



Research Article

Role of Probiotics on Oral Health - A Randomized, Double Blind, Placebo-Controlled Microbiological Study

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Abstract

The aim of this study was to investigate commercially available combined probiotic formulation for its effect on plaque, gingivitis and salivary *Streptococcus mutans* levels in subjects with chronic gingivitis. A total of 36 subjects were finally enrolled in a double blind placebo controlled randomized trial. Selected subjects were randomly divided in two groups; Group A (Control group) with 17 subjects, receiving placebo tablets twice daily and Group B (Probiotic group) with 19 subjects receiving probiotic tablets twice daily. The study consisted of two 2 weeks periods, a two week intervention period (T1-T2) and a two week post treatment period (T2-T3). Clinical parameters and bacterial counts of salivary *Streptococcus mutans*, were evaluated at the baseline (T1), at the completion (T2) of medication and 2 weeks after medication (T3). On comparative evaluation between the two groups, results indicated that Group-B (Probiotic group) exhibited statistically significant reduction in Plaque Index, Gingival index and *Streptococcus mutans* level than Group-A (Control group) over the entire span of the study. Our results indicated that probiotic could be useful in the improvement/ maintainance of oral health.

Keywords: Probiotic, *Streptococcus mutans*, caries, gingivitis.

Introduction

Dental plaque, a biofilm of microorganisms on the tooth surface, is an important etiological factor in the development of most common oral diseases – dental caries, gingivitis and destructive periodontal diseases.²²In dental practice, the control of oral inflammation typically involves the use of mechanical or chemical means to remove

biofilm. Despite the best efforts by dental professionals to sterilize the oral cavity, the oral cavity will inevitably repopulate with oral bacteria. In unfavourable oral environment the disease persists and more therapeutic measures will be required in future. Little attention has been paid to the identification of health associated and potentially beneficial bacterial species that may reside in the oral cavity.

Bacteriotherapy in the form of probiotics seems to be a natural way to maintain health and protect oral tissues from disease. This article endeavors to introduce the concept of probiotics in the oral cavity.

The basic principle of probiotics is to use good bacteria to compete against pathogenic bacteria. The term probiotic, which literally means “for life” was first coined in the 1960’s by Lilly and Stillwell.²² According to the World Health Organization, probiotic bacteria are defined as live micro-organisms which, when administered in adequate amounts, confer a health benefit on the host.⁶ The interest in such an alternating way to combat infections is rapidly growing especially in the light of the frequent use of antibiotics with a subsequent risk for development of resistant strains.

Bacterial endotoxins and antigens, together with some metabolic by-products produced by the pathogens in the dental biofilm, invariably lead to gingivitis and subsequently periodontitis.

Streptococcus mutans has been implicated as one of the major and most virulent of the caries producing microorganisms.^{1,12} It can colonize the tooth surface and initiate biofilm formation by their ability to synthesize extracellular polysaccharides from sucrose, mainly water-insoluble glucan, using the enzyme glucosyltransferase.²⁴ Inhibiting the colonization of *Streptococcus mutans* on the tooth surface is believed to prevent the formation of biofilm and development of dental caries.

In the oral cavity, probiotics adhere to dental tissues as a part of biofilm, acting as a protective lining. Such a biofilm keeps bacterial pathogens off oral tissues by filling a space which could have served as niche for pathogens in future; and competing with cariogenic bacteria and periodontal pathogens growth.⁴

To be able to exert probiotic properties in the oral cavity, however, it is essential for the microorganism to resist the oral environmental conditions, to be able to

adhere to saliva coated surfaces, to colonize and grow in the mouth, and to inhibit oral pathogens. The putative probiotic species also needs to be safe for the host. Kligler and Cochrane (2008) summarize that significant adverse effects after the use of probiotics are rare.¹⁰

The most commonly used and studied probiotics are lactic acid bacteria, in particular *Lactobacillus* spp., and *Bifidobacterium* spp.²⁶

To determine the effects of probiotics on oral health promotion, we conducted a double blind, placebo-controlled randomized clinical trial in healthy subjects with gingivitis. The specific aim of this study was to investigate the effect of combined probiotic formulation on plaque, gingivitis and caries during and after discontinuation of administration.

Objectives

To clinically evaluate the effect of probiotics on

- Plaque accumulation
- Gingival inflammation
- Caries by estimating the level of *Streptococcus mutans* in saliva.

Materials and Methods

Probiotic Product

The study product (capsule BifilacHp) contained *Lactobacillus sporogenes* 100 million, *Streptococcus faecalis* T-110 JPC 60 million, *Clostridium butyrium* TO-A 4 million, *Bacillus mesentericus* TO-A JPC 2 million. Combination of probiotic strains has been used that may act synergistically and enhance the possibility for permanent installation. This probiotic capsule was tested against a placebo tablet from the manufacturer that contained only microcrystalline cellulose but was of identical taste, texture, and appearance. The dose was two tablets taken orally every day throughout the test period.

Subjects

40 students suffering with chronic gingivitis were recruited from amongst National Dental College and Hospital, Derabassi. The mean age was 21 years with uneven sex distribution (30F, 10M).

Subjects were outwardly healthy and further confirmed to meet the following criteria:

- Not currently visiting their dentist.
- Not taken antibiotic within last month.
- Not using probiotic supplements

All subjects provided written informed consent, and the study was approved by Research Ethics Committee constituted by National Dental College and Hospital, Derabassi.

Study Design

The study was performed as a double blind placebo controlled randomized trial over a 4-weeks test period. Subjects selected were divided in two groups, with 20 subjects in each group.

Group A: Control group

Group B: Probiotic group

Subjects in the Control group consumed two placebo capsules every day. Those in the Probiotic group consumed two capsules containing probiotic (BifilacHp) every day. Subjects in each group were directed to place capsules in mouth and swallow it with water. They were also instructed not to change their oral hygiene regimens throughout the test period. Neither professional prophylaxis nor toothbrushing instructions were performed during or before the study period. The study consisted of two 2 weeks periods, a two weeks intervention period (T1-T2) and a two weeks posttreatment period (T2-T3). Clinical parameters and bacterial counts of salivary *Streptococcus mutans*, were evaluated at the baseline (T1), at the completion (T2) of medication and 2 weeks after medication (T3) (Fig-1). Four subjects were lost due to consumption of antibiotics during the follow-up period. Finally, 36 subjects, 17 in the Control and 19 in the Probiotic group were analysed.

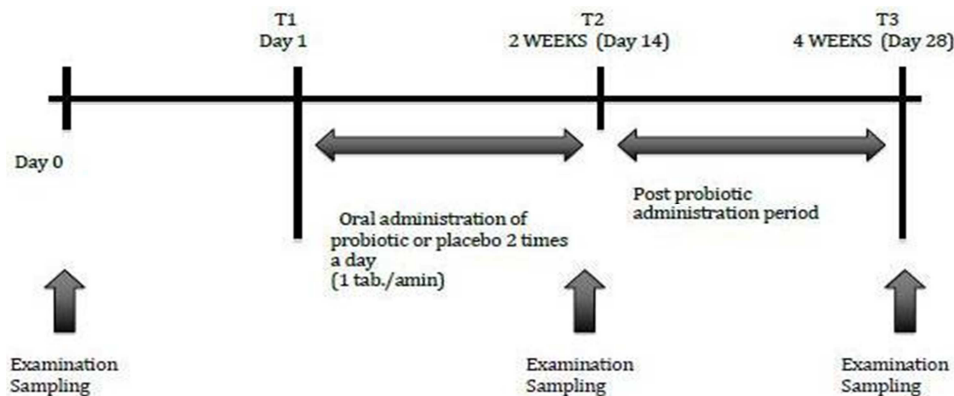


Fig. 1: Experimental Schedule

Clinical Measurements

The gingival condition was assessed by gingival index (GI; Loe and Sillness 1963).¹⁹Supragingival plaque was scored by plaque index (PI; Sillness and Loe 1964). Both the indices were obtained from

Ramford's six teeth (16,21,24,36,41, and 44 in the FDI two-digit notation system) and considered representative of the whole dentition.^{27,29}When one of the selected teeth was missing in the oral cavity, parameters were obtained from the adjacent tooth in the same area of the jaw.

Saliva Sampling

After the clinical measurements, saliva samples were obtained. The subjects were asked not to eat anything for one hour before collection of sample. The subjects were asked to chew sterile cotton swabs to stimulate saliva production. 2 – 3 ml of saliva was collected in sterile disposable container.

Laboratory Procedure

Within half an hour of collection of samples, culture was done. Using 4 mm diameter inoculation loop, 10 ul of sample was streaked on Mitis Salivarius Bacitracin (MSB) Agar containing 1% potassium tellurite and 10 % sucrose.⁷ This medium contains selective agents – crystal violet, potassium tellurite and bacitracin. These agents inhibit most gram negative bacilli and most gram positive bacteria except Streptococci. Sucrose is incorporated in 10% concentration which is utilised as energy source by Streptococci.

MSB plates were incubated in 5 % CO₂ candle jar for 24 hours at 37°C. Following

incubation, colonies of Streptococcus mutans were identified by gram stain, culture morphology and Biochemical test.

On gram stain Streptococcus mutans appear as gram positive cocci arranged in short chains.

Biochemical tests: Streptococcus mutans is Catalase negative, Mannitol and Sorbitol fermentation positive

On culture - Streptococcus mutans forms raised, convex, undulate, opaque, pale blue colonies, granular frosted glass appearance sometimes exhibiting glistening bubble on surface of colony due to excessive synthesis of glucan from sucrose (Fig 2).

Colony count was done with a magnifying glass and count of Streptococcus mutans was expressed as number of colony forming units per ml of saliva (CFU / mL)

Semiquantitation of number of colonies was done by multiplying the actual colony with 1×10^2

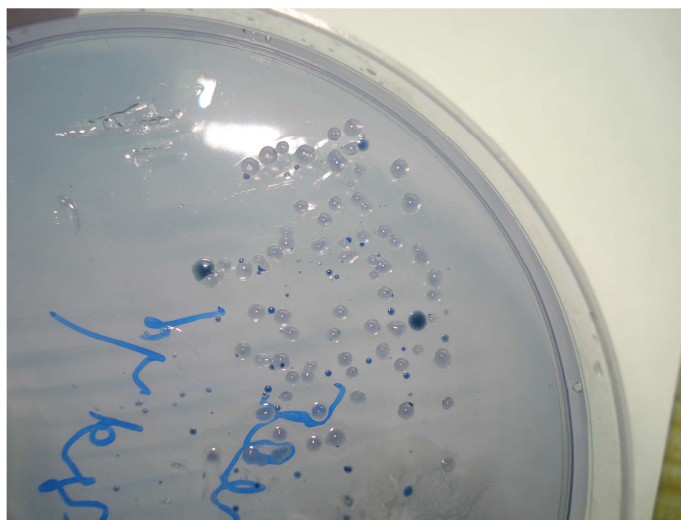


Fig. 2: Closer View of MSB Agar: Showing Convex, Pale Blue Colonies, Granular Frosted Glass Appearance of Streptococcus Mutans Colonies

Results

All subjects tolerated the surgical procedures well, experienced no post-

operative complications, although three subjects in the control group and one subject in the test group were withdrawn during the follow-up period. These subjects

took antibiotics during the study protocol and stopped the intake of tablets.

Clinical parameters and bacterial counts of salivary *Streptococcus mutans*, were evaluated at the baseline (T1), at the completion (T2) of medication and 2 weeks after medication (T3). The observations recorded were subjected to statistical analysis. No difference in results was found between male and female subjects.

The mean values of plaque index (PI) [Table-1], gingival index (GI) [Table-2], *Streptococcus mutans* levels [Table-3] at three points in time were evaluated. The efficacy of the two treatment modalities at T2 and T3 post-operatively were evaluated during the paired Student's t-test because the observations at the two points in time were expected to be closely related to each other. The two groups, Group A (Control group) and Group B (Probiotic group) were then comparatively evaluated over the

three time intervals using the independent Student's t-test.

On analyzing the change in PI, and GI of the two groups, it was seen that there had been significant reduction in PI and GI in both the groups at all the three points of time.

The *Streptococcus mutans* level for both the groups was statistically significant at all the three points of time.

On comparative evaluation between the two groups, results indicated that Group-B (Probiotic group) exhibited statistically significant reduction in PI [Table-4] and *Streptococcus mutans* level [Table-5] than Group-A (Control group) over the entire span of the study.

Group-B (Probiotic group) provided statistically highly significant reduction in GI [Table-6] at T2 and T3, than Group-A.

Table1: Plaque Index of Group-A (Control Group) and Group-B (Probiotic Group) (in mm)

Group-A				Group-B		
	T1	T2	T3	T1	T2	T3
Mean±S.Em	0.53±0.04	0.41±0.03	0.43±0.04	0.51±0.03	0.05±0.03	0.52±0.032

S.Em: Standarderror of mean

Table2: Gingival Index of Group-A (Control Group) and Group-B (Probiotic Group) (in mm)

Group-A				Group-B		
	T1	T2	T3	T1	T2	T3
Mean±S.Em	0.49±0.03	0.38±0.024	0.39±0.27	0.53±0.30	0.54±0.03	0.54±0.031

S.Em: Standarderror of mean

Table3: Streptococcus Mutans Count of Group-A (Control Group) and Group-B (Probiotic Group) (in CFU/mL)

Group-A				Group-B		
	T1	T2	T3	T1	T2	T3
Mean±S.Em	24.14±23.13	19.7±17.5	20.04±17.55	22.6±16.85	23.4±16.55	20.08±16.75

S.Em: Standarderror of mean

Table4: Comparison of mean Plaque Index scores between Group-A (Control Group) and Group-B (Probiotic Group)

	T1 - T2	T1 - T3	T2 -T3
Group A mean	0.12± 0.016	0.10 ± 0.016	-0.016 ± 0.0069
Group B mean	-0.0058± 0.0070	-0.014 ± 0.0053	-0.0084± 0.0052
P - value	0.00**	0.00**	0.35*

P: Level of significance.

** Values are statistically highly significant at both 5% and 1% probability level.

* Values are statistically significant at both 5% probability level.

Table5: Comparison of Mean Gingival Index Scores between Group-A (Control Group) and Group-B (Probiotic Group)

	T1 - T2	T1 - T3	T2 -T3
Group A mean	0.105 ± 0.013	0.09 ± 0.012	0.0065 ± 0.0047
Group B mean	-0.0074 ± 0.0082	-0.0137 ± 0.0090	-0.0063 ± 0.0042
P - value	0.00**	0.00**	0.98 ^{NS}

P: Level of significance.

** Values are statistically highly significant at both 5% and 1% probability level.

NS: Values are statistically non-significant at both 5% and 1% probability level.

Table6: Comparison of Mean Streptococcal Mutans Counts between Group-A (Control Group) and Group-B (Probiotic Group)

	T1 - T2	T1 - T3	T2 -T3
Group A mean	24.40± 23.61	24.09 ± 23.63	-0.34 ± 0.29
Group B mean	-14.86± 13.10	18.51±18.97	33.37± 23.36
P - value	0.28*	0.29*	0.18*

P: Level of significance.

• Values are statistically significant at both 5% probability level.

Discussion

The paradigm that oral inflammation is the root cause of not only periodontal disease and caries, but cardiovascular disease, diabetes, arthritis and many more is rapidly emerging.

Treating the oral infection by reducing the number of pathogenic oral microorganism and establishing a favourable oral environment to promote predominantly remineralization of tooth structure over time will stop the disease process.⁸

Probiotic technology represents a breakthrough approach to maintain oral health by utilizing natural beneficial bacteria commonly found in healthy mouths 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 by modifying the protein composition of salivary pellicle. Probiotic bacteria could modify the protein composition of the pellicle by two different methods, namely binding to and the degradation of salivary proteins.¹⁷

Probiotic lower the pH so that microorganism cannot form dental plaque and calculus that causes oral inflammation. Polonskaya first described the phenomena that probiotic such as *Lactobacilli acidophilus* strains may inhibit the invitro growth of other bacteria.⁹

Lactobacilli can produce low molecular weight bacteriocins with an inhibitory effect against a wide range of bacterial species including oral *Streptococci*, causative agent of caries and gingivitis.^{28,30}

Fusiform nucleatum has been regarded as a chain microorganism in dental plaque formation, possessing the ability to co-aggregate with the majority of other bacteria in the oral cavity. *Lactobacilli* co-aggregate with *F. nucleatum* at nearly 90%, thus modulating composition of oral biofilms.¹¹

Lactobacilli had been shown to be the strongest inhibitor of *A-Actinomyces comitans*, *P. gingivalis* and *P. intermedia*, potent microorganisms responsible for periodontal destruction.²⁰

Probiotic microorganisms do not act exclusively by affecting the microbiota, but also protect the oral cavity through the promotion of a beneficial host response. They exert effects either by modulating immunological parameters, epithelial permeability and bacterial translocation, or by providing bioactive or regulatory metabolites.⁵ The latter effects are appealing for periodontal healthcare because current evidence shows that the destruction of the periodontium is substantially mediated by the host and driven by the bacterial challenge.²³ Therefore, probiotics might not only suppress the emergence of endogenous pathogens or prevent the superinfection with exogenous pathogens but they might also protect the oral cavity through the promotion of a beneficial host response.²¹

Probiotic bacteria or their products (e.g. metabolites, cell wall components and DNA) can be recognized by host cells such as epithelial cells and immune cells.¹⁶ It is known that probiotics can regulate the

expression of phagocytosis receptors in the neutrophils of healthy individuals and enhance natural killer cell activity.^{18,31}

In the present study, beneficial impact of probiotic bacteria is well established by statistical significant reduction in plaque index (PI) and level of *Streptococcus mutans* in Group-B (Probiotic group) as compared to Group-A (Control group).^{2,3} The observed improvement in clinical status may be attributed to the reduced levels of cariogenic as well as periodontal pathogens and effective colonization of the probiotic bacteria within the oral cavity. Significant reduction is also observed in gingival index (GI) in Group-B (Probiotic group) as compared to Group-A (Control group) at T2 and T3. Non significant change between T2-T3 signify that improvement in gingival condition tend to remain stable after cessation of probiotic intake. Residence time of probiotics in oral cavity after treatment withdrawal is yet not known. The results did not suggest that a permanent installation can take place in person with established microflora.^{13,14} But, their mechanism of action suggested that they need not to permanent colonize their host; even repeated daily use of probiotic over a long period of time will support its increased levels in the oral cavity. Since it seems unlikely that probiotics have any significant residual effect after discontinuation of intake, daily intake seems to be a prerequisite for potential action.^{14,15}

Conclusion

Probiotics used for the management of oral disease may reduce the cost of conventional therapy and prevention programs. The idea of replacing harmful microorganisms with non-harmful, inactivated or genetically modified bacteria is attractive. With the focus on disease prevention and optimal health for all ages, the potential for probiotics use is enormous. In this study improvement in gingival health and decrease in *Streptococcal mutans* levels in Probiotic group warrant its further exploration in prevention of gingival diseases and caries. Efforts should be made to increase the awareness of the general

dental practitioner with this aspect of oral disease therapy and encourage the implementation of the concept of "food rather than medicine". Much more scientific developments are needed to have a better understanding of these tiny forms of lives in order to broaden their potential applications.

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