Original Article

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Evaluation of the Effect of Short-Term Consumption of Probiotic (*Bacillus coagulans*) and Ordinary Cake on Salivary *Streptococcus mutans*: A Pilot Study

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ABSTRACT

BACKGROUND AND OBJECTIVE: Consumption of food causes changes in oral flora. Foods that create an appropriate media for *Streptococcus mutans* and other cariogenic microflora, cause increase the rate of tooth caries. The aim of this study was the assessment of short-term consumption of probiotic cake (contain *Bacillus coagulans*) on salivary *Streptococcus mutans* count and comparison with ordinary cake.

METHODS: A cross over blind study was conducted on 30 healthy adult volunteers in two groups (16 males and 14 females). The first group ate probiotic cake (75 grams daily) for 1 week and after 2 weeks wash out period, they ate ordinary cake. The second group first ate ordinary cake and after 2 weeks wash out period, they ate probiotic cake (75 grams daily). Non-stimulating salivary samples, before (as baseline) and after eating probiotic and ordinary cake, were collected. Then counting of *Streptococcus mutans* was done with colony counter.

FINDINGS: Of the 30 patients, 16 (53.33%) were male and 14 (46.67%) were female with an average age of 40.86±17.15 years. Number of *Streptococcus mutans* in baseline saliva samples was $(7.872\pm1.430)10^6$ CFU/ml and in the saliva samples after consumption of probiotic cake was $(4.652\pm0.841)10^6$ CFU/ml and in saliva samples after consumption of ordinary cake was $(21.386\pm3.895)10^6$ CFU/ml. There was no significantly difference between mutants count before and after eating probiotic cake (p=0.769) but after consumption of ordinary cake than probiotic cake the count of streptococcus increased significantly (21.3±39.9 compared with 4.65±0.84) (p=0.032).

CONCLUSION: Based on the results of this study, the addition of probiotic bacteria to sweet and high-consumption foods can reduce the adverse effects of foods such as cakes on oral health.

KEY WORDS: *Saliva, Bacillus coagulans, Streptococcus mutans, Probiotics*.

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Introduction

Probiotics are a group of living microorganisms that can have beneficial effects on host health when used as dietary supplements (1-3). Probiotics have direct antibacterial effects and prevent the growth of bacterial strains (4). Oral cavity as an extremely complex ecosystem contains a variety of bacteria and creatures whose reduction or increase in each of them has different effects on the health of the oral cavity (5). This increase and decrease are generally very complicated mechanisms and probiotic bacteria can play an important role in regulating oral flora (4,6). *Streptococcus mutans* (*S. mutans*) is an acidogenic bacterium and is one of the major etiologic factors in dental caries in human being (7,8). In past studies, dairy products have been used as carriers of probiotic bacteria and their effects on dental caries and pH of the oral environment have been investigated (9-11). On the other hand, studies on commercial probiotics and its effect on the count of *Streptococcus mutans* in laboratory samples containing sucrose have been performed, suggesting a decrease in *S. Mutans* count and suggesting doing further studies in this field on human specimens (12,13). *Bacillus coagulans "Lactobacillus spurogenesis"* is a gram-positive strain and rod-shaped (14).

Resistance and high stability of this bacterium spore against heat, pressure and difficult environmental conditions have caused this probiotic bacterium to be selected in non-dairy products in comparison with other bacterial species (15). This bacterium has been confirmed by FDA (16,17) and the antimicrobial properties of the proteins that are produced by this bacterium have been confirmed (18, 19). Due to the association of microbial flora of different parts of the human body with each other, oral microbial flora is likely to be altered after intake of *Bacillus coagulans* "Lactobacillus spurogenesis" (20). On the other hand, according to studies that have been carried out on the addition of this bacterium to starchy products such as bread (15), as well as the successful results of the use of probiotic bacteria in foods containing sucrose (21,22), snack foods such as cake as a food containing starch and sucrose, which has plenty of appeal for consumers, were used. Although decay is a multifactorial phenomenon, it cannot be stated certainly that decrease in *S. Mutans* count will result in less caries, but reducing this bacterium can be one of the factors affecting oral health. Since clinical research has not been conducted to evaluate the effect of probiotic Bacillus coagulance on carriers of decaying food such as cake and its effect on *S. Mutans* counting as one of the factors affecting oral health, this study was conducted to investigate the effect of short-term consumption of probiotic cake containing Lactobacillus spurogenesis on the count of *Streptococcus mutans* in comparison with conventional cakes and also before use of any cake (baseline).

Methods

This cross-sectional study was conducted as a pilot study after registration in dental ethics committee of Tehran University of Medical Sciences with code IR.TUMS.DENTISTRY.REC. 275.1396. Using the results of previous studies and accuracy of 5%, 95% confidence interval, second type error β=0.2 and standard deviation of 30 for discovery of minimum difference of 16 units, the sample size was estimated to be 30. For this purpose, 55 healthy adult volunteers aged 20-70 years were selected (23). People with a history of any systemic illness, taking medication, especially antibiotics, corticosteroids and oral contraceptives during three months prior to onset of the study, habitual consumption of probiotic products and chewing gum containing xylitol and pregnant women (24) were excluded. In addition, before the study, there was no active dental infection, gingivitis and periodontitis in individuals. According to inclusion and exclusion criteria, among the volunteers, 30 individuals entered the study. All subjects were explained verbally about the study and after having informed consent, they expressed their consent to participate in the study in written form. Prior to the first sampling, toothbrushes, toothpastes and similar dental floss were given to the participants for 2 weeks. In addition to oral health instruction, they were asked to complete oral health care (25). Then, in order to reduce unwanted errors, subjects were divided into two groups. In both groups, the initial

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sampling was done to determine the *S. Mutans* baseline before beginning of the study. Before sampling, subjects were asked to avoid doing actions that affect saliva secretion, such as eating and drinking. Given the circadian rhythm, sampling was done at a specified time (7-8 AM in the morning) (26).

Samples of saliva in pre-weighed containers were collected with spitting method (for 60 seconds, the subjects collect saliva in their mouths and then spit in a sterile container and do this for 5 minutes) (27) and then the samples were stored at -20 °C.

In the first group, 75 grams of probiotic cake (containing Bacillus coagulance probiotic bacteria), which had the same date of consumption, was consumed as breakfast for one week each day. This group did not know the probiotic content of the cake and its difference with the ordinary cake (commercial label was covered with a marker and the study was done as single blind). After one week of using probiotic cake, the second saliva samples were collected as described before. Two weeks were considered as washout period and none of these products were used. In this group, in the fourth week, the consumption of ordinary cakes as breakfast began in a blind manner and after a week, a third saliva sample was collected. This procedure was also performed for the second group, with the difference that during the first week of the ordinary cake, and then for two weeks as washout period, then after the fourth week of probiotic cake, saliva sampling was done according to the procedure. (Fig 1).

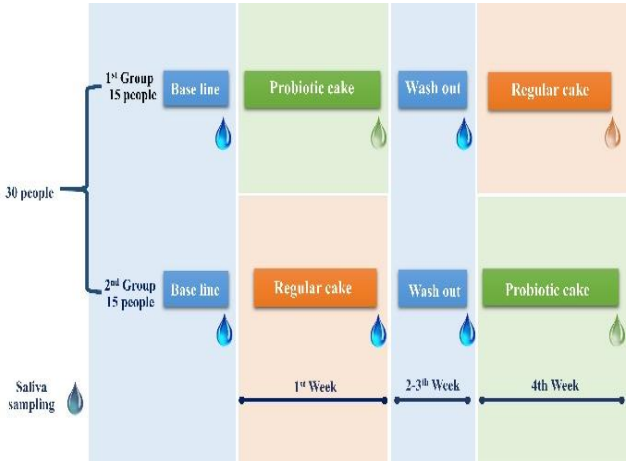

Figure 1. Schematic of salivary collection method in different stages of both groups (30 people)

Saliva samples that was collected in different stages were cultured for *Streptococcus mutans* count. For *S. Mutans* counting, bacteria, 100 µL of saliva was prepared in 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4} and 10^{-5} dilutions. Saliva samples were grown Agar and culture plates were stored in 37°C for 48 hours in a jar. Number of *S. Mutans* was counted using colony counter and their average number was reported as the final number. For statistical analysis, IBM SPSS statistics for Windows version 21.0 was used. Paired t-test was used to compare the count of *Streptococcus mutans* before and after consumption of probiotic cake and ordinary cake. $p<0.05$ was considered significant.

Results

Of the 30 patients, 16 (53.33%) were male and 14 (46.67%) were female with an average age of 40.86 ± 17.15 years. The minimum age of participants was 20 years and the maximum age was 68 years. The number of smokers participating in the study was 8, and the number of people taking alcohol was 11. The bacteria count increased significantly after the consumption of ordinary cakes (p=0.021). The *S. Mutans* count after consumption of probiotic cake increased very little. This increase was not statistically significant. There was a significant difference between the mean of *S. Mutans* after consumption of probiotic cake compared to ordinary cake (p=0.032). *S. Mutans* count after ordinary cake compared to probiotic cake showed a significant increase (Table 1).

Table 1. The average count of *Streptococcus mutans* and results of saliva pH measurements

Variable	pH	Salivary *Streptococcus mutans* count (*10^6)(CFU/ml)
Baseline	7.125±0.563	7.872±1.430
Probiotic cake	6.910±0.441	4.652±0.841
Ordinary cake	6.700±0.640	21.386±3.895
Total	6.908±0.548	5.968±2.050

Finding have shown that baseline bacterial count is not significantly different in smokers and non-smokers (Fig 2). Also, the consumption of ordinary cakes in

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alcoholic participants will increase the count of bacteria. Similar to smokers, in alcoholic people, with conventional cakes compared to probiotic cake, the bacterial count increases significantly (Fig 3)

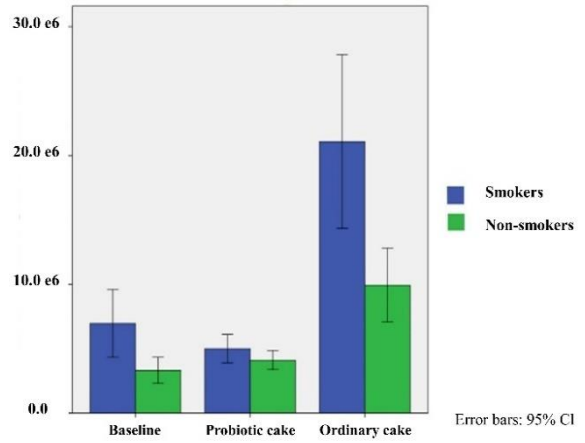

Figure 2. Comparison of the average saliva *S. Mutans* count of smokers and non-smokers after consuming ordinary cakes and probiotic cake compared to baseline.

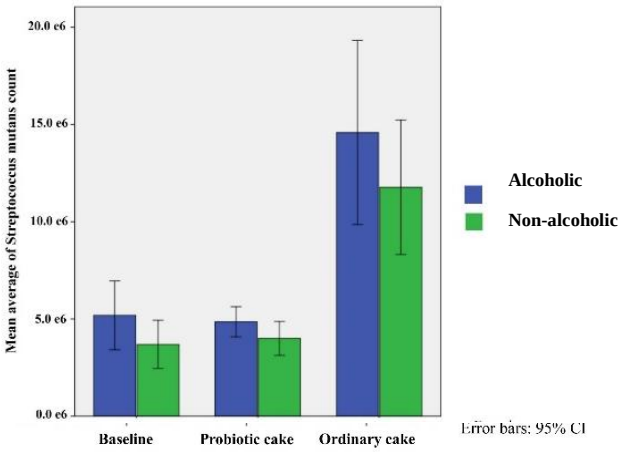

Figure 3. Comparison of the mean *S. Mutans* count in each ml of saliva of alcoholic and non-alcoholic individuals after consumption of ordinary cake and probiotic cake compared to baseline

Discussion

The results of this study showed that adding probiotic bacteria (*Bacillus coagulans)* to cake in the short term (one week) did not significantly increase *S. Mutans* count in saliva, while the short term consumption (one week) of ordinary cake caused a significant increase in *S. Mutans* count in saliva. In addition, comparing these two groups (probiotic cake and ordinary cake consumers) showed a significant difference between the two groups and the consumption of ordinary cake compared to probiotic cake would greatly increase the salivary *S. Mutans* count. In some studies, the consumption of dairy products containing probiotic bacteria has reduced the *S. Mutans* count. (25, 28-30). Also, the use of lotions with *Lactobacillus* probiotic bacteria significantly reduced the *S. Mutans* count (31), which is consistent with the results of the present study. The difference between these studies and the present study is the use of dairy products as carriers of probiotic bacteria and probiotic bacterial species.

In some studies, using of products containing probiotic bacteria such as milk, yogurt, pills and oral food lotions has not significantly reduced the *S. Mutans* count (32,33). This difference is due to the difference in the time of consuming compounds containing probiotic bacteria, the culture medium of bacteria and food containing probiotic bacteria, the design of the study, the age and sex of the subjects, the comparison and selection of the baseline and the type of probiotic bacteria regarding to the effect of strains specific probiotics. In the present study, there was no significant difference in the baseline count in smokers and non-smokers.

This finding is consistent with the findings of some studies that using tobacco does not significantly alter the count of salivary mutans streptococci (34,35), although there are studies that claim that using tobacco reduces the *Streptococcus mutans* count. However, the later studies have been carried out on laboratory samples (36, 37), as opposed to Sheth studies, as in the current study on a human sample.

Eating ordinary cake in smokers was associated with a higher increase in *S. Mutans* count than non-smokers. In smokers and non-smokers, eating probiotic cake result in significantly decrease in *S. Mutans* count. Therefore, the effect of using probiotic cake compared to ordinary cake was higher on *S. Mutans* count in non-smokers. The baseline *Streptococcus mutans* count in alcoholic subjects was higher than non-alcoholic subjects, although this conclusion contradicts the results of Sheth et al. (34), which can be due to the type of alcohol used, type of tobacco used and the difference in the design of the study. Considering the results of this

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study and the tendency of people to use snacks such as cakes, use of probiotic cake compared to ordinary cakes is recommended because daily use of probiotic cake does not significantly increase the count of *S. Mutans* in saliva. However, daily consumption of ordinary cakes significantly increases the count of *S. Mutans* in saliva, so adding probiotic bacteria to sweet and snack foods such as cake can reduce the adverse effects of potentially cariogenous foods such as cakes on oral health.

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