Comparative evaluation of different probiotic products on salivary *Streptococcus mutans* and *Lactobacillus* level in caries risk population

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**Abstract**

**Introduction:** Dental caries is a multifactorial disease with the main causative organism being *Streptococcus mutans* and *Lactobacillus* spp. “Probiotics” are defined as living microorganisms, principally bacteria, which are safe for human consumption and when ingested in sufficient quantities, have beneficial effects on human health, beyond basic nutrition. These can be used to replace the pathogenic strains of bacteria with the nonpathogenic type in the oral cavity thus can help prevent dental caries.

**Aims:** To evaluate and compare the role of probiotic milk and lozenges on *S. mutans* and *Lactobacillus* spp. count on patients who are exposed to probiotics continuously for 7 days.

**Materials and Methods:** Sixty volunteers who were prone to caries were divided into three equal groups. Experimental groups were given probiotics daily for 7 days. In Group A, patients were given milk without any probiotics (control); in Group B, probiotic milk is given; and in Group C, probiotic lozenges are given. Saliva samples were assessed on the 1st day and after 7 days of intervention. The collected saliva samples were inoculated on the selected culture media and estimation was done by measuring the colony-forming unit.

**Statistical Analysis Used:** Statistical analysis was performed using Student’s paired *t*-test and multiple comparisons by Tukey’s honest significant difference test.

**Results:** There was a significant reduction in salivary *S. mutans* level in both experimental groups after 7 days (*P* < 0.05). However, there was no significant difference in *Lactobacillus* count before and after the intervention.

**Conclusions:** Probiotics have a role in reducing salivary *S. mutans* count. Probiotics lozenges showed greater efficacy in reducing salivary *S. mutans* count than probiotic milk.

**Keywords:** Dental caries; *Lactobacillus casei*; probiotics; *Streptococcus mutans*

**INTRODUCTION**

Over the years, dental caries being proved as a multifactorial disease has a strong relation with microbial flora of the oral cavity. Shafer defined dental caries as an irreversible microbial disease of the calcified tissues of the teeth, characterized by demineralization of the inorganic portion and destruction of the organic substance of the tooth, which often leads to cavitation. Even though these living beings are normally present in food and water, they can likewise be intentionally added during the processing...
of food, for example, cheddar, yogurt, and matured milk items. For a very long time now, probiotics have been added to a certain food because of their benefits for human health. The bacteria in yogurt and fermented milk items establish the most imperative source of probiotics for people. By far most probiotic bacteria belong to the genera *Lactobacillus*, *Bifidobacterium*, *Propionibacterium*, and *Streptococcus*. Few clinical studies have suggested potential applications of probiotics in the treatment of cardiovascular disease, urogenital infections, oropharyngeal infections, and cancers.[2,3]

The term probiotic, which means “for life,” was first coined in the 1960s by Lilly and Stillwell. Probiotics are defined as living microorganisms, principally bacteria, that are safe for human consumption and, when ingested in sufficient quantities, have beneficial effects on human health, beyond basic nutrition.

A carious lesion begins with the establishment of a combination of specific bacterial population, which is capable of demineralizing enamel under specific modified environment in the oral cavity.[4] Different changes appear in the homeostasis of the oral ecosystem leading to the proliferation of the bacterial biofilm, composed notably of streptococci from the mutans group. To have a beneficial effect in limiting or preventing dental caries, a probiotic must be able to adhere to dental surfaces and integrate into the bacterial communities making up the dental biofilm. It also competes with and antagonizes the cariogenic bacteria and thus prevents their proliferation. Finally, the metabolism of food-grade sugars by the probiotic should result in low acid production.

The development of caries was believed to be caused by only a few Gram-positive bacterial species, such as *Streptococcus mutans* and *Streptococcus sobrinus*, and progression is caused by *Lactobacillus* spp.[5] This understanding was based on cultivation studies by isolating these bacteria and determining their cariogenic properties. It became evident that a caries lesion could happen in the absence of these putative pathogens. Current evidence states that population groups and individuals are susceptible to dental caries with a low level of *S. mutans* and vice versa.

The Global Burden of Disease Study 2017 estimated that oral diseases affect close to 3.5 billion people worldwide, with caries of permanent teeth being the most common condition. Globally, it is estimated that 2.3 billion people suffer from caries of permanent teeth and more than 530 million children suffer from caries of primary teeth.[6] Different methods such as diet modification, use of the fluoridated product, and use of oral hygiene aids have been advocated for the prevention of dental caries. One such preventive measure is probiotics which inhibit the growth of caries causing bacteria.

A thorough review of the literature showed that there are very minimum interventional studies regarding the role of probiotics in the oral cavity. Poorni *et al*. in their systematic review also mentioned that there are in vitro studies regarding probiotics that reduce caries; clinical studies are very few to know the clear clinical outcome.[7] Hence, the present study was undertaken to evaluate and compare the role of probiotic milk and lozenges on *S. mutans* and *Lactobacillus* spp. count on patients who are exposed to probiotics continuously for a period of 7 days.

**MATERIALS AND METHOD**

18-30 years old patient who are healthy, non-smokers and haven’t undergone any preventive treatment in last six months were selected for this study. Patients with lactose intolerance, who used xylitol products in the last 3 weeks, or who have undergone antibiotic treatment in the last 3 months were excluded from the study. The selected...
patients underwent a second round of screening using the Saliva-Check Buffer (GC) kit to detect buffer capacity and pH of saliva [Figure 1a and b]. Using a pipette, a saliva sample was taken and 1 drop was placed on each of the three test pads. The test pads began to change color immediately, but the final color was only detected after 2 min. Then, the result was calculated by adding the points according to the final color of each pad: green – 4 points, green/blue – 3 points, blue – 2 points, red/blue – 1 point, and red – 0 points. All points were counted and the result was determined: 0–5 points as very low buffering ability, 6–9 points as low, and 10–12 points as normal/high. Patients with salivary pH of <5.8 and very low buffer capacity were included in the study as they were more prone to develop dental caries. Sixty such patients were selected and included in this study.

The patients were then randomized into three equal groups, and instruction for oral hygiene maintenance was given. Sampling of saliva was carried out between 9 and 10 a. m. before breakfast. Two ml of unstimulated saliva was collected on the next day after the study period of 7 days, inoculated the same as for baseline data, and colonies were counted after incubation of 48 h. The data were recorded.

The saliva samples underwent microbial analysis by taking 10 μL samples with a micropipette and inoculated in both freshly prepared Tryptone yeast cysteine sucrose agar (TYCSB agar) with bacitracin for selective growth of S. mutans [Figure 1c] and in Man, Rogosa, and Sharpe (MRS) agar for selective growth of Lactobacillus species for incubated for 48 h [Figure 1d]. After 48 h, colonies were identified by Gram staining and were counted using a manual colony counter. Colonies were expressed as the number of colony-forming units per ml (CFU/ml) of saliva. By multiplying the actual colony count by $1 \times 10^5$, semiquantification of the number of colonies was done. The readings obtained were tabulated and considered as baseline data.

For the next 7 days, Group A was given 20 ml of milk without any probiotic (placebo control). Group B was given 65 ml of probiotic drink (Yakult). Group C was given adult’s dental care probiotic lozenges (Nature Plus) twice daily after breakfast and dinner. Patients were advised to take the probiotic medication after having food and also instructed to not have water or rinse the mouth for an hour. The probiotic drink used had 6.5 billion Lactobacillus casei strain Shirota, manufactured by Yakult Danone India Pvt. Ltd. (New Delhi, India). Probiotic lozenges that were used in the study contained 4 billion Lactobacillus acidophilus M23 strain and 2 billion Streptococcus salivarius M18 strain manufactured by Natural Organics Laboratories, Amityville, New York, USA.

Unstimulated saliva was collected on the next day after the study period of 7 days, inoculated the same as for baseline data, and colonies were counted after incubation of 48 h. The data were recorded.

## RESULTS

The data obtained were tabulated and subjected for statistical analysis using Student’s paired t-test, and Turkey’s honest significant difference test was applied for comparison between groups. Statistical analysis was performed with the help of Epi Info (TM) 7.2.2.2 software. Epi Info is statistical software for epidemiology developed by Centers for Disease Control and Prevention (CDC) in Atlanta, Georgia (US).

The result of the study showed that there was a significant reduction of salivary mutans levels in both experimental groups after a study period of 7 days [Table 1 and Figure 2]. Although patients who were under probiotic lozenges showed comparatively more reduction in salivary S. mutans level compared to probiotic milk, it was nonsignificant. This may be because probiotic lozenges had more contact time with oral mucosa than probiotic milk, which caused a greater reduction of salivary S. mutans count.

In Group B, reduction in Lactobacillus count is seen, but it was statistically nonsignificant. The postexperimental lactobacilli levels in Group C did not differ from baseline [Table 2].

The comparisons suggested that both probiotic milk and probiotic lozenges were able to reduce salivary S. mutans level. Although probiotic milk showed a reduction in Lactobacillus count, it was nonsignificant.

### Table 1: Average Streptococcus mutans counts at baseline and after 7 days

| Study period (days) | Group | n   | Mean  | SD    | $t$, $P$ |
|---------------------|-------|-----|-------|-------|----------|
| 0                   | A     | 20  | 82.25 | 8.13  | 0.379,   |
| 7                   | A     | 20  | 81.56 | 10.77 | 0.708 (NS) |
| 0                   | B     | 20  | 83.14 | 12.24 | 3.715, <0.001 |
| 7                   | B     | 20  | 72.97 | 12.32 | (significant) |
| 0                   | C     | 20  | 83.76 | 9.33  | 5.962, <0.001 |
| 7                   | C     | 20  | 71.32 | 10.54 | (significant) |

SD: Standard deviation, NS: Not significant

### Table 2: Average Lactobacillus counts at baseline and after 7 days

| Groups            | n   | Lactobacilli count (cfu/ml) | $P$ |
|-------------------|-----|-----------------------------|-----|
|                    |     | $<10^4$                     | $<10^5$ | $<10^6$ | $>10^6$ |
| Group I: baseline | 20  | 6                           | 8     | 4     | 2     | NS     |
| End               | 20  | 8                           | 8     | 4     | 0     |        |
| Group II: baseline| 20  | 8                           | 0     | 6     | 6     | NS     |
| End               | 20  | 12                          | 2     | 4     | 6     |        |
| Group III: baseline| 20  | 6                           | 2     | 4     | 8     | NS     |
| End               | 20  | 6                           | 4     | 4     | 6     |        |

NS: Not significant
DISCUSSION

Being a multifactorial disease, treatment or prevention of dental caries requires a multimodal approach. There are various methods available for caries prevention such as maintenance of oral hygiene, fluoride application, diet modification, and pit and fissure application. One of the approaches is antimicrobial, a probiotic-enriched product such as milk, yogurt, cheese, and lozenges, are gaining popularity these days. Probiotic products contain Bifidobacterium and Lactobacillus spp. as living microorganisms. The hypothesized mechanisms of action include direct interactions in the dental plaque with the interference of biofilm formation, plaque ecology, competing with oral microbes for the available substrate, and production of antimicrobial substances and indirect actions including modulation of systemic immune function, local immunity, the effect on nonimmunologic defense mechanisms, regulation of mucosal permeability, and oral colonization by less pathogenic species. The present study was conducted to observe the inhibitory effect of consumption of probiotic milk containing L. casei and probiotic lozenges containing S. salivarius and L. acidophilus on salivary S. mutans count and Lactobacillus count.

Previously, Bafna et al. [9] have done a study on the effect of short-term consumption of probiotic yogurt containing L. acidophilus La5 and Bifidobacterium lactis Bb12 on salivary S. mutans count in high caries risk individuals. They found that there was a statistically significant reduction of salivary S. mutans, which was recorded after probiotic yogurt consumption with minimal residual effect.

Saliva Check (GC Asia Dental Pvt. Ltd., India) was used in this study to select the patients with caries risk. Studies have shown that patients with high caries activity have resting pH below 5.5. A study conducted by Ericsson (1959) [11] showed that salivary buffering capacity has an inverse relationship with caries incidence. Thus, in the present study, only patients with a higher risk of getting dental caries, i.e., salivary pH below 5.8 (which is the lowest pH that can be detected using Saliva Check Kit), and patients with very low salivary buffering capacity were selected.

In our study, we found that patients who were given probiotic lozenges or milk showed a significant reduction in salivary S. mutans count after 7 days.

Previously Sutula J et al. revealed a significant but temporary and consumption-dependent presence of Lactobacillus casei in saliva and tongue plaque samples from healthy dentate individuals during the probiotic intervention phase. [12] The probiotic strain L. casei belong to the species Lactobacillus paracasei according to recent reclassification. [13] A double-blind, placebo-controlled trial demonstrated that consuming yogurt with Lactobacillus reuteri significantly reduced the oral carriage of mutans streptococci, compared with the placebo yogurt. [14]

Different strains of S. salivarius have been shown capable of counteracting the growth of mutans streptococci and, of these, the strongest clinical potential has been shown by strain M18. [15,16] On this basis, Francesco Di Pierro et al. conducted a study and found that 90 days of treatment with S. salivarius M18 oral probiotic has increased the chances of avoiding new cavities in children. [17] Strain M18 has specific anticariogenic characteristics, after colonizing in the oral mucosa it can release bacteriocins, limiting the growth of S. mutans and S. sobrinus, and can release the enzymes dextranase and urease which catalyzes the breakdown of dextran (aiding solubilization of plaque) and the hydrolysis of urea (increasing saliva pH). [17]

According to Wade et al., Tryptone Yeast Cysteine Sucrose medium with bacitracin is superior to mitis salivarius bacitracin agar in isolating S. mutans from saliva. [18] De Man, Rogosa, Sharpe agar, often abbreviated as MRS, is a selective culture medium designed to favor the growth of lactobacilli remained laboratory standard to this day. [19,20] Thus, these two media were selected to culture S. mutans and Lactobacillus spp. in this study.

In this study, in patients who consumed probiotic milk, there was a significant reduction of S. mutans level after the study period. Similar result shown in the study done by Sutula et al. [12]

Patients who consumed lozenges with S. salivarius M18 strain have also shown a decrease in salivary S. mutans count. The result is according to a study by Di Pierro et al. [17]

When probiotic milk and lozenges were compared, though lozenges showed more reduction in salivary S. mutans count, it was nonsignificant. These changes may be because probiotic lozenges allow more direct and thorough contact with the oral mucosa and biofilm compared with probiotic milk which explains such a result. Another probable mechanism is there may be a synergistic effect of S. salivarius strain and L. acidophilus strain which is better in reducing salivary S. mutans than L. casei strain alone. Though more studies with increased sample sizes required to confirm effect of probiotics on oral microflora.

Regarding Lactobacillus spp. count, we did not find any significant changes in pre- and post-treatment samples. This may be because the intervention of only 7 days is too less to mark any changes in salivary Lactobacillus count. This result is according to a previous study done by Singh et al. [21]
The limitation of the study may be the oral hygiene status of the patients was not standardized, which could be avoided by performing oral prophylaxis for each patient. The patient’s dietary habits were also not considered in this study.

CONCLUSIONS

Within the limitation of the study, it can be concluded that short-term daily ingestion of probiotics can significantly reduce the level of salivary S. mutans. Furthermore, probiotic lozenges performed better than probiotic milk in reducing S. mutans level.

Thus, it can be said that probiotic holds the bright prospect as a novel approach to reduce salivary S. mutans level in caries risk patients ultimately reducing caries incidence. Though more long-term studies with more sample size are needed to confirm their efficacy on oral microflora. Since the current study focused on only two types of bacteria, further investigations are required to ascertain the clinical efficacy of the bacterium in reducing various other caries causing microorganisms. Thus, the potentially beneficial influence of probiotics with a different vehicle on the complex oral microflora may be justified.

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Conflicts of interest
There are no conflicts of interest.

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