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Original Research

Evaluation of *Streptococcus mutans* Levels in Saliva before and after Consumption of Probiotic Milk: A Clinical Study

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Abstract:

Background: Probiotic bacteria are thought to reduce the risk of some infectious diseases. Recently, the probiotics and their effect on oral health have been a focus of numerous trials. The documented trials have been reported from developing countries, such as India, with its focus on pediatric population. This study was done to evaluate the effects of short term consumption of probiotic milk containing *Lactobacillus casei* Shirota, and its effects on the levels of salivary *Streptococcus mutans* in children.

Materials and Methods: A cross-sectional study was performed on 20 healthy subjects and was followed for 7 days. The assessment of *S. mutans* in saliva was done at baseline, 1 h after consumption of probiotic milk and after 7-day of intervention period using mitis salivarius bacitracin agar. The colonies of *S. mutans* were identified and counted using a digital colony counter.

Results: A statistically significant reduction of salivary *S. mutans* was recorded after consumption of the probiotic milk. The comparison between baselines versus follow-up salivary *S. mutans* count after consumption of probiotic milk was found to be statistically significant ($P \leq 0.05$).

Conclusion: Based on observations of this study - Short term consumption probiotic milk reduced the growth of salivary *S. mutans* levels. Efforts should be made to increase the awareness of the dental practitioners about this aspect of oral therapy and encourage the implementation of the concept of "food rather than medicine."

Key Words: *Lactobacillus casei* shirota, probiotics, saliva, *Streptococcus mutans*, Yakult milk

Introduction

Due to increase in the cost of health care system and widespread use of antibiotics has led to research and development in the area of functional foods having potential positive effect on health beyond basic nutrition. Although, the concept of functional food was introduced long back by Hippocrates as their motto was "let food be your medicine."[1]

The term probiotic is a Greek word which means "for life." The concept of probiotics was introduced by Nobel Prize winner, Ukrainian bacteriologist Elie Metchnikoff in 1908, and the term probiotics was coined by Lilly and Stillwell in 1965.[2] According to World Health Organization probiotics are live microorganisms when administered in adequate amounts confers health benefits to the host.[3]

The *Streptococcus mutans* are considered as one of the most virulent caries producing microorganisms as they can colonize and initiate plaque formation on the tooth surface by its ability to synthesize extracellular polysaccharides from sucrose, mainly water soluble glucan, using the enzyme glucosyltransferase. It is believed that inhibiting the colonization of *S. mutans* on tooth surface prevents the formation of dental plaque which in turn reduces the chances of development of dental caries.[4] To combat infection, administration of probiotic bacteria is the most common therapy which beneficially affects the host by improving its intestinal microbiological balance.[5] Dental caries is one of the most common childhood infectious disease which can occur at any age and it is preventable. Various approaches for prevention of dental caries are chemo prophylactic agents, antibiotics, caries vaccine, sugar substitutes, fluoride, bacteriotherapy, or replacement therapy.

India is a developing country where major portion of population lacks basic health facilities. Oral health is largely neglected across all the age groups. Dental caries causes impaired function, affects aesthetics and ultimately results in global burden. There is a need to target preventive measures and spread awareness that dental caries is preventable by adopting simple oral health prevention measures.[6]

Probiotics and its possible impact on oral health is less explored till date. There is a need to identify the ideal administration vehicle for probiotic bacteria for which various dairy products such as milk, yoghurt and cheese can be considered, but the ideal administration vehicle which is suitable for all ages has yet to be identified. It has been suggested that probiotic exposure in early life may facilitate permanent installation of health promoting strains. Products, such as milk, curd, and ice-cream, are considerable probiotic foods as they are popular and universally liked.[7]

The present study was conducted to examine whether short-term consumption of milk containing *Lactobacillus casei* Shirota (LcS) can affect the levels of salivary *S. mutans* count in children, thus assessing probiotic as a preventive tool against the development of dental caries.

Materials and Methods

The present study was carried out in Sri Ram Public School Shiksha Samiti, Mathura, Uttar Pradesh, India. The research protocol was reviewed and approved by the Institutional Ethical Committee of Kanti Devi Dental College and Hospital. Consent forms were signed by school authorities, prior to patient enrolment in the study. This study compares *S. mutans* levels in saliva before and after consumption of probiotic milk.

For this study, 20 subjects were randomly selected from Shri Ram Public School Shiksha Samiti, Mathura. The commercially available probiotic fermented milk Yakult (supplied by Yakult UK Ltd.) were provided to the subjects for consecutive 7-day period. Each bottle of 65 ml Yakult fermented milk contained minimum of 6.5×10^9 viable cells of LcS.[8]

Inclusion criteria

- Children in the age group of 6-9 years
- Children without a history of antibiotic intake preceding 1 month
- No clinically detectable caries
- No history of any preventive dental treatment.[6]

Exclusion criteria

- Presence of any congenital abnormalities, mental disorders, systemic disorders, chronic debilitating diseases
- Children undergoing orthodontic treatment.[6]

The randomly selected subjects were equally divided into two groups comprising of 10 children in each group. Subjects in Groups 1 and 2 were provided with 65 ml of probiotic milk and plain milk respectively for 7 days. Subjects were encouraged to maintain good oral hygiene throughout the study period. The assessment of saliva samples were done at baseline, just after an hour and after 7-days of intervention period by using mitis salivarius bacitracin agar medium.

Following a thorough clinical examination, collection of saliva was done. To clear the residual saliva subjects were asked to swallow the preexisting saliva and were made comfortable. Subjects were provided with sterile plastic containers and were asked to spit the saliva into it. During the period of sample collection and processing the samples were precoded. For the purpose of analysis, the samples were handed over on the same day to the Rangeshwar Pathology Lab, Mathura city. The precoding of the samples was not disclosed to the technicians.

All plates were cultivated on mitis salivarius bacitracin agar medium at 37°C under anaerobic conditions for 72 h shown in Figure 1. Confirmation of *S. mutans* was performed under light microscopy after staining a heat fixed smear. Microbial counts were expressed as colony-forming units per ml of saliva.[9] The laboratory staff and clinicians evaluating the culture plates were blinded to the subject's group assignment. Assessment of saliva was done at baseline, after 1 h and on the 7th day. The colonies of *S. mutans* were identified on the basis of their morphology and counted using a digital colony counter and subjected to unpaired *t*-test.

Statistical analysis

The Statistical Package for Social Sciences version 16 was used to determine the mean and standard deviation for *S. mutans* in samples. The change in microbial counts before and after intervention was assessed using Unpaired Student *t*-test. The confidence level of the study was kept at 95%, hence a $P \leq 0.05$ indicated a statistically significant difference.

Results

In this study, mean salivary *S. mutans* count at baseline for probiotic milk and plain milk groups was found to be 289 ± 30.7 and 286.5 ± 46 respectively. Mean salivary mutans streptococci count at 1 h after consumption of probiotic milk and plain milk was 230 ± 38.6 and 291 ± 40.4 respectively. When compared after 7 days, mean salivary *S. mutans* count after consumption of probiotic milk and plain milk was found to be 194 ± 16.1 and 290 ± 44.6 respectively. When comparison of the mean

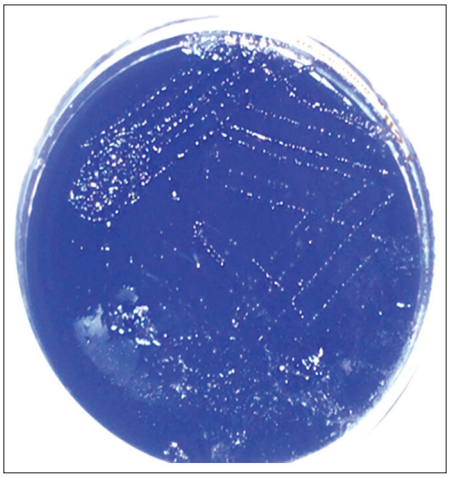


Figure 1: Growth of *Streptococcus mutans* on mitis salivarius bacitracin agar.

salivary *S. mutans* was done at 1 h and 7 days after consumption of probiotic milk and plain milk, the results were found to be statistically significant (Table 1 and Graph 1).

Discussion

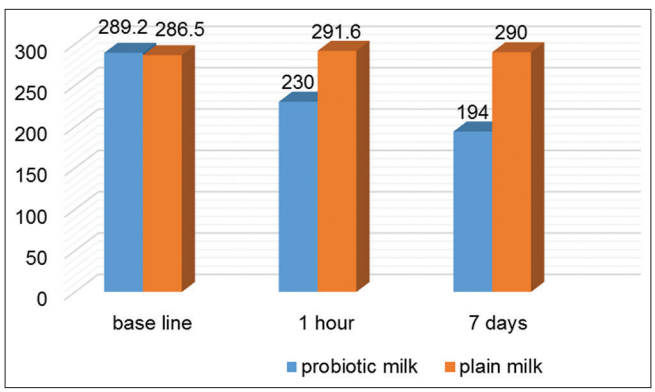
Pathogenic microorganisms could be displaced by probiotic bacteria. In order to prevent enamel demineralization probiotic products could be used which provide a natural defense against the harmful bacteria in oral cavity. Probiotics seems to be a promising approach in maintaining the oral health by using the natural beneficial bacteria found in healthy mouth.[4] There is a need to change approach for community based prevention and oral health promotion programmes for developing countries, a successful research requires focus on easy availability and cost effectiveness of products. To best of our knowledge, there are few studies till date to validate the beneficial effects of probiotic milk on the oral ecology. Therefore, this study was conducted to estimate the beneficial effects of probiotic milk on salivary *S. mutans*.

Sudha *et al.*[10] conducted study on 5-13 years age group which showed higher prevalence of dental caries in 5-7 years age group compared to that of 8-9 and 11-13 years age group. In our study, children of 6-9 years were taken as at this age the permanent teeth are erupting, so there are chances that the new surfaces would be colonized by pathogenic bacteria. Therefore preventive measures taken at this time might be helpful in long run. Also a positive compliance could be expected from a child of this age group. According to Jean Piaget, 7 years of age largely corresponds to increase in cognitive development. Therefore, an opportunity existed and prompted us to take up present study with an age group of 6-9 years.

Table 1: Comparison of mean salivary *S. mutans* after consumption of probiotic and plain milk at base line, after 1 h and after 7 days.

Time interval	Milk	Mean±SD	*P*-value
Baseline	Probiotic plain	289.2±30.7	0.87
		286.5±46	
After 1 h	Probiotic plain	230±38.6	0.002*
		291.6±40.4	
After 7 days	Probiotic plain	194±16.1	0.0001*
		290±44.6	

*: $P \leq 0.05$, *S. mutans: Streptococcus mutans*, SD: Standard deviation



Graph 1: Progressive decline in the levels of *Streptococcus mutans* at baseline, 1 h and after 7 days of probiotic milk consumption which was statistically significant.

Various probiotic products available in India are probiotic preparations of milk, curd, yogurt, chocolate and ice cream. Poureslami *et al.*[11] concluded that enamel demineralization can be prevented by increasing the calcium content of dental plaque which can be effectively done by consuming dairy products. Among all dairy products, milk was chosen for the present study as they have anti cariogenic property due to natural presence of casein, calcium and phosphorus. Above all milk is popular, universally liked and well accepted by the subjects.

The levels of salivary *S. mutans* found in the present study were statistically significant ($P \leq 0.05$) when compared at baseline, after 1 h, and after 7 days of probiotic and plain milk consumption as shown in Graph 1. The results found in this study was in accordance with the study done by Chinnappa *et al.*[7] in which after 1 h and 7 days, their results were statistically significant. Marked reduction in salivary *S. mutans* ($P \leq 0.05$) was found in our study after consumption of probiotic milk, which was also in accordance with the previous studies done by Caglar *et al.*,[5] Caglar *et al.*,[12] Zhu *et al.*,[13] Ahola *et al.*[14] and Jindal *et al.*[6] in which a statistically significant reduction of salivary *S. mutans* was recorded after probiotic yogurt consumption ($P \leq 0.05$). Singh *et al.*[15] also reported that probiotic ice-cream brought a statistically significant reduction in *S. mutans* count ($P \leq 0.05$). Similarly, Jindal *et al.*[6] reported a statistically significant reduction ($P \leq 0.05$) in salivary *S. mutans* counts after probiotic ingestion, whereas our results were in contrast to the study done by Chuang *et al.*[16] in which no differences were found in the counts of *S. mutans* between probiotic and control groups. However, Montalto *et al.*,[17] found oral administration of probiotics increased the salivary lactobacilli counts but *S. mutans* counts remained unchanged.

The marked reduction in salivary levels of *S. mutans* in the present study can be due to possible mechanism of action of probiotics that is bacterial adhesion, ability to early colonize in oral cavity, interspecies interaction and immunomodulation.[18] Grover and Luthra[19] reported that the hypothetical mechanism of probiotic action in oral cavity is by various direct and indirect means. The possible direct interactions in dental plaque includes binding of oral microorganisms to protein, production of chemicals that inhibits oral bacteria, action on plaque formation and its complex ecosystem by competing and intervening with bacterial attachments. Various indirect probiotic actions are modulation of systemic immune function, enhances local immunity, regulates mucosal permeability and effects on the non-immunologic defense mechanism. It also functions as antioxidants and neutralizes free electrons thus prevents plaque formation.

S. mutans can synthesize extracellular polysaccharides from sucrose, mainly insoluble glucan using the enzyme

glucosyltransferase and it has the ability to initiate plaque formation.[4] Therefore in the present study, probiotic product Yakult milk was tested against the most virulent caries producing microorganism, since inhibiting the colonization of *S. mutans* on the tooth surface is believed to prevent the formation of dental plaque and development of dental caries.

In the present study, only short term administration of probiotics was assessed - However, it resulted in significant reduction of cariogenic bacterial count of *S. mutans*. It seems plausible that prolonged administrations of probiotic preparations may have a preventive role against caries development. Therefore, long term studies on effects of probiotic milk in inhibiting the bacterial count of *S. mutans* would be valuable.

Conclusion

In accordance with the results of present study following conclusions can be drawn:

Use of probiotic milk could be an alternative strategy of displacing pathogenic microorganisms by probiotic healthy bacteria and can thus prevent enamel demineralization and development of dental caries. Current findings on the potential use of probiotics against inhibition of *S. mutans* are very encouraging. However, more research with a larger sample size and long term clinical trials are needed. It is important to realize that the suggested health benefit and efficacy of one strain cannot be implied on the whole species rather each bacterial strain should be studied individually.

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