Comparison of the effect of regular and probiotic cake (*Bacillus coagulans*) on salivary pH and *Streptococcus mutans* count

Porównanie wpływu spożywania ciastek zwykłych i probiotycznych (*Bacillus coagulans*) na pH śliny i liczbę *Streptococcus mutans*

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Abstract

Background. Dental caries is considered the most common infectious disease in humans worldwide. Cariogenesis is the outcome of a complex interaction between the host's oral flora and diet. The consumption of snacks such as cake, which have the potential to promote dental caries, has increased.

Objectives. The aim of this study was to investigate the effect of including probiotic bacteria (*Bacillus coagulans* – *B. coagulans*) in consumed snack cake on the *Streptococcus mutans* (*S. mutans*) count and salivary pH.

Material and methods. We conducted a randomized, double-blind, cross-sectional cohort study on 40 healthy volunteers. The subjects were divided into 2 groups. In the 1st group, the subjects consumed probiotic cake as breakfast for 1 week and then, following a 4-week wash-out period, consumed regular cake as breakfast for 1 week. In the other group, the administration of probiotic and regular cake was reversed. For both groups, samples of at least 5 mL of non-stimulated saliva were collected using the spitting technique before and after the 1st and the 6th week. A colony counter was used to determine the number of *S. mutans* colonies. Salivary pH was measured before eating (8–9 a.m.).

Results. We detected no statistically significant difference in the *S. mutans* count before and after the consumption of probiotic cake, but noted a statistically significant difference in the count before and after the consumption of regular cake. We did not detect a significant difference in salivary pH with respect to the consumption of probiotic and regular cake, although the consumption of both foods caused a drop in salivary pH.

Conclusions. The addition of probiotic bacteria to sweet snack cake caused a minimal increase in the salivary count of *S. mutans*, a bacterial species with a definite role in cariogenesis, but did not impact salivary pH. Since probiotic cake has a slight impact on the *S. mutans* count, it is preferred over regular cake as a snack food.

Key words: salivary pH, *Streptococcus mutans*, *Bacillus coagulans*

Słowa kluczowe: pH śliny, *Streptococcus mutans*, *Bacillus coagulans*

Introduction

Probiotics are a group of live microorganisms that can have salutary effects on the host's health when properly supplemented or added to food.[1] Probiotics have a variety of effects, including immunity enhancement.[2] The latter may be achieved by stimulating the phagocytic leukocytes, increasing the secretion of immunoglobulin A (IgA), and affecting the production and activity of enzymes. In the gastrointestinal tract, probiotics help maintain the balance within the digestive tract and improve mucosal immunity.[3,4] The benefits of probiotics are facilitated through several mechanisms, including the alteration of the flora composition and immunomodulation.[5] The oral cavity is an extremely complex ecosystem with 700 bacterial species, 30 fungal species, several species of protozoa, and intracellular viruses.[6] Any factor perturbing the balance of these species can potentially affect oral health. Multiple studies have confirmed the potential impact of probiotics on regulating oral microflora, supporting a role for probiotics in the prevention of gingivitis, periodontitis, recurrent aphthous stomatitis, oral candidiasis, and dental caries.[7–9]

Streptococcus mutans (*S. mutans*) is an acidogenic bacterium and one of the major etiologic factors in dental caries.[10] Probiotic species, such as *Lactobacillus rhamnosus*, *Lactobacillus acidophilus*, *Lactobacillus casei*, *Lactobacillus reuteri*, and *Bifidobacterium animalis* subsp. *lactis* (*Bb-12*) can suppress the growth of *S. mutans*.[11] An in vitro study confirmed the growth inhibition of salivary *S. mutans* evoked by sucrose-containing commercial probiotics.[12] There is also an in vivo study supporting the effect of probiotic ice cream on the reduction of the *S. mutans* count.[13] In vivo studies using dairy products also demonstrated a significant reduction in the salivary *S. mutans* count.[14,15]

There is evidence that lactic acid-producing bacteria, and especially the *Bacillus* species, remain stable in heat and maintain their activity after baking.[16] The stability of these bacteria is facilitated by the presence of heat-resistant spores.[17] *Bacillus coagulans* – B. coagulans (*Lactobacillus sporogenes*) is a gram-positive, spore-forming, facultative anaerobe, which resists high pressure and temperature, and can function as a probiotic of choice in non-dairy products.[16] Other probiotic microorganisms, such as *Lactobacillus reuteri*, *Lactobacillus acidophilus* and *Bifidobacterium* are not spore-forming, and therefore are sensitive to temperature, pressure and stomach acid. *Bacillus coagulans* is a probiotic approved by the U.S. Food and Drug Administration (FDA) for use in animals and humans.[18] *Bacillus coagulans* has been shown to increase the antimicrobial properties of proteins as well as to decrease the clinical signs of induced rheumatoid arthritis in rats.[19] In this novel study, we used cake as the carrier of *B. coagulans* in order to evaluate the impact of probiotics on salivary pH and the *S. mutans* count.

Material and methods

This study was reviewed and approved by the Ethics Committee of Tehran University of Medical Sciences (IR.TUMS.DENTISTRY.REC.1396.2754). The people involved in this study were informed about the study and all of them signed the informed consent forms. All volunteers were from the same geographical region and had a comparable socioeconomic status. The study was a randomized, double-blind, crossover trial. Following informed consent, a total of 40 healthy adults without gingivitis, periodontal disease or active caries were enrolled. Excluded from participation were pregnant females, women using contraceptives, and subjects using xylitol gum, probiotic products, corticosteroids, or antibiotics within 3 months of the study initiation. Prior to the start, the subjects were provided oral hygiene instruction, issued toothbrushes, toothpaste and dental floss, and asked to maintain thorough oral health care for 2 weeks. A questionnaire was used to collect information about demographics, oral and general health, oral hygiene practices (frequency of brushing, flossing, mouthwash use, etc.), nutritional habits (consumption of sweet and sour foods), and social history (tobacco and alcohol consumption) from each participant. Our study followed a rigorous selection process and recorded all the behaviors considered likely to affect the *S. mutans* count.

The subjects were divided into 2 groups. Prior to the start of the project in both groups, a minimum of 5 mL of saliva was collected from each subject using the spitting method (the subjects allowed saliva to gather in the mouth for 60 s and then spit it into a sterile container; this process continued for a total time of 5 min). These specimens were used to determine pH and the *S. mutans* count at baseline. The subjects were asked to refrain from actions that affect the secretion of saliva, such as eating, drinking and smoking. Sampling was performed before breakfast and at a specific time (8–9 a.m.) to maintain consistency with the circadian rhythm. The latter is known to impact the salivary flow rate, microbial dilution and salivary composition (IgA, cortisol, etc.). In the 1st group, the subjects consumed 70 g of probiotic cake containing the probiotic bacteria *B. coagulans* (Dorna Food Industrial Group, Tehran, Iran) as breakfast for 1 week. After that time, saliva samples were collected in the manner previously described and used to measure salivary pH and the *S. mutans* count. The 2nd–5th weeks of the study were considered as a wash-out period with no pertinent data collection. Following the wash-out period, baseline saliva samples were collected and the subjects consumed regular cake as breakfast for 1 week. The saliva samples were collected for the 4th time after the 6th week and were used to measure pH and the *S. mutans* count. This protocol was also followed for the 2nd group, but the order of probiotic and ordinary cake consumed was reversed (regular cake consumed during the 1st week and probiotic cake consumed during the 6th week). The subjects in both

groups were blinded to the content of the cake consumed. Figure 1 provides a flowchart of the study protocol. The collected samples were stored at –70°C, and 100 µL of the specimen were used to make 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4}, and 10^{-5} dilutions in phosphate buffered saline (PBS) and in the calibrator diluent buffer, respectively. To culture the saliva samples, the specimen was streaked on 96-well plates containing the Mitis Salivarius Agar medium (Merck, Darmstadt, Germany). The agar plates were incubated at 37°C for 48 h. The bacterial colonies were counted with a colony counter using different dilutions; their mean value was reported as the final *S. mutans* count. The GC pH strips (900 200; GC America, Inc., Alsip, USA) were used for the measurement of salivary pH. A single sheet of the GC pH strips was dipped into saliva for 40 s to allow complete wetting of the strip. The color change in the GC pH strip was compared with the standard chart to determine salivary pH.

Statistical analysis

Statistical analysis was carried out using the IBM SPSS Statistics software, v. 21.0 (IBM Corp., Armonk, USA) for Windows. The data was analyzed using the *t*-test and the paired samples method. Differences were considered significant at $p < 0.05$.

Results

A total of 40 subjects were enrolled in the study. Twenty-one (52.5%) subjects were male and 19 (47.5%) were female. The age range of the participants was 15–73 years with an average of 41.67 ±16.80 years. Eleven subjects were smokers (at least 1 cigarette per day) and 17 participants reported having consumed alcohol regularly (at least once a day). Ten subjects were both smokers and regular alcohol drinkers and 18 of the 40 neither smoked nor drank alcohol regularly. The mean value of the *S. mutans* count was 6.42×10^6 CFU/mL at baseline, 6.95×10^6 CFU/mL after the weekly consumption of probiotic cake and 1.23×10^7 CFU/mL after eating regular cake for 1 week (Table 1). The mean value for salivary pH at baseline, after the consumption of probiotic and regular cake, was 7.125, 6.902 and 7.000, respectively (Table 1).

Table 1. Mean and standard deviation (*SD*) of salivary pH and the *Streptococcus mutans* (*S. mutans*) count at baseline and after 1-week consumption of probiotic and regular cake

Status of cake consumption	pH (mean ±*SD*)	*S. mutans* count (×10^6) [CFU/mL] (mean ±*SD*)
Baseline	7.125 ±0.493	6.42 ±13.53
Probiotic cake	6.902 ±0.231	6.95 ±10.42
Regular cake	7.000 ±0.472	1.23 ±20.16

Table 2 summarizes the results of the statistical analysis for the salivary *S. mutans* count and pH. Following the consumption of regular cake, the *S. mutans* count increased in comparison with the baseline *S. mutans* count and the difference was statistically significant ($p = 0.027$). Although the *S. mutans* count increased slightly after the consumption of probiotic cake in comparison with the baseline value, the difference was not statistically significant ($p = 0.795$). The mean *S. mutans* count after
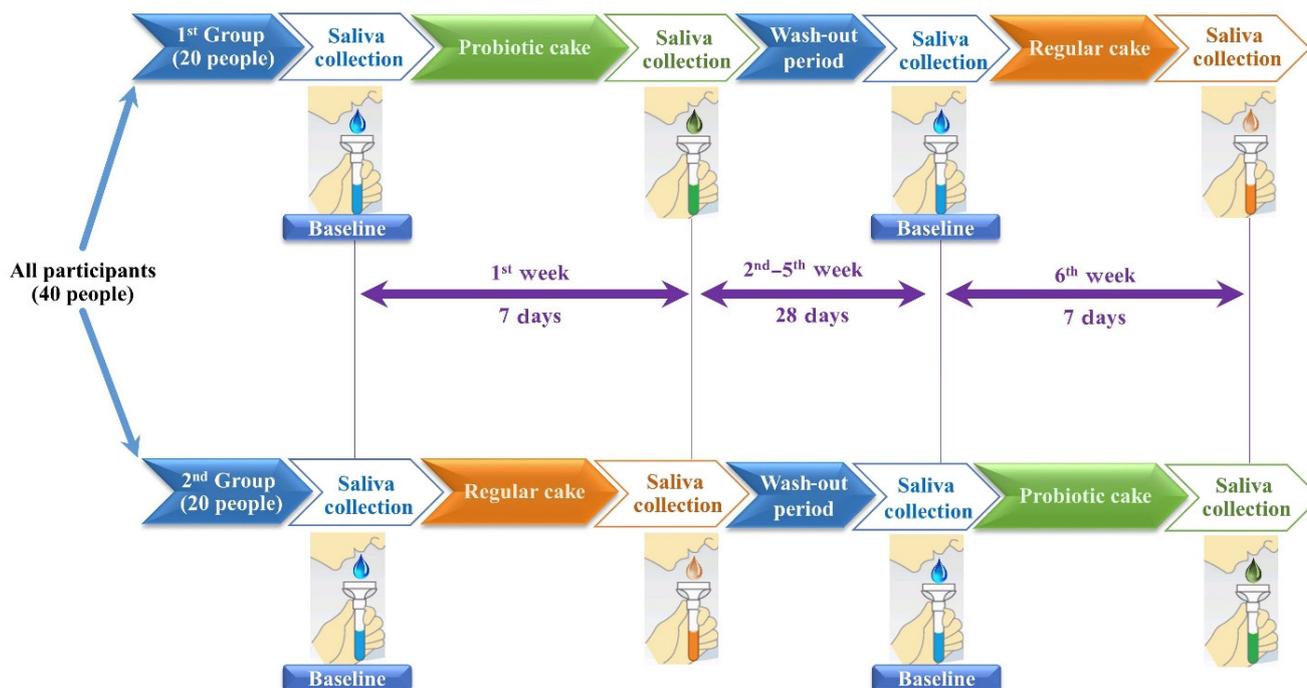

Fig. 1. Flowchart of saliva collection in the 1st and the 2nd group

Table 2. Statistical analysis of the *Streptococcus mutans* (*S. mutans*) count and salivary pH at baseline and after probiotic and regular cake consumption

Comparison between groups		Paired samples correlation (*p*-value) *S. mutans* count		Paired samples test Sig(2-tailed) (*p*-value) *S. mutans* count	Paired samples test Sig(2-tailed) (*p*-value) pH
		correlation	Sig	*S. mutans* count	pH
Pair 1	average *S. mutans* count at baseline average *S. mutans* count probiotic cake	0.439	0.005	0.795	0.068
Pair 2	average *S. mutans* count at baseline average *S. mutans* count regular cake	0.139	0.392	0.027	0.723
Pair 3	average *S. mutans* count probiotic cake average *S. mutans* count regular cake	0.204	0.208	0.030	0.183

eating regular cake was significantly higher than the mean *S. mutans* count following the consumption of probiotic cake ($p = 0.030$).

We did not detect a statistically significant difference in salivary pH after eating probiotic or regular cake in comparison with the baseline level, although both food types caused a minor drop in salivary pH (Table 2).

Discussion

Utilizing probiotics as a supplement in foods that are potentially cariogenic is an interesting approach for improving oral health, as seen in a number of recent studies aimed at controlling caries, periodontal disease and oral candidiasis.[20] In this study, we chose cake as a carrier of *B. coagulans* to reduce the *S. mutans* count. This choice is consistent with the prevalent consumption of sucrose-containing snacks in modern societies, especially among children.

Our results showed that short-term consumption of probiotic cake did not significantly increase the *S. mutans* count in saliva, while week-long consumption of regular cake caused a significant increase in the *S. mutans* count. The results of previous studies show a similar trend. In contrast, Keller et al. demonstrated that consuming probiotic-containing tablets did not significantly reduce the *S. mutans* count.[21] Siddiqui et al. showed that the consumption of probiotic-containing dairy products decreased the *S. mutans* count.[22] In several studies, a reduction in the *S. mutans* count with the consumption of probiotic-containing dairy products or lozenges was significant.[23,24] Our study differs from previous research with respect to the design, choice of probiotic species and the carrier food, period when the probiotic food was consumed, and culture medium. In addition, the dairy products utilized in earlier studies did not contain glucose or sucrose, both of which are shown to increase the *S. mutans* count.[22,25]

Two review studies, by Laleman et al. and Seminario-Amez et al., provide support for the efficacy of probiotics in reducing the *S. mutans* count.[11,20] The role of *S. mutans* in cariogenesis is well-established and despite the inadequacy of evidence for caries prevention properties

of probiotics, a reduction in the *S. mutans* count is a step forward.[7] We did not find a significant difference in the baseline *S. mutans* count between smokers and non-smokers. This observation is consistent with the findings of Sheth et al. and Voelker et al.[26,27] Our results show that the consumption of regular cake in smokers greatly increased the count of *S. mutans* bacteria and the rate of increase in smokers was much higher than in non-smokers. Compared to regular cake, the consumption of probiotic cake caused a much smaller change in the *S. mutans* count in both smokers and non-smokers. The difference in the *S. mutans* count after the consumption of probiotic cake compared to regular cake was greater in smokers than in non-smokers. The consumption of probiotic cake in smokers led to a much smaller increase in the *S. mutans* count compared to that observed with regular cake, while the consumption of probiotic cake in non-smokers caused a reduction in the *S. mutans* count. A plausible explanation for the lower efficacy of probiotic cake in reducing the *S. mutans* count in smokers is tobacco smoke-induced disturbance of oral microflora and its interactions with the probiotic species.[28,29]

Our results show a higher *S. mutans* count among the subjects who regularly consumed alcohol compared to those who did not regularly drink alcohol in all groups. This finding contradicts the results reported by Sheth et al.[26] It may be attributed to the difference in the study design, amount and type of alcohol as well as duration of drinking. In the subjects who regularly consumed alcohol, the consumption of regular cake increased the *S. mutans* count compared to the baseline. In comparison with regular cake, the *S. mutans* count increased slightly with the consumption of probiotic cake, irrespective of alcohol use, and the increase in the *S. mutans* count was more pronounced in the subjects who were not habitual users of alcohol. This difference may be attributed to the bacteriostatic effects of some types of alcohols on oral microflora. The consumption of regular cake by the users of both substances (alcohol and tobacco) increased the *S. mutans* count. A similar but less pronounced trend was also observed with the probiotic cake consumption in the subjects who both smoked and used alcohol regularly. Cigarette smoking reduces salivary pH, which predisposes the hard and soft tissues of the oral cavity to a variety

of diseases over time.[30] The highest level of *S. mutans* at baseline was observed in the non-smoker alcoholic subjects and the lowest level of *S. mutans* at baseline referred to the non-alcoholic smoker subjects. The highest level of *S. mutans* after the consumption of regular cake was observed in smokers that simultaneously consumed alcohol. The highest value of the *S. mutans* count after eating probiotic cake was observed in smokers who simultaneously consumed alcohol as well. In all groups, except for the non-smoker alcoholics, the consumption of regular cake led to a significant increase in the *S. mutans* count.

Conclusions

Although the development of dental caries depends on a complex interaction between the host's oral flora and diet, the role of *S. mutans* in cariogenesis is well-accepted. The addition of probiotic bacteria (*B. coagulans*) to sweet snack cake caused a minimal increase in the salivary count of *S. mutans*, but did not impact salivary pH. As probiotic cake has a low impact on the *S. mutans* count, it is preferred over regular cake as a snack food. Considering the frequent consumption of snacks such as cakes, in modern societies, the addition of probiotic flora (*B. coagulans*) to cakes may offer a strategy for reducing the *S. mutans* count in the oral cavity.

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