus mutans and Lactobacilli and reported that probiotic bacteria could reduce the salivary level of Streptococcus mutans significantly. [17]

Steckson-Blicks et al. (2009) reported that daily use of probiotic milk and fluoride decreases the caries in pre-school children. [18] Moreover, Caglar et al. (2006) investigated the effect of the probiotic bacterium Lactobacillus reuteri ATCC 5573 on salivary mutans streptococci and Lactobacilli and demonstrated that a short-term consumption of Lactobacilli-derived probiotics reduced the level of salivary mutans streptococci in young adults. [19] In another study, Singh et al. (2011) showed that probiotic ice-cream could decrease the count of Streptococcus mutans, but its effect on Lactobacilli count has not been significant. [20]

Regarding the findings of some studies that have demonstrated the probiotics to be useful in reducing the cariogenic flora of mouth and also the availability of probiotic yogurt, the present study is aimed to evaluate the effect of probiotic yogurt consumption on the cariogenic flora of children. We were also interested to explain whether the effect of probiotics would remain during the predefined four different periods.

Materials and Method
This randomized double-blind study recruited 49 healthy children with the age range of 6-12 years old (mean age=9.2±1.7) referred to the School of Dentistry, Shiraz University of Medical Sciences. Participants had not used any probiotic products. Those who were receiving Xylitol-containing products or fluoride treatments were excluded from the study. Using any fluoride products was forbidden.

Participants were instructed to brush their teeth twice a day and maintain their normal oral hygiene. Using chewing gums containing Xylitol as well as probiotic yogurt during the study was forbidden. Any changes in health and using any medicine were considered. An experienced dentist examined the oral health of the participants before the study.

This study had two parallel groups; 24 healthy children in the case group and 25 healthy children in the control group. Both groups were matched on the following attributes: age, gender and DMFT. All of the children in the study were followed up over four periods. Periods 1 and 3 were wash-out periods with duration of 1 and 2 weeks, respectively. During periods 2 and 4 (2 weeks each), the participants were asked to eat 200g yogurt, containing Bifidobacterium lactis, 10⁶ colony forming units (CFU)/mL, once daily at lunch. Tooth brushing was forbidden for at least 1 hour after eating probiotic yogurt. Since the most frequently available probiotic bacteria are Lactobacillus and Bifidobacterium,
Lactobacilli count before starting the study
L2: Lactobacilli count after the 2nd period
L3: Lactobacilli count after the 3rd period
L4: Lactobacilli count after the 4th period

Figure 1a: Changes in Lactobacilli count

Streptococcus count before starting the study
S2: Streptococcus count after the 2nd period
S3: Streptococcus count after the 3rd period
S4: Streptococcus count after the 4th period

b: Changes in Streptococcus mutans count

[21] we used a probiotic yogurt containing Bifidobacterium in this study.

Sampling of saliva was done after each phase and the participants were prevented from eating and drinking 3 hours before sampling. Then, 5mL non-stimulated saliva was collected in a sterile graded test tube (Falcon; China). The colonies of streptococci mutans were counted after culture and incubation period of 48 hours in blood agar (Merk; UK) plates at 37°C. Meanwhile, lactobacilli were cultured in tomato juice agar (HiMedia, India) anaerobically and their colonies were counted with a stereomicroscope (Zeiss; German) after an incubation period of 48 hours at 37°C. Statistical analysis was carried out using SPSS Software, version 14.0. Data were analyzed using t-test and paired-samples method. Differences were considered significant at \( p < 0.05 \).

Results

Figures 1 illustrate the levels of Streptococcus mutans and Lactobacilli both case and control groups in different phases. As shown in the Figures, from phase 1 to phase 4, there was no significant difference in Lactobacilli counts between case and control groups \( (p = 0.383) \); while the Streptococcus mutans counts exhibited a statistically significant difference between the two groups \( (p = 0.003) \). Although there was a reduction in Streptococcus mutans and Lactobacillus counts between phases 1 and 4 in control group, the count reduction was significant and prominent only for Streptococcus mutans \( (p = 0.009) \). In the case group, neither the Streptococcus mutans count nor the Lactobacilli count was significantly reduced after consumption of probiotic yogurt.

Normal yogurt decreased the Streptococcus mutans level significantly between period 1 and 2, also between period 3 and 4, but probiotic yogurt could not reduce the Streptococcus mutans count between these periods. Distributions of salivary mutants streptococci and Lactobacilli in the case and control groups are summarized in Table 1.

Discussion

The findings of this study showed that short-term consumption of probiotic yogurt could decrease neither Streptococcus mutans nor Lactobacillus counts. Caglar et al. (2009) investigated the colonization of Lactobacillus reuteri ATCC 55730 in the oral cavity after discontinuation of probiotic yogurt.

Table 1: Distribution of salivary mutants streptococci and lactobacilli counts at the end of 4 different periods in the case group

|                  | S1          | S2          | S3          | S4          | L1          | L2          | L3          | L4          |
|------------------|-------------|-------------|-------------|-------------|-------------|-------------|-------------|-------------|
| Number of cases  | 24          | 24          | 24          | 24          | 24          | 24          | 24          | 24          |
| Mean count of microorganisms | 60083       | 49750       | 55416       | 50416       | 32416       | 33451       | 34666       | 32452       |
| SD               | 40696       | 32647       | 35604       | 30684       | 16895       | 18996       | 20580       | 31112       |
| Minimum count of microorganisms | 0           | 0           | 0           | 0           | 0           | 0           | 0           | 0           |
| Maximum count of microorganisms  | 200000      | 120000      | 140000      | 100000      | 60000       | 80000       | 100000      | 160000      |

S1: streptococcus count after the 1st period
S2: streptococcus count after the 2nd period
S3: streptococcus count after the 3rd period
S4: streptococcus count after the 4th period
L1: Lactobacilli count after the 1st period
L2: Lactobacilli count after the 2nd period
L3: Lactobacilli count after the 3rd period
L4: Lactobacilli count after the 4th period
with this bacterium. The results showed that Lactobacillus reuteri had not been colonized permanently in the oral cavity after a period of 2 weeks consumption.

In accordance with most studies conducted before, this study showed no reduction of salivary Lactobacilli count after consumption of probiotic yogurt. There was even an increase in Lactobacilli growth in some cases. It can be concluded that the types of microorganisms in probiotic yogurt could not decrease and compete with the oral normal Lactobacilli flora.

Ahola et al. (2002) reported that all samples within the probiotic group revealed even increased Lactobacilli counts. [23] In the present study, after a short term consumption of probiotic yogurt, there was no statistically significant reduction in Streptococcus mutans and Lactobacilli counts. Ahola et al. used Edam cheese, containing Lactobacillus rhamnosus GG and Lactobacillus rhamnosus LC 7.5 to 18-35 year-old participants and found that the Streptococcus mutans count decreased in 20% of participants. [23] The difference between our findings and that of Ahola et al. may be explained by the difference of age range and the type of the probiotic bacteria.

In the control group, consumption of yogurt reduced the Streptococcus mutans and Lactobacilli count, but for Streptococcus mutans, the reduction was significant after period 4. The type of Lactobacillus present in the control group yogurts may be similar or almost similar to the oral Lactobacilli which can compete with them and hence reduce the member of oral normal flora of Lactobacilli to some degrees. Furthermore, the presence of some microbial products or other formulas found in control group yogurt, but not in the probiotic samples, might be responsible for the inhibition of the Streptococcus mutans proliferation. The type of probiotic microorganism used in the yogurt (Bifidobacterium lactis) did not have any inhibitory effect on Streptococcus mutans proliferation. Caglar et al. (2005) and Cildir et al. (2009) reported reduction of the salivary level of Streptococcus mutans after a short-term consumption of probiotic yogurt. [24-25] However, their studies differed from the present study, both in terms of the age range of participants and the type of probiotic bacteria used.

Our data revealed that usage of common yogurt could decrease bacterial level more than probiotic yogurt in case group. This finding is comparable with that of Petti et al. (2001) that demonstrated the effect of yogurt containing Lbulgaricus on the salivary microflora. [26] It suggests that normal yogurt can decrease the count of mutans streptococci and lactobacilli and can be used as a caries-preventing food.

In a systematic review, Cagetti et al. evaluated several papers about the use of probiotic strains in caries prevention. [27] This assessment revealed that all studies were generally small or medium in sample size and majority of them (80%) were short-term interventions. The quality of published papers recorded using the Consort score was 4 excellent, 9 good and 10 poor. These evidences have limited the results obtained about the efficacy of probiotics usage in caries prevention. Although administration of probiotics for prevention of caries has ascertained promising results, only few studies have shown clear clinical outcome; hence, the scientific data is still poor.

Owing to brevity of consumption period of probiotic product in this study and the limitation of probiotic microorganism type, more investigations are required to show the effect of longer consumption period of probiotic products on the salivary level of streptococcus mutans. Until the time of this study, none of the studies, conducted on the role of probiotic in caries prevention,

| Table 2: Distribution of salivary mutans streptococci and lactobacilli counts at the end of 4 different periods in the control group |
|---------------------------------|----------------|----------------|----------------|----------------|----------------|----------------|----------------|
| S1: streptococcus count after the 1st period | S2: streptococcus count after the 2nd period | S3: streptococcus count after the 3rd period | S4: streptococcus count after the 4th period | L1: Lactobacilli count after the 1st period | L2: Lactobacilli count after the 2nd period | L3: Lactobacilli count after the 3rd period | L4: Lactobacilli count after the 4th period |
| Number of cases | 25 | 25 | 25 | 25 | 25 | 25 | 25 |
| Mean count l of microorganism | 129200 | 120160 | 112080 | 64960 | 33120 | 30000 | 26080 | 23040 |
| SD | 97891.1 | 89489.1 | 84418.1 | 62198.9 | 18412.7 | 15874.5 | 15721.5 | 14249.2 |
| Minimum l count of microorganism | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Maximum count of microorganism | 360000 | 340000 | 360000 | 240000 | 40000 | 70000 | 60000 | 80000 |
have evaluated the effect of variable pH of probiotic yogurts on salivary cariogenic microflora, although changes of yogurt pH within the consumption period should be considered. Therefore, assessing the efficacy of different probiotic yogurt with variable pH on the risk factors of caries is recommended.

**Conclusion**

Based on the findings of this study, short-term daily consumption of probiotic yogurt containing *Bifidobacterium lactis* could not reduce the levels of *salivary streptococci mutans* and *lactobacilli* in 6 to 12 year-old children while the normal yogurt could significantly reduce the number of *streptococcus mutans*.

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**Conflict of Interest**

The authors have no conflict of interests to declare.

**References**

[1] Patro BK, Ravi Kumar B, Goswami A, Mathur VP, Nongkynrih B. Prevalence of dental caries among adults and elderly in an urban resettlement colony of New Delhi. Indian J Dent Res 2008; 19: 95-98.

[2] Hicks J, Garcia-Godoy F, Flaitz C. Biological factors in dental caries enamel structure and the caries process in the dynamic process of demineralization and remineralization (part 2). J Clin Pediatr Dent 2004; 28: 119-124.

[3] Bonifait L, Chandad F, Grenier D. Probiotics for oral health: myth or reality? J Can Dent Assoc 2009; 75: 585-590.

[4] Chuang LC, Huang CS, Ou-Yang LW, Lin SY. Probiotic Lactobacillus paracasei effect on cariogenic bacterial flora. Clin Oral Investig 2011; 15: 471-476.

[5] Chopra R, Mathur S. Probiotics in dentistry: A boon or sham. Dent Res J (Isfahan) 2013; 10: 302-306.

[6] Gibson GR, Roberfroid MB. Dietary modulation of the human colonic microbiota: introducing the concept of prebiotics. J Nutr 1995; 125: 1401-1412.

[7] Bhardwaj A, Bhardwaj SV. Role of Probiotics in Dental Caries and Periodontal Disease. Arch Clin Exp Surg 2012; 1: 45-49.

[8] Bhushan J, Chachra S. Probiotics- Their Role in Prevention of Dental Caries. J Oral Health Comm Dent 2010; 4: 78-82.

[9] Parvez S, Malik KA, Ah Kang S, Kim HY. Probiotics and their fermented food products are beneficial for health. J Appl Microbiol 2006; 100: 1171-1185.

[10] Doron S, Gorbach SL. Probiotics: their role in the treatment and prevention of disease. Expert Rev Anti Infect Ther 2006; 4: 261-275.

[11] Isolauri E, Kirjavainen PV, Salminen S. Probiotics: a role in the treatment of intestinal infection and inflammation? Gut 2002; 50 Suppl 3: III54-9.

[12] Saini R, Saini S, Sharma S. Potential of probiotics in controlling cardiovascular diseases. J Cardiovasc Dis Res 2010; 1: 213-214.

[13] Chong ES. A potential role of probiotics in colorectal cancer prevention: review of possible mechanisms of action. World J Microbiol Biotechnol 2014; 30: 351-374.

[14] Reid G, Bruce AW, Fraser N, Heinemann C, Owen J, Henning B. Oral probiotics can resolve urogenital infections. FEMS Immunol Med Microbiol 2001; 30: 49-52.

[15] Raman M, Ambalam P, Kondepudi KK, Pithva S, Kohi C, Patil AT, et al. Potential of probiotics, prebiotics and synbiotics for management of colorectal cancer. Gut Microbes 2013; 4: 181-192.

[16] Nâse L, Hatakka K, Savilahla E, Saxelin M, Pönskä A, Poussa T, et al. Effect of long-term consumption of a probiotic bacterium, Lactobacillus rhamnosus GG, in milk on dental caries and caries risk in children. Caries Res 2001; 35: 412-420.

[17] Caglar E, Kavaloglu SC, Kuscu OO, Sandalli N, Holgerson PL, Twetman S. Effect of chewing gums containing xylitol or probiotic bacteria on salivary mutants streptococci and lactobacilli. Clin Oral Invest 2007; 11: 425-429.

[18] Stecksén-Blicks C, Sjöström I, Twetman S. Effect of long-term consumption of milk supplemented with probiotic lactobacilli and fluoride on dental caries and general health in preschool children: a cluster-randomized study. Caries Res 2009; 43: 374-381.

[19] Caglar E, Cildir SK, Ergeneli S, Sandalli N, Twetman S.
Salivary mutans streptococci and lactobacilli levels after ingestion of the probiotic bacterium Lactobacillus reuteri ATCC 55730 by straws or tablets. Acta Odontol Scand 2006; 64: 314-318.

[20] Singh RP, Damle SG, Chawla A. Salivary mutans streptococci and lactobacilli modulations in young children on consumption of probiotic ice-cream containing Bifidobacterium lactis Bb12 and Lactobacillus acidophilus La5. Acta Odontol Scand 2011; 69: 389-394.

[21] Saxelin M, Tynkkynen S, Mattila-Sandholm T, de Vos WM. Probiotic and other functional microbes: from markets to mechanisms. Curr Opin Biotechnol 2005; 16: 204-211.

[22] Caglar E, Topcuoglu N, Cildir SK, Sandalli N, Kulekci G. Oral colonization by Lactobacillus reuteri ATCC 57730 after exposure to probiotics. Int J Paediatr Dent 2009; 19: 377-381.

[23] Ahola AJ, Yli-Knuuttila H, Suomalainen T, Poussa T, Ahlström A, Meurman JH, et al. Short-term consumption of probiotic-containing cheese and its effect on dental caries risk factors. Arch Oral Biol 2002; 47: 799-804.

[24] Caglar E, Sandalli N, Twetman S, Kavaloglu S, Ergeneli S, Selvi S. Effect of yogurt with Bifidobacterium DN-173010 on salivary mutans streptococci and lactobacilli in young adults. Acta Odontol Scand 2005; 63: 317-320.

[25] Cildir SK, Germec D, Sandalli N, Ozdemir FI, Arun T, Twetman S, et al. Reduction of salivary mutans streptococci in orthodontic patients during daily consumption of yoghurt containing probiotic bacteria. Eur J Orthod 2009; 31: 407-411.

[26] Petti S, Tarstitani G, D’Arca AS. A randomized clinical trial of the effect of yoghurt on the human salivary microflora. Arch Oral Biol 2001; 46: 705-712.

[27] Cagetti MG, Mastroberardino S, Milia E, Cocco F, Lingström P, Campus G. The use of probiotic strains in caries prevention: a systematic review. Nutrients 2013; 5: 2530-2550.