

ORIGINAL ARTICLE

Salivary mutans streptococci and lactobacilli modulations in young children on consumption of probiotic ice-cream containing Bifidobacterium lactis Bb12 and Lactobacillus acidophilus La5RICHA (POLKA) SINGH¹, SATYAWAN GANGARAM DAMLE² & AMRITA CHAWLA³

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Abstract

Objectives. To compare the levels of mutans streptococci and lactobacilli in saliva of school children, before and after consumption of probiotic and control ice-cream. **Materials and methods.** A double-blind, cross-over, placebo-controlled trial was carried out in forty, 12–14 year-old children, with no clinically detectable caries. The selected children were randomized equally into two groups I and II. Following an initial run-in period of 1 week, children in group I and II were given ice-creams 'A' and 'B', respectively, for 10 days. Being a cross-over study, the ice-creams were interchanged in the two groups after a 2-week wash-out period. Saliva samples at baseline and follow-up were assessed using Dentocult SM and Dentocult LB kits. **Results.** On statistical evaluation, it was seen that probiotic ice-cream brought about a statistically significant reduction (p -value = 0.003) in salivary mutans streptococci levels with no significant effect on lactobacilli levels. **Conclusion.** In conclusion, probiotic ice-cream containing Bifidobacterium lactis Bb-12 ATCC27536 and Lactobacillus acidophilus La-5 can reduce the levels of certain caries-associated micro-organisms in saliva.

Key Words: probiotics, caries prevention, bacteria

Introduction

Public appetite for novel strategies or therapies that are perceived to be natural has recently increased. One such novel strategy for prevention against dental caries involves manipulation of resident oral microbiota by ingestion of probiotic bacteria to reduce the cariogenic load. According to WHO, probiotics are 'Live microorganisms which when administered in adequate amounts confer a health benefit on the host' [1]. The background thinking behind probiotics is replacement of the pathogenic species with non-pathogenic species such as strains of lactobacilli or bifidobacteria, which are the most common types of microbes used as probiotics [2].

Use of probiotics in children to improve oral health may lead to non-pathogenic bacteria replacing cariogenic bacteria early in life, thereby preventing their

colonization in the oral cavity and thus decreasing caries risk. Dairy foods like cheese, yoghurt and milk are considered useful vehicles for probiotic bacteria, but an ideal administration vehicle has yet to be identified [2]. Ideally, the probiotic vehicle should be suitable for all ages and especially for young children, since it has been suggested that exposure early in life may facilitate a permanent installation of health promoting strains [3,4]. In this context, ice-cream is an interesting probiotic food [5,6], as it is popular and universally liked.

Studies carried out to validate the beneficial effect of probiotic ice-cream on oral ecology are few and limited to adults. Caglar et al. [7] in 2008 assessed the short-term effect of ice-cream containing Bifidobacterium lactis Bb-12 on the number of salivary mutans streptococci (MS) and lactobacilli (LB) in adults. They observed a significant reduction in salivary

MS levels after consumption of probiotic ice-cream. Another study with significant reduction in salivary MS levels after consumption of yoghurt containing *Bifidobacterium* DN 173010 was reported by Caglar et al. [8] in 2005.

To the best of our knowledge, the effect of ice-cream containing a combination of *Bifidobacterium lactis* Bb-12 and *Lactobacillus acidophilus* La-5 probiotic strains, on caries associated micro-organisms in the oral cavity has not been reported in children or adults. Thus, as an embarkment into this field, a study was planned and carried out with the aim to compare the levels of salivary mutans streptococci and lactobacilli in 12–14 year old children with no clinically detectable caries, before and after consumption of probiotic and control ice-cream. The study was based on the null hypothesis: there would be no alteration in bacterial levels after consumption of probiotic ice-cream.

Materials and methods

Prior to commencement of the study, ethical clearance was obtained from the ethics committee of Maharishi Markandeshwar University's Medical College and Hospital, Mullana, Ambala. The study design was explained thoroughly to the principal and health officer of the school from where the children were to be selected and subsequently permission was obtained.

Forty children were selected as a sample for the study. Sample size was estimated using the power calculation $\alpha = 0.05$ and $\beta = 0.20$ based on previous reported findings by Caglar et al. [7]. Children in the age group of 12–14 years with all permanent teeth erupted (except 3rd molars); no clinically detectable caries according to WHO criteria [9]; no history of any preventive dental treatment and no antibiotic medication for the past 6-months were included in the study. Children undergoing orthodontic treatment and medically compromised children were excluded from the study. The selected children were residents of Chandigarh since birth, with fluoride content of 0.38 ppm in municipal water supply. A written consent was obtained from the parents and the selected children, who were then equally divided by simple randomization into two groups, Group I and II. The method of distribution consisted of writing 40 slips of equal size and shape, 20 marked with 'I' and 20 with 'II'. The slips were folded and pooled in a bowl and shuffled. Each child was asked to pick up a slip from the bowl. Those with mark 'I' were allocated to Group I and similarly with mark 'II' were in group II. To facilitate identification, children in both groups were further numbered I(1), I(2), I(3) ... up to I(20) and II(1) to II(20) respectively. Children in both groups were instructed to brush twice daily and refrain from consumption of curd and xylitol chewing gums.

The study was carried out in two phases: (1) Active study phase and (2) Evaluation phase.

Active study phase

The probiotic ice-cream used in the study was prepared by adding a freeze-dried culture of probiotic strains of *Bifidobacterium lactis* Bb-12 and *Lactobacillus acidophilus* La-5 and was manufactured by Amul India Pvt. Ltd. (Anand, Gujarat, India). The probiotic ice-cream weighing ~54 g, contained 1×10^6 CFU of each probiotic bacterial strain per gram.

The study was double blind in nature. This was possible as the ice-creams, control and test had similar taste (vanilla) and were distributed in similar white cups (~54 g) marked 'A' or 'B'. The content was not known to the children or the investigator. The codes were sealed and given to the chief supervisor of the study. The sanctity of the double-blind trial was strictly maintained and decoding done only after statistical evaluation of all parameters. The active study phase was conducted in four consecutive stages.

Stage I was the pre-experimental stage. A 7-day gap was kept before taking the baseline sample [7]. At the end of the 7-day run-in period, a baseline sample of saliva was collected to estimate mutans streptococci and lactobacilli. Care was taken to obtain saliva samples as per the standard protocol.

Stages II and IV marked the intervention stages in which ice-creams were given to the children, while Stage III was the wash-out stage. It was a cross-over study, i.e. after consumption of the allotted ice-creams by children in the two groups, a wash-out period of 2 weeks was observed, following which children in Group I having had 'A' ice-cream were then given 'B' and children in Group 'II' having had 'B' were given 'A' ice-cream. The ice-creams (one cup-daily weighing 54 g) were distributed during the school lunch-break at ~12:00 noon. The compliance of the children regarding consumption of ice-creams was good. At the end of each of the four stages microbial evaluation was carried out.

Sample collection, incubation and microbial evaluation.

During the course of the study, a total of four saliva samples were obtained. Dentocult SM and Dentocult LB (Orion Diagnostica, Espoo, Finland) were used to estimate the levels of mutans streptococci and lactobacilli in saliva, respectively. Saliva samples were obtained between 9:30–11 am, as brushing and intake of food, etc. could affect the results for 1–2 h [10]. Mutans streptococci levels in saliva were assessed as per the 'Strip mutans' method described by Jensen and Bratthall [11] in 1989, based on the use of a selective culture broth and adherence of mutans streptococci on the test strip, while lactobacilli levels

in saliva were assessed using dipslides covered on both sides with modified Rogosa medium.

Evaluation phase

Density of the colonies on the rough surface of the Dentocult SM strip was interpreted using the company's manual and accordingly allocated a score of 0, 1, 2 or 3. The colony density on the LB dipslide was compared with the manufacturer's model chart and accordingly densities of 10^3 , 10^4 , 10^5 or 10^6 CFU/ml were allocated. Scores of 1, 2, 3 and 4 were allocated to the densities, respectively. The intra-examiner reliability was found to be significant (0.82) using kappa test of agreement.

To assess the difference between time-related variables, i.e. bacterial scores at baseline and after consumption of control and probiotic ice-cream, Wilcoxon Signed Ranks Test was used. A p -value < 0.05 was taken to be statistically significant.

Maintaining the sanctity of the double-blind protocol, the test and control ice-creams were decoded after statistical evaluation of all parameters. Decoding revealed that 'A' ice-cream was normal ice-cream without any viable bacteria which served as control, while 'B' ice-cream was the test product containing *Bifidobacterium lactis* Bb-12 and *Lactobacillus acidophilus* La-5.

Results

The baseline and follow-up scores of MS and LB in saliva are given in Tables I and II. Findings of the study revealed a significant reduction in salivary (MS) scores of 14 children, after consumption of probiotic ice-cream as compared to baseline (Table III). A certain decline of high mutans streptococci counts was also evident after intake of control ice-cream, but the difference compared to baseline was not statistically significant. One child dropped out of the study, citing medical reasons, during Stage II of the study.

Table I. Salivary MS scores at baseline and at follow-up after intake of control and probiotic ice-cream.

Mutans Streptococci score	Ice-cream			
	Control (n)		Probiotic (n)	
	Baseline	Follow-up	Baseline	Follow-up
0	3	4	2	4
1	6	5	6	9
2	15	17	15	16
3	15	13	16	10

n = number of subjects (1 drop-out).
Score 0 $< 10^3$ CFU/ml; Score 1 $< 10^4$ CFU/ml; Score 2 = 10^4 – 10^5 CFU/ml; Score 3 $> 10^5$ CFU/ml.

Table II. Salivary LB scores at baseline and at follow-up after intake of control and probiotic ice-cream.

Lactobacilli score	Ice-cream			
	Control (n)		Probiotic (n)	
	Baseline	Follow-up	Baseline	Follow-up
0	24	23	22	19
1	7	9	6	11
2	5	4	7	6
3	3	3	4	3
4	0	0	0	0

n = number of subjects.

Score 0 = No growth; Score 1 = 10^3 CFU/ml; Score 2 = 10^4 CFU/ml; Score 3 = 10^5 CFU/ml; Score 4 = 10^6 CFU/ml.

The comparison between baseline vs follow-up salivary MS counts after consumption of probiotic ice-cream was found to be statistically significant (p -value = 0.003), whereas the comparison was non-significant after consumption of control ice-cream.

The baseline levels of Lactobacilli (LB) were similar prior to the two intervention periods and no statistically significant changes appeared between baseline and follow-up LB scores.

Discussion

Bacteriotherapy is a novel and promising concept for combating infections and preventing dental caries. Various studies have been performed to validate the survival and positive effects of probiotic bacteria *Bifidobacterium 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 findings of the present study revealed a reduction in salivary Mutans Streptococci (MS) levels in 36% of children, after consumption of 'probiotic' ice-cream. The null hypothesis was, hence, rejected for mutans streptococci. A reduction in salivary MS levels was also observed after consumption of 'control' ice-cream in 13% of the children. This can be attributed to the anti-cariogenic property of milk due to the presence of casein, calcium and phosphorus. Findings of the study are in accordance with previous studies [7,8].

The counts of salivary mutans streptococci and lactobacilli were estimated using simple chair-side tests that correlate well with conventional laboratory methods with selective media [14,15]. The intra-examiner reliability was found to be significant (0.82) using kappa test of agreement.

The probiotic ice-cream used in the study contained 6% fat and 28% total solid and sucralose as

Table III. Sample distribution and salivary MS scores, at baseline and after intake of 'probiotic' ice-cream and 'control' ice-cream.

Sample distribution			Probiotic salivary MS		Control salivary MS	
Subject code	Age	Sex	Baseline	Follow-up	Baseline	Follow-up
I-1	12	F	2	2	1	2↑
I-2	12	M	2→	1	2	2
I-3	12	F	1	1	1	1
I-4	13	M	1	1	0	0
I-5	12	M	3	3	3	3
I-6	13	F	2	2	2	2
I-7	14	M	0	0	0	0
I-8	13	M	2	2	1	2↑
I-9	13	F	2	2	2	2
I-10	13	F	2→	1	2	2
I-11	13	F	2	3↑	2	2
I-12	14	F	3→	1	3→	2
I-13	13	M	3	3	3	3
I-14	14	M	3→	2	3	3
I-15	13	M	3→	2	2→	0
I-16	13	M	3→	2	2	2
I-17	14	F	3	3	3	3
I-18	13	M	2→	1	2	2
I-19	12	M	W	W	W	W
I-20	12	M	3	3	3	3
II-1	12	F	3	3	3	3
II-2	12	M	2→	1	3	3
II-3	13	F	1	1	1	1
II-4	12	M	1→	0	2	2
II-5	13	M	1	2↑	2	2
II-6	13	F	3	3	3→	2
II-7	13	F	3	3	3	3
II-8	13	F	0	0	0	1↑
II-9	13	F	3→	2	3→	2
II-10	14	M	3	3	3	3
II-11	14	M	1→	0	2→	0
II-12	13	M	2	2	2	2
II-13	14	F	2→	1	1	1
II-14	14	M	2	2	2	3↑
II-15	13	M	2	2	2	2
II-16	14	M	3→	2	3	3
II-17	13	M	3→	2	3	3
II-18	13	M	3	3	3	3
II-19	12	M	2	2	1	1
II-20	13	M	2	2	2	2

Code I (1–20) = Group I; F = female; M = male; W = withdrawn due to medical condition.

Salivary MS score 0 = $<10^3$; 1 = $<10^4$; 2 = 10^4 – 10^5 ; 3 = $>10^5$ CFU/ml.

Horizontal arrows → depict one-step decrease in MS scores (13 children); →→ depict a two-step decrease in MS scores (1 child).

Vertical arrows ↑ depict increase in MS scores (2 children).

the sweetener. It was prepared by adding a freeze-dried culture of probiotic strains *Bifidobacterium lactis* Bb-12 and *Lactobacillus acidophilus* La-5. Probiotic ice-cream weighing ~ 54 g contained 1×10^6 CFU of each bacterial strain per gram. Although a statistically significant reduction (p -value = 0.003) in MS levels on consumption of probiotic ice-cream was observed, a number of children especially with high scores (Score 3) did not exhibit any change. This might indicate that the intervention period of 10 days, in our study, was too short to alter oral ecology favourably for the host in children with high MS levels, and needs to be increased in future trials of the kind. Also the difference between the first and second baseline scores of salivary mutans streptococci and lactobacilli in saliva was very close to significance (p -value = 0.058); a wash-out period of 2-weeks was short and should be extended in future cross-over trials. Two subjects showed a reduction in salivary MS from score 2 to 0 and score 1 to 0 after the 1st intervention stage, but after the 2-week wash-out period the baseline levels of MS showed a steep rise from score 0 to 3 and 0 to 2, respectively. This initial decline could be attributed to the fact that the subjects were prescribed antibiotics during Stage II and failed to report it to the investigator during the study.

A variety of bacterial strains have been explored for their potential probiotic properties. The most widely used species belong to the genera *Lactobacillus* and *Bifidobacteria* [16–19]. The focus is on these species as they are already produced in the dairy industry and are rarely implicated in any human infections.

A probiotic bacterium should possess the ability to adhere to tooth surfaces, become part of the biofilm that develops on teeth and compete with cariogenic bacteria. Comelli et al. [20] examined 23 microorganisms used in the dairy industry for potential probiotic properties with respect to the prevention of dental caries. They showed that two *S. thermophilus* species and two *L. lactis* species were able to adhere to hydroxyapatite. The authors concluded that such a property in a non-pathogenic dairy bacterial strain might prove beneficial in modulating the establishment of cariogenic dental plaque. Various other authors have stated that probiotic bacteria are only transiently harboured in the mouth and, after discontinuation of intake, they disappear quite rapidly [21–23].

Most often, probiotic products contain a single bacterial strain, but it is important to underline that the efficacy of one lactobacilli strain does not necessarily imply that other strains will be equally efficacious [1,2,24], while it has been postulated by Juntunen et al. [25] in their gastrointestinal studies that a combination of probiotic strains can enhance adherence in a synergistic manner. The possibility of a

similar mechanism could be anticipated in the oral cavity as well. Thus, two strains, *Bifidobacteria* Bb12 and *Lactobacilli* La5, were selected. A dairy-based vehicle was chosen for the probiotic bacteria as its buffering effect would hamper the bacterial acidogenicity.

The present investigation was an attempt to explore new avenues and vistas for prevention of dental caries by using a commonly consumed product liked by one and all, especially children, under natural circumstances. Results were encouraging in spite of low titres of probiotic bacteria used (2×10^6 CFU/g) compared to previous studies [7,8] which have used higher levels of bacteria (10^7 – 10^8 CFU/g) with the same effect. The synergistic effect of combined low level titres of probiotic bacteria thus needs to be further explored. Theoretically, a long-term reduction of mutans streptococci could thus imply a reduced risk for initial lesions [26]. In conclusion, this is an intermediate end-point and this alternative strategy of displacing pathogenic bacteria by probiotic bacteria can thus be exploited for the prevention of enamel demineralization.

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