Research Article

Effects of probiotics consumption on salivary pH and *Streptococcus mutans* counts

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ABSTRACT

Background: Probiotics play an important role in pathogenesis in oral cavity and also improve in gastrointestinal health problems. Antimicrobial methods which control dental caries that includes probiotic agents play an important role in establishing caries control in children from moderate to high risk for developing dental caries. **Aim:** This study aims to analyze and compare the usage of probiotic products and its effect on salivary pH and *Streptococcus mutans*. **Objective:** The objective of this study was to compare the effect of short-term consumption on probiotic curd and normal curd. **Materials and Methods:** This study was carried out in 10 human volunteers who are caries free, examination period is done for 20 days. It is examined as two periods, that is, before probiotic and after probiotic, it is also tested using pH strips to denote saliva pH before and after consumption of probiotic curd. *Mutans* agar was used to enumerate colonies. Method - dilute the saliva to 1:20 dilution. 1 ml of saline with 50 µl saliva then transfer to 10 µl to *mutans* agar and incubate at 37*C and count. **Reason:** Probiotic agents are believed to play a role in dental caries. A shift of pH range will determine the risk of inhibition of the growth of *S. mutans* counts.

KEY WORDS: Antimicrobial, Dental caries, Oral cavity, Probiotics, Salivary pH, *Streptococcus mutans*

INTRODUCTION

Probiotics are defined in terms as live microorganisms which when administered in adequate amounts confer a health benefit on the host (FAO/WHO). The concept of probiotics was introduced in the early 20th century. Probiotics are also being used for preventing tooth decay and for preventing or treating other oral health problems such as gingivitis and periodontitis. However, the rapid growth in marketing and consumer interest and use has outpaced scientific research on the safety and efficacy of probiotics for specific health applications.[1] It appears following changes in the homeostasis of the oral ecosystem, leading to 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.[3]

Probiotic products containing different bacterial strains are commercially available such as lozenges, suckling tablets, chewing gums, and dairy products such as milk, ice cream, cheese, and yogurt. Probiotics are used in the treatment of several intestinal and systemic diseases, after ingestion, it integrates with bacterial microflora to improve homeostasis and intestinal defenses, increase resistance to colonized by pathogens, induce productivity of antibacterial action, stimulate local immunity, and modulate the inflammatory response.[4]

Probiotic technology represents a breakthrough approach to maintain oral health by utilizing the natural beneficial bacteria commonly found in healthy mouth to provide natural defense against those bacteria thought to be harmful to teeth and gums.

Probiotic may affect the oral ecology by specifically preventing the adherence of other bacteria and

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by modifying the protein composition of salivary pellicle. Probiotic bacteria could modify the protein composition of the pellicle by two different methods, namely by binding to and degradation of salivary proteins.[5]

MATERIALS AND METHODS

This study was carried out in 10 human volunteers who were caries free, no systemic illness, or any other oral problems. The subjects were examined for 20 days.

Inclusion Criteria

The following criteria were included in the study:
Age group selected of 18–20 years.
Caries free.
No systemic disorders.

Exclusion Criteria

Severely ill people, medically compromised people, people who had been on medication in the past 6 months, and those undergoing orthodontic treatment were excluded from the study.

Selection of Samples

Before probiotic consumption, salvia was collected from 10 human volunteers on 3 days, respectively. The salvia was expectorated directly into Eppendorf tubes. During the intake course of probiotic for 10 days, samples were collected on day 5 and day 10.

pH Determinants

Using pH paper, pH of salvia has been known.

Microbiological Analysis

A serial dilution of the collected salivary sample was done in saline. Diluting the saliva to 1:20 ie 1 ml of saline with 20 µl saliva then transfer to 10 µl of *mutans* agar. Using inoculating loop, the diluted salivary sample was streaked on *mutans* agar selective media for *Streptococcus mutans*. The plates were incubated at 37*C for 24 h and the number of colonies was counted based on the colony characteristics.

RESULTS

Before Probiotic

Sample	pH	*Mutans* count
Day 1	6.60±0.966	49,085±44,636
Day 5	6.10±0.738	22,103±27,830.616
Day 10	6.60±1.17	18,238.80±25,251.8

After Probiotic

Samples	pH	*Mutans* counts
Day 5	5.60±0.699	31,540±54,267.98
Day 10	5.6±0.699	48,550±25,641.2

Before the consumption of probiotics, normal salvia is collected from individuals as day 1 sample whose mutant counts give mean and standard deviation as 49,085 ± 44,646 and pH gives 6.6 ± 0.96. On day 5, 22,103 ± 27,830.6 for mutant counts and pH shows 6.10 ± 0.73. On day 10, mutant counts show 18,238 ± 25,251 and for pH is 6.60 ± 1.174.

After the consumption of probiotics, saliva is collected on day 5 mutant counts 31,540 ± 54,267 and for pH 5.6 ± 0.699.

On day 10, mutant counts show 48,550 ± 25,641 and for pH is same as the day 5.

DISCUSSION

Dental caries being a multifactorial disease process often requires a multimodal approach to treatment and control. Probiotic products contain living microorganisms such as *Bifidobacterium* and lactobacilli.[1] Lactobacilli play a key role in the etiopathogenic mechanisms, leading to the development of dental caries. The absence of significant variations in the salivary counts of *S. mutans* emphasizes the specificity of the lactobacilli increase.

S. mutans is a Gram-positive anaerobic bacterium. Since it is anaerobic, it is mostly found between adjacent teeth or in the deep crevices on occlusal (the biting surface) of teeth. It is as mentioned before the main cavity and halitosis-causing bacteria in the mouth (although there have been hundreds of other bacteria as well as other microorganisms identified). The entire genome of *S. mutans* of over 2 million base pairs has been completely identified and sequenced.

S. mutans has been implicated as one of the major and most virulent of the caries-producing microorganisms. It can colonize the tooth surface and initiate plaque formation by its ability to synthesize extracellular polysaccharides from sucrose, mainly water-insoluble glucan, using the enzyme glucosyltransferase. Inhibiting the colonization of *S. mutans* on the tooth surface is believed to prevent the formation of dental plaque and development of dental caries.[6-8]

On comparing the mean salivary *mutans* streptococci at baseline, after 1 h, and after 7 days of consumption of probiotic and normal curd, the results were found to be statistically significant after 1 h ($P \leq 0.05$) and after 7 days ($P \leq 0.05$) period. This was in accordance with the study done by Chinnappa *et al*. in which after 1 h and 7 days, statistically significant ($P \leq 0.05$) results were obtained.

Significant reduction in salivary *mutans* streptococci ($P \leq 0.05$) was found in our study after consumption of probiotic curd, which was in accordance to the previous studies done by Chinnappa *et al*., Caglar *et al*., Caglar

et al., and Zhu *et al*. in which a statistically significant reduction of salivary *mutans* streptococci was recorded after probiotic yogurt consumption ($P \leq 0.05$). Singh *et al*. also reported that probiotic ice cream brought about a statistically significant reduction in *mutans* streptococci count ($P \leq 0.05$). Similarly, Jindal *et al*. concluded that statistically significant reduction ($P < 0.05$) in salivary *mutans* streptococci counts was recorded after probiotic ingestion. Whereas our results were in contrast to the previous study done by Chuang *et al*., in which no differences in the counts of *mutans* streptococci between probiotic and control groups were found.[9-13]

Use of probiotics may reduce the cost of conventional therapy and prevention programs for the management of oral disease. It is an attractive idea of replacing harmful microorganisms with non-harmful, inactivated, or genetically modified bacteria. With the focus on disease prevention and optimal health for all ages, the potential for probiotics' use is enormous. Awareness should be created about the aspect of oral disease therapy and encourage the implementation of the concept of "food rather than medicine." The existence of probiotics in the indigenous oral microflora of humans should be explored because these bacteria offer the advantage of being perfectly adapted to the oral ecosystem.

CONCLUSION

Probiotic curds containing *Lactobacillus acidophilus* may be used as adjuncts, for prevention of dental caries, especially as a part of dietary modification in children at varying risk for dental caries. The effects of probiotic strains in the prevention of diarrhea caused by enterotoxigenic microorganisms have been studied in subjects' traveling to a number of destination. From this study, we found that the pH level of saliva is lower before the consumption of the probiotic drink, whereas the mutant count was comparatively higher.

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