

Comparative Study of Probiotic Ice Cream and Probiotic Drink on Salivary *Streptococcus mutans* Levels in 6-12 Years Age Group Children

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Abstract:

Background: Dental caries is one of the most common health problems in the world. Probiotics are one the various preventive methods to reduce dental caries. The aim of this study is to compare the effectiveness of probiotic ice cream and drink on salivary *Streptococcus mutans* levels in children of 6-12 years age group.

Materials and Methods: A three phase study was carried out in children ($n = 50$) of 6-12 years age with zero decayed missing filled teeth (dmft)/DMFT. They were randomly divided into two equal groups. Saliva samples were collected before the consumptions of probiotic ice cream and probiotic drink. Colony count obtained was recorded as baseline data. For both groups probiotic ice cream and drink was given randomly for 7 days and a washout period of 90 days were given and then the saliva samples were collected and colony counting was done.

Results: Statistical analysis was performed using Student's paired *t*-test and multiple comparisons by Tukey's honest significant difference test which showed, there is a significant reduction in salivary *S. mutans* level in both groups after 7 days period. However, after washout period only probiotic ice cream showed reduction whereas drink did not. Also, there was no significant difference between probiotic ice cream and drink.

Conclusion: Probiotic organisms definitely have a role in reducing the salivary *S. mutans* level and ice cream would be a better choice than drink. However, the prolonged use of the agents and their effects on caries is still to be determined.

Key Words: Dental caries, *Lactobacillus casei* shirota, probiotics, saliva, *Streptococcus mutans*

Introduction

Since the introduction of caries by Miller, over the past 115 years there has been remarkable changes in understanding dental caries.¹ Traditional approaches to management of caries like extraction, restoration, and modern methods like fluorides, minimal invasive dentistry, remineralizing agents has been tried. This helped in removal or reconstruction of the tooth structure.²

It is been proved that *Streptococcus mutans* are the main causative organism for dental caries.³ Traditional approaches does not reduce caries causing organisms in the oral cavity. Hence, a newer approach being tried and been successful in reducing caries causing organisms is the use of probiotics.⁴

"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.⁵ Their ability to survive in acidic environment, which is present during carious attack on the tooth made probiotics a choice to interact with these organisms.⁶

The most studies and proved beneficial probiotic organisms are Lactobacilli and Bifidobacteria. Probiotics basically replace the pathogenic bacteria by non-pathogenic. Hence, this is also called as replacement therapy.⁷

Probiotic organisms are mainly delivered through dairy products which are easily available and it should be easily accepted by people and especially children. Various studies compared usage of probiotics in dairy products such as cheese, yogurt, ice cream, and milk but compared with placebos or similar non-probiotic products. Ideal suitable vehicle is yet to be identified.⁸

Comparative studies of probiotic products are very less and also benefits of probiotic organisms on adult oral cavity are less explored. In this study, we compare two probiotic products ice cream and drink on salivary *S. mutans* level in children of 6-12 years age group.

Materials and Methods

Ethical clearance was obtained from the educational board. The study design was explained to the District Educational Officer

and also the school headmaster and permission was taken to conduct the study. General screening was done in children of age group 6-12 years of urban school and 50 children were included for the study and consent for the same was taken from the parents. Selected children were from the same locality and had a single source of drinking water supply with 0.4 ppm of fluoride.

The children with any recent history of dental treatment, systemic antibiotics or topical fluoride treatments 3 months prior to baseline, habitual use of dairy probiotics, xylitol chewing gums, severe medical conditions, and who are allergic to dairy products were excluded from the study. 50 children between 6 and 12 years of age and with zero decayed missing filled teeth (DMFT)/dmft index based on WHO criteria⁹ were selected as a sample for the study.

These children were randomized into two groups and diet instructions; oral hygiene maintenance was given and told to continue during the study period. On the next day 2 ml of unstimulated saliva was taken from each of the participant in 5 ml disposable sterile container during 10-11 am of school hours.

The saliva sample collected was subjected to microbial analysis by taking 10 ul of saliva sample in 4 mm internal diameter inoculation loop and streaking on freshly prepared mitis salivarius agar culture plates and inoculated for 48 h.

Colonies were identified by Gram-staining and were counted using 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 1×10^3 semi quantification of the number of colonies was done. The readings obtained were tabulated and considered as baseline data (Table 1).

For the next 7 days Group A was given 54 g of probiotic ice cream (Amul[®]) and Group B was given 65 ml of probiotic drink (Yakult[®]) continuously. The probiotic ice cream used in this study was prepared by adding a freeze dried culture of probiotic strains of *Bifidobacterium lactis* Bb-12 and *Lactobacillus acidophilus* La-5 and manufactured by Amul India Pvt. Ltd., (Anand, Gujarat, India). The probiotic ice cream contained 1×10^6 CFU of each probiotic strains.¹⁰ The probiotic drink used had 6.5 billion *Lactobacillus casei* strain Shirota, manufactured by Yakult Danone India Pvt. Ltd. (New Delhi, India).

Un-stimulated saliva was collected next day after the study period, inoculated, colonies were counted after incubation. Data were recorded. A washout period of 90 days were given and in this period children were instructed to maintain their oral hygiene as per the instructions given. After washout period, saliva samples collected again inoculated, incubated and colony counting was done.

The data thus obtained subjected to statistical analysis using Student's paired *t*-test and Turkey's honest significant difference (HSD) test was applied for multiple comparisons. The results were tabulated using SPSS version 17 software.

Results

Data obtained from baseline, after 7 days and 90 days of washout period were arranged and tabulated (Table 1). The study revealed that there was significant reduction of salivary *S. mutans* levels in both groups after study period ($P < 0.001$), but after washout period there was no significant difference or decline in the *S. mutans* levels. Though in children who consumed probiotic ice cream even after the washout period there was a significant reduction of salivary *S. mutans* levels compared to the baseline readings (Table 2). There was no significant difference between the groups over the study period.

Multiple comparisons were done by Turkey's HSD test which showed that there was a significant reduction of salivary *S. mutans* from baseline to 7 days after the ingestion of probiotic ice cream and drink? After the washout period, there was no significant reduction in *S. mutans* levels in both the groups.

When compared to the baseline data in children who consumed probiotic ice cream there was a significant reduction in salivary *S. mutans* levels. However, there was no significant reduction seen in children who consumed probiotic drink (Table 2).

These comparisons suggest that both probiotic ice cream and drink reduced the salivary *S. mutans* level in the children during the study period. However, after the washout period both the groups showed no significant reduction (Graph 1).

Table 1: Average *S. mutans* counts at baseline, 7 days, and after 90 days.

Study Period	Group	N	Mean	SD	t
0 days	Group A	25	72.24	12.33	0.012, P=0.0991 ns
	Group B	25	72.20	11.60	
7 days	Group A	25	63.32	12.28	0.0465, P=0.0644 ns
	Group B	25	64.84	10.78	
90 days (3 months)	Group A	25	62.56	10.84	1.1261, P=0.244 ns
	Group B	25	66.28	10.02	

P<0.001: Significance, N: Number of subjects, t: Students paired t-test value, ns: Not significant, Group A: Ice cream, Group B: Drink, SD: Standard deviation

Table 2: Multiple comparisons of the groups.

Group	(I) time	(J) time	Mean difference (I-J)	P
Group A (N=25)	Base line	7 days	8.92	0.026 sig
		90 days	9.65	0.014 sig
Group B (N=25)	7 days	90 days	0.76	0.976
		Base line	7.36	0.049 sig
	90 days	7 days	5.92	0.136
		7 days	90 days	-1.44

P<0.001: Significance, N: Number of subjects, ns: Not significant, sig: Significant

Moreover, also both probiotic ice cream and drink showed no significant difference in their effects in reducing the *S. mutans* levels in the oral cavity.

Discussion

Probiotic microorganisms such as some *Lactobacillus* spp. have been described as promoting a healthy digestive system and immune modulation. There is reasonable evidence that some probiotic species may also have a beneficial effect on oral health.^{10,11} *L. casei* strains Shirota (LcS) is one of the most studied probiotic strains with scientifically proven health benefits.¹¹

Various studies have been performed to validate the survival and positive effects of probiotic bacteria, *B. lactis* Bb-12 within the human body, including immune response and gastrointestinal health in young children,^{12,13} but research concerning probiotics and dental health is limited.

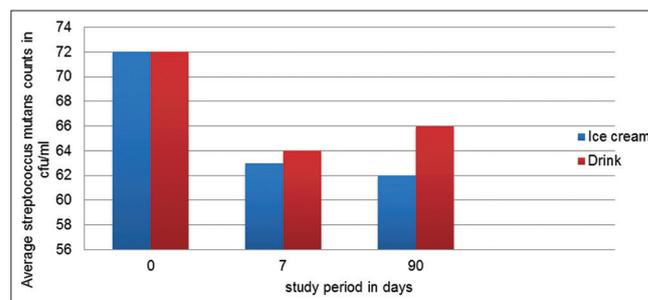
The main probiotic preparations currently on the market are the lactic acid-producing bacteria are *Bifidobacterium* and *Lactobacillus*.⁶ *Lactobacillus rhamnosus* is safe and useful in its intended uses as a functional food or ingredient its storage stability in refrigerated milk-based foods was proved excellent.¹⁴ Probiotics are mainly supplied in various dairy products such as yogurt, ice cream cheese, and drink. The acceptance of these products by children or adults depends on the taste and availability.

Probiotics create a biofilm which acts as a protective lining for tissues and keep pathogens away, with no host environment for pathogens.¹⁵ Studies done on probiotic approach were first to the alteration of the gut flora and were successful. Similarly, it was tried to alter the microbial flora of the oral cavity, in reducing the oral diseases, especially caries and found successful mainly in reducing the levels of *S. mutans* species.¹³ When probiotics were added while oral biofilm formation, *Lactobacillus* species strongly inhibited the growth of the *S. mutans*.^{14,15}

Limited studies have been conducted on the comparison of various probiotic organisms or the foods on caries causing bacteria. Hence in our study, we compared the efficacy of two probiotic food products and their effects on bacteria levels in the oral cavity, which mostly causes dental caries.

The prevalence of dental caries is rising in India in children with age groups 6-12, hence we selected that age group for the study.¹⁶ To avoid any bias we included children with zero dmft/DMFT score. Even though, the ideal vehicle is not concluded for probiotics ice cream is one of the most liked dairy products by children and to compare with another probiotic non-dairy product the probiotic drink is used.

By conducting the study, we found out that in children who consumed probiotic ice cream showed significance



Graph 1 : Average *S. mutans* counts during study periods in both groups

reduction ($P < 0.001$) of salivary *S. mutans* levels after 7 days of the study period. After the washout period of 90 days, there was a significant reduction of *S. mutans* level compared to baseline, but after study period there was no significant reduction (Table 2). This suggests that the effects of probiotic organisms reduced after the washout period. This result is similar to other studies done using ice cream as vehicle.¹⁷⁻¹⁹

Sutula *et al.* found in their study that utilization of the LcS select medium revealed a significant but temporary and consumption-dependent presence of LcS in saliva and tongue plaque samples from healthy dentate individuals.²⁰ 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.²¹ Studies done on probiotic containing milk showed that reduction in *S. mutans* levels significantly after use.²²⁻²⁴

In this study, of children who consumed probiotic drinks, there was a significant reduction of *S. mutans* level after the study period (Table 2). However, there was no significant reduction when compared to the baseline data or levels after the washout period. This also shows that the effects of probiotics reduced after the study period. Similar to the study by Sutula *et al.*²⁰

When comparison of efficiency of ice cream and drink being used as vehicle both showed no significant difference in their effects after study period and also after washout period (Table 1) When baseline data were compared after 90 days of washout period there was significant reduction in *S. mutans* levels in children who consumed probiotic ice cream but this was not seen in the case of drink (Table 2).

Conclusion

It can be said that probiotic organisms have a definitive effect in reducing salivary *S. mutans* level and can be used as a caries control method. As the vehicle in this study ice was better and effective than drink and it was more liked by children. Only short time studies are available now. long term studies with more population is needed and long term use of ice cream as a vehicle have to be justified.

References

1. Miller W. Micro-Organisms of the Human Mouth. Philadelphia: SS White; 1890.
2. Meurman JH, Stamatova I. Probiotics: Contributions to oral health. *Oral Dis* 2007;13(5):443-51.
3. Loesche WJ. Role of *Streptococcus mutans* in human dental decay. *Microbiol Rev* 1986;50(4):353-80.
4. Anderson MH, Shi W. A probiotic approach to caries management. *Pediatr Dent* 2006;28(2):151-3.
5. Report of a Joint FAO/WHO Expert Consultation on evaluation of health and nutritional properties of probiotics in food including powder milk with live lactic acid bacteria. 1-4 October; Cordoba, Argentina; 2001; Available on internet, Available from: <ftp://www.ftp.fao.org/docrep/fao/009/a0512e/a0512e00.pdf>.
6. Meurman JH. Probiotics: Do they have a role in oral medicine and dentistry? *Eur J Oral Sci* 2005;113(3):188-96.
7. Sameer A. The synergism of probiotics in dentistry. *Saudi Dent J* 2007;17:3.
8. Singh RP, Damle SG, Chawla A. Salivary *Streptococcus mutans* and *Lactobacilli* modulations in young children on consumption of probiotic ice-cream containing *Bifidobacterium lactis* Bb12 and *Lactobacillus acidophilus* La5. *Acta Odontol Scand* 2011;69(6):389-94.
9. World Health Organization. Oral Health Surveys Basic Methods. 4th ed. Geneva: World Health Organization; 1997. p. 40-4.
10. Bonifait L, Chandad F, Grenier D. Probiotics for oral health: Myth or reality? *J Can Dent Assoc* 2009;75(8):585-90.
11. Devine DA, Marsh PD. Prospects for the development of probiotics and prebiotics for oral applications. *J Oral Microbiol* 2009;1.
12. Fujimoto J, Matsuki T, Sasamoto M, Tomii Y, Watanabe K. Identification and quantification of *Lactobacillus casei* strain Shirota in human feces with strain-specific primers derived from randomly amplified polymorphic DNA. *Int J Food Microbiol* 2008;126(1-2):210-5.
13. Saavedra JM, Abi-Hanna A, Moore N, Yolken RY. Effect of long term consumption of infant formulas with bifidobacteria and *S. thermophilus* on stool patterns and diaper rash in infants. *J Pediatr Gastroenterol Nutr* 1998;27:483.
14. Ranganathan R, Vaidya R. Preventing dental caries the probiotic approach. *J Ahmadabad Dent Coll Hosp* 2011;2:60-5.
15. Lee SH, Kim YJ. A comparative study of the effect of probiotics on cariogenic biofilm model for preventing dental caries. *Arch Microbiol* 2014;196(8):601-9.
16. Joshi N, Rajesh R, Sunitha M. Prevalence of dental caries among school children in Kulasekharam village: A correlated prevalence survey. *J Indian Soc Pedod Prev Dent* 2005;23(3):138-40.
17. Ashwin D, Ke V, Taranath M, Ramagoni NK, Nara A, Sarpangala M. Effect of probiotic containing ice-cream on salivary mutans streptococci (SMS) levels in children of 6-12 Years of Age: A randomized controlled double blind study with six-months follow up. *J Clin Diagn Res* 2015;9(2):ZC06-9.
18. Hekmat S, McMahon DJ. Survival of *Lactobacillus acidophilus* and *Bifidobacterium bifidum* in ice cream for use as a probiotic food. *J Dairy Sci* 1992;75(6):1415-22.
19. Caglar E, Kuscu OO, Selvi Kuvvetli S, Kavaloglu Cildir S, Sandalli N, Twetman S. Short-term effect of ice-cream containing *Bifidobacterium lactis* Bb-12 on the number of salivary mutans streptococci and lactobacilli. *Acta Odontol Scand* 2008;66(3):154-8.
20. Sutula J, Coulthwaite LA, Thomas LV, Verran J. The effect of a commercial probiotic drink containing *Lactobacillus casei* strain Shirota on oral health in healthy dentate people. *Microb Ecol Health Dis* 2013;24.
21. Ghasempour M, Sefidgar SA, Moghadamnia AA, Ghadimi R, Gharekhani S, Shirkhani L. Comparative study of Kefir yogurt-drink and sodium fluoride mouth rinse on salivary mutans streptococci. *J Contemp Dent Pract* 2014;15(2):214-7.
22. Nikawa H, Makihira S, Fukushima H, Nishimura H, Ozaki Y, Ishida K, et al. *Lactobacillus reuteri* in bovine milk fermented decreases the oral carriage of mutans streptococci. *Int J Food Microbiol* 2004;95(2):219-23.
23. Näse L, Hatakka K, Savilahti E, Saxelin M, Pönkä A, Poussa T, et al. Effect of long-term consumption of a probiotic bacterium, *Lactobacillus rhamnosus* GG, in milk on dental caries and caries risk in children. *Caries Res* 2001;35(6):412-20.
24. Juneja A, Kakade A. Evaluating the effect of probiotic containing milk on salivary mutans streptococci levels. *J Clin Pediatr Dent* 2012;37(1):9-14.