



Original Research

Comparative evaluation of change in the pH and *Streptococcus mutans* count in dental plaque after use of probiotic curd – an in vivo microbiological study

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ABSTRACT

Background: Numerous studies have demonstrated the decline in *Streptococcus mutans* level following consumption of Probiotic. However, most of them have tested the salivary levels of microbes.

Aim: The aim of present study was to assess the effect of probiotic curd on pH and colony count of *Streptococcus Mutans* in Dental Plaque.

Subjects and methods: This randomized control trial was conducted on 20 healthy children of 6-12 years of age. The subjects were randomly divided into group 1- probiotic group; who consumed probiotic yogurt for 15 days and group 2 – control group without any additional supplement. The pH and colony count of *Streptococcus mutans* in dental plaque was recorded at baseline and after the intervention period of 15 days.

Results: The drastic decline in the *Streptococcus mutans* level as well as drop in pH of plaque was noticed among the study group whereas no significant difference was seen in the control group.

Conclusion: Even a short-term consumption of probiotics has detrimental effect on caries producing organism. Thus, it can be used as a preventive measure to control dental caries with added advantage on the general health.

Keywords: *microbiological study, pH, Probiotic curd, Streptococcus mutans*

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INTRODUCTION

Oral health is an important entity to general well-being of a person. As high as 1000 bacterial species¹ resides harmoniously in dynamic equilibrium of synergistic and antagonistic microbial interactions.² Disturbance of this homeostasis can lead to shifts in microbial composition and eventually cause common bacterial diseases in humans, i.e., dental caries and periodontal disease.¹

To counteract these issues various measures were adopted like fluoride² or casein phosphopeptide amorphous calcium phosphate, personal hygiene products³, antibiotics, antimicrobial therapy with chlorhexidine or povidone iodine, or enamel surface attenuation with an argon laser⁴. However, these measure had drawbacks like fluoridation requires frequent visit to dentist and causes discoloration of teeth⁴; antimicrobial therapy results in indiscriminate destruction of both commensal and potentially harmful bacteria with risk of development of resistance strains³. Added to, dental caries continues to remain a “silent epidemic” in the world and an economic burden on health care system.^[3]

A breakthrough was achieved when it was understood that rather than targeting the pathogen one can indirectly affect it by preventing disturbance² or interfering with the ecological pressure responsible for the selection of the pathogen and re-establish a healthy microbial homeostasis via the use of probiotics.⁵ It was discovered by Eli Metchnikoff in 1908 as live microbial food supplements; defined by Food and Agriculture Organization/World Health Organization, 2001 as living microorganisms that, when administered in adequate amounts, confer health benefits to the host.¹

Initially probiotic was used to enhance the general health of the subjects. Inspired by the beneficial effect of probiotic on general health, the interest in probiotic therapies to prevent and control oral diseases has grown significantly. Use of probiotic was possible because they have an ability to survive in acidic environment which will be present especially in dental caries.⁵ Most microorganisms identified to date as probiotics are gram-positive, rarely implicated in infections in humans⁶ and belong to the genera *Lactobacillus* and *bifidobacteria*^{5,1}. The mechanism by which probiotic hinders the growth of cariogenic bacteria is not well known but usually considered to be a combination of local and systemic immune responses as well as non-immunological defense mechanisms.⁶ But the most plausible explanation are direct impact on the oral microbiome⁷ by antagonistic effect against specific groups of organisms³ by production of antimicrobial substances such as bacteriocins¹ or posing a competition for nutrients and adhesion¹ or by stimulation of immunity by immune modulation.⁷

The benefits of probiotics were demonstrated by numerous studies but most of them used salivary count *Streptococcus mutans* to evaluate the efficiency. However, it is the dental plaque which forms the mode for interaction between teeth and microbe resulting in development of caries. Therefore, in the present study the use of *Streptococcus mutans* count and pH in dental plaque was done to evaluate the effect of probiotic containing *Lactobacillus acidophilus* and *Bifidobacterium bifidum* on oral health.^{4,7}

MATERIALS AND METHODS

SAMPLE SELECTION

A descriptive cross-sectional observational study was conducted by the Department of Pedodontics and Preventive Dentistry, College of Dental Sciences, Davangere after obtaining ethical clearance was obtained from the Ethical committee of College Of Dental Sciences, Davangere

A total of 68 children of 9 to 12 years of age irrespective of sex age and socio economic status was selected from outpatient clinic of the Department of Pedodontics and Preventive dentistry, College of Dental Sciences, Davangere, Karnataka, India

Inclusion criteria

1. Good general health with no significant medical history.
2. No anti-inflammatory or antibiotic medications taken in the month before the study.
3. No chewing gum or mouthwash used in the last week and during the study period.
4. Habit of brushing twice daily with fluoridated toothpaste.

Exclusion criteria

1. Children with decayed teeth.
2. Children under antibiotic treatment or topical fluoride or antiseptic mouthwash for three weeks before and during the course of the study.
3. Children on any other probiotic supplements during the course of the study.
4. Children on use of any xylitol products for 3 weeks before and during the course of the study.
5. Children on use of any antiseptic mouthwash.

Material used in the study were basic examination kit consisting of disposable mouth mask and gloves, mouth mirror, explorer, tweezers and sterile cotton, Amul probiotic yogurt – Flavvyo, Digital ph meter, Mitissalivarius bacitracin (MSB) agar (hi-media), Calibrated inoculating loop and Bunsen burner, Incubator and Autoclave.

Using simple random sampling (lottery method) the sample was allocated to either test or control groups. The subjects were encouraged to maintain their normal oral hygiene habits and to continue to brush their teeth.

Group 1: The subjects were provided by the probiotic yogurt, 200 ml of which they were supposed to consume daily at dinnertime. No tooth brushing was allowed for at least 1 hour after yogurt consumption.

Group 2: Consisted of patients who received no probiotic treatment (control group). They were encouraged to maintain oral hygiene and follow the routine dietary pattern.

All subjects will be asked to brush twice daily with their regular fluoridated toothpaste. The patients were instructed to avoid chewing gums, mouthwashes, and antibiotics during the study. Those subjects who fell sick and required antibiotic therapy were dropped from the study and replaced by other children. Samples were collected twice: before the study began and 15 days.

ESTIMATION OF PLAQUE PH:

Plaque specimens were collected from the labial surfaces of the maxillary teeth with a sterilized scaler. A digital ph meter was used to analyse the pH of collected plaque.

ESTIMATION OF STREPTOCOCCUS MUTANS COUNT:

The rest of the plaque was dispersed in sterile containers containing transport media and carried to the microbiologic laboratory for analysis of Streptococcus mutans count. Plaque Streptococcus mutans were enumerated immediately before (baseline) and after (follow-up) period. (Figure 1 and 2)



Figure 1: MSB agar with *streptococcus mutans* growth in study group

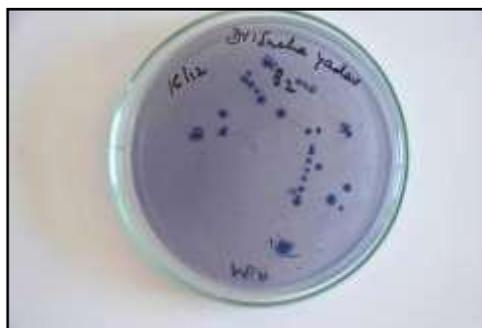


Figure 2: MSB agar with *streptococcus mutans* growth in control group

STATISTICAL ANALYSIS:

The readings obtained were tabulated and compared. The results were derived using SPSS and Excel software for data entry and statistical analysis. The results were averaged (mean + standard deviation) for each parameter and both groups. Statistical analysis was done using paired t-test to compare within each group and unpaired t-test for intergroup comparison.

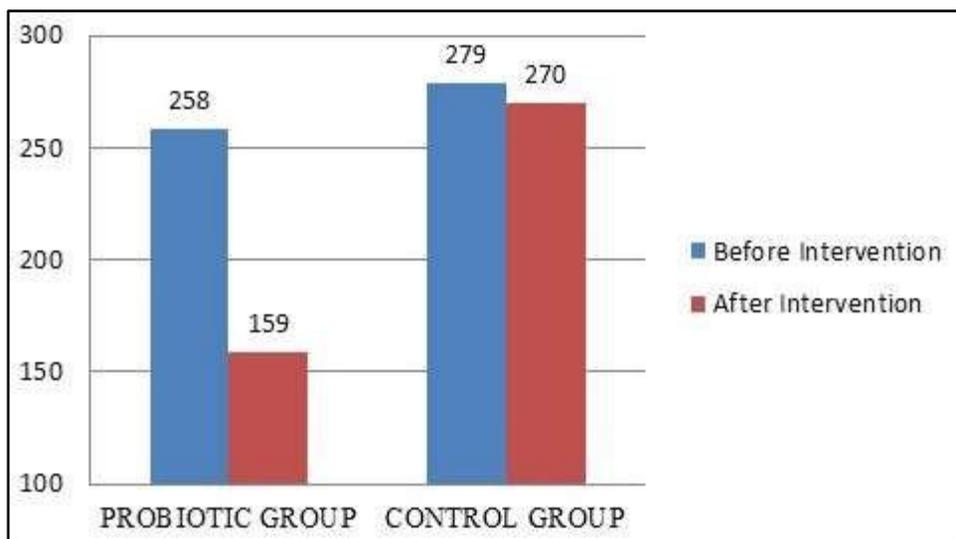
RESULTS

CHANGES IN THE PH:

In the study group as well as the control group, a drop in the pH was recorded. However the drop was significant in the study group, while that in the control group was statistically non-significant (As shown in table 1 and graph 1).

	Mean pH at baseline	Mean pH after 15 days	t value	p value
Probiotic group	6.069 ± 0.6	5.9 ± 0.5	2.38	0.04 S
Control group	6.291 ± 0.35	6.069 ± 0.26	2.78	0.3 NS

Table 1: Comparison of the pH between test group and control group at baseline and at 15 days interval



Graph 1: Mean pH values in the test group and control group at baseline and 15 days interval

When the change in the pH of the two groups was compared, the mean pH of study group was lower in study group as compared to control, but the difference was statistically non-significant. (p-value = 0.062) (As shown in table 2 and graph 1)

	Mean	Standard Deviation	t value	p value
Probiotic group	5.9 ± 0.5	0.5		
Control group	6.069 ± 0.26	0.26	-3.37	0.062

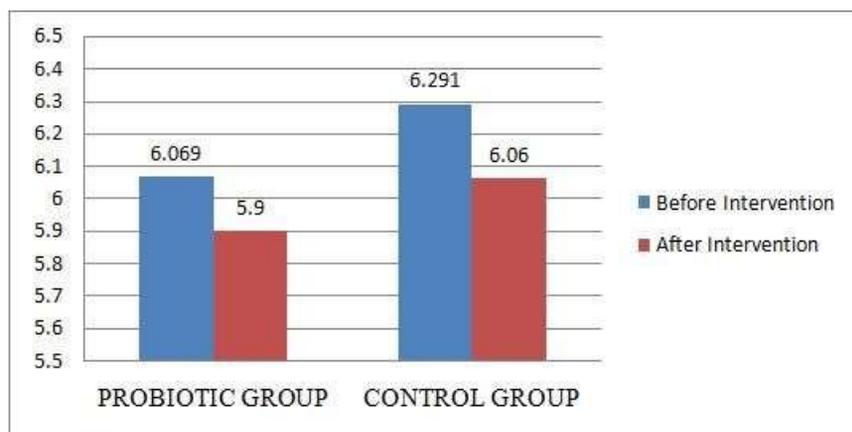
Table 2: Comparison of pH between test group and control group at 15 days interval

CHANGE IN STREPTOCOCCUS MUTANS COLONY COUNTS:

The drastic decline in the colony of Streptococcus mutans in the plaque was recorded after the consumption of probiotic yogurt. On other hand the control group showed no significant difference (As mentioned in table 3 & graph 2).

	Mean of count at Baseline	Mean of count after 15 days	t value	p value
Probiotic group	258.4 ± 58.77	159.8 ± 53.40	9.51	0.00 HS
Control group	279.9 ± 83.54	270.2 ± 88.67	1.06	0.3 NS

Table 3: Comparison of the number of colonies between test group and control group at baseline and at 15 days interval



Graph 2: Mean number of colonies in test group and control group at baseline and 15 days interval.

	Mean	Standard Deviation	t value	p value
Probiotic group	159.8 ± 53.40	53.40		
Control group	270.2 ± 88.67	88.67	-1.98	0.03

Table 4: Comparison of the number of colonies between test group and control group at 15 days interval

On comparing the colony count of the two groups a highly significant decrease was seen in the experimental group as compared to control (As mentioned in table 4 & graph 2).

DISCUSSION

Dental caries is a multifactorial microbial disease, so the treatment also needs to be multimodal. One cannot sterilize the mouth or can even come close to it, another promising alternative of balancing the harmony of microbial environment, such that the pathogenic organism remains in control by the presence of antagonizing bacteria. Though seems tricky, it can be easily done by what is known as Replacement therapy, i.e. the consumption of probiotics.⁸ It has the power to selectively remove only the (odonto) pathogen while leaving the remainder of the oral ecosystem intact.⁹

Discovery of probiotics is credited to Elen Metchnikoff who acknowledged the yogurt the reason for the healthy life of Bulgarian peasants.¹⁰ The terms PROBIOTIC which was an antonym to the term antibiotic¹¹ and meant 'For Life' was coined by Lilly & Stillwellin in 1965.⁵ Its benefit to oral health was first described by Polonskaya by demonstrating the inhibition of plaque forming bacteria- *Fusiform nucleatum* by the use of probiotic *Lactobacilliacidophilus* strains.¹² Thereafter series of studies began, to check the effect of probiotic on oral health.

The present study included children of 6-12 years of age as this age group shows maximum prevalence of dental caries. Children with zero or minimum dmft/DMFT score, residing in same locality and having common source of drinking water were selected to avoid any bias.

The probiotic culture was given in the form of an yogurt (Amul India Pvt. Ltd., Anand, Gujarat, India) which was prepared by adding a freeze dried culture of probiotic strains of *Lactobacillus acidophilus* La-5 and *Bifidobacterium bifidum* with the concentration of 1×10^8 CFU/ml of each probiotic strains. Though probiotic is available in various forms, Yogurt was chosen as the vehicle because it is readily available, a common dietary constituent used in several different combinations that are acceptable to the paediatric age group. Also the brand is one of the common commonly used one in Indian market for dairy products. Added advantage of using yogurt is that it has a high buffering capacity, non-erosive nature and

low cariogenic potential as it contains mineral like calcium.¹³ Its semisolid consistency helps in its retention in the oral cavity for a longer period of time with an extended beneficial effect.¹⁴ The comparison done by Sutulael al. and Montalto M et al¹⁵ between different means of probiotics showed no significant difference in the effect and it was thus concluded that direct contact with oral tissues is not a prerequisite for probiotics to have a beneficial effect; purely systemic administration of a probiotic could enhance *Lactobacillus* proliferation in the oral cavity.⁶ The microbes used in the present study are one of the most commonly used organisms. *Lactobacillus acidophilus* acid resistance and known probiotic potential³ in form of promoting a healthy digestive system, immune modulation⁸ and secretion of compounds such as organic acids, hydrogen peroxide, and bacteriocins or bactericidal proteins.³ On the other hand *Bifidobacterium* has proved its usefulness within the human body, through enhancing immune response and benefitting gastrointestinal health in young children.⁸ Though these organisms have been implicated in the progression of dental caries especially in dentin, the buffering capacity of the yogurt to which it is added makes the consumption safe.¹⁴ Also this aciduric and lactic acid-producing species plays a role only in deep dentine caries progression but not in enamel demineralization.¹³

Majority of the studies are been conducted to check the count of microbes in saliva. However the major drawback of using saliva is that the count of microbes is not specific to tooth surface but a sum total from previous carious lesions, the tongue, and other sites that harbor the organisms. On the other hand plaque remains adhered to the teeth and gives rise to caries and some studies have shown a difference between the *Streptococcus mutans* counts in saliva and in plaque.⁴ Thus, in the above study plaque samples were used to determine the change in the pH and *Streptococcus mutans* count. Specifically *Streptococcus mutans* were checked as their role is the most virulent caries producing organism.¹⁴

A decrease in the pH of the plaque was seen in the study group. We assume the drop in the pH is due to the acidic nature of the yogurt. However, this drop cannot be considered harmful as it is well above the critical pH given by Stephan's curve (5.2-5.5). Above all, the properties of yogurt of buffering acidity and high mineral content prevent occurrence of caries or any further progress of it. This finding was in accordance to that of Sudhira et al¹⁴ where salivary pH and *Streptococcus mutans* was recorded after use of probiotic curd.

The colony count in the study group showed high significant difference whereas that in the control group the change was non-significant. A drop in the count of *Streptococcus mutans* after 15 days of yogurt consumption made it clear that the probiotic bacteria can cause reduction in the survival of streptococcal strain. This theory was supposed by the in vitro study done by Simark-Mattsson et al.¹⁶ who proved that *Lactobacilli* have an inhibitory effect on the growth of *Streptococcus Mutans* and *S. Sobrinus* both in dental plaque and saliva. Other studies which were in accordance to the present study were that of Nase et al.,¹⁷ 2001 and Ahola et al¹⁸. They proved that *Lactobacillus* not only negatively affects the number of *Streptococcus mutans* in the oral cavity but also declines the risk of caries in children. Similar results were seen in their in vivo study done by Sudhira et al¹⁴, Ghasempour¹⁹ and Juneja et al, on salivary *Streptococcus mutans* count.²⁰ This finding reinforced the results of previous studies done by Caglar et al 2005, 2007, 2008^{21,22,23} and Singh RP et al²⁴ on salivary level of *Streptococcus mutans*. When the effect of probiotic was checked on patients undergoing orthodontic treatment by Cildir and Mahanteshaa drop in salivary *mutans* level was discovered.¹³

The role of probiotic in hampering the growth of pathogenic bacteria in oral cavity is not fully understood. The hypotheses about the mechanism of action are as follows: (a) Direct Interactions - interference of biofilm formation, plaque ecology via synergistic and antagonistic interaction, competing with oral microbes for the available substrate and production of antimicrobial substances,²⁵ modification

of protein composition of the pellicle by degradation of salivary proteins¹² and (b) Indirect actions - which includes modulation of systemic immune function such as of elevations of the numbers of killer cells, T-cells, and interferon¹³ and secretion of antimicrobial compounds called bacteriocins: eg, reuterin.⁴

However Sudhir et al attributed the reduction seen to the general antimicrobial activity of curd rather than a specific competitive activity of curd microorganisms.¹⁴ Also it is believed that the drop in pH due to probiotic prevent the formation of dental plaque and calculus that causes oral inflammation.¹⁰

Most of the authors used saliva for comparison; however in the present study plaque was used for inspection and results were in favor of probiotics. The study done by Jose⁴ was in accordance to our finding. He investigated level of s mutans in the plaque around the brackets in orthodontic patients by using curd and toothpaste. In this case also a reduction in the level was seen, irrespective of the vehicle chosen. The systemic review done by Laleman²⁶ and Cagetti²⁷ concluded that the use of probiotic reduces the S Mutans count in saliva and plaque but its effect on dental caries needs to be examined.

In spite of numerous studies concluding the fact that probiotics results in competitive inhibition of pathogenic bacteria some studies like that conducted by Montaltoet al¹⁵ and Sotiria Gizani,²⁸ showed contradictory findings. However, the author explained the contradictory results on the basis of suboptimal daily dosage, duration of the intervention, age of the participants, and a para mount cariogenic challenge.

CONCLUSION

Considering various aspects including literature as well as findings of this study, it was concluded that probiotic have a beneficial effect on the oral health by reducing the count of cariogenic bacteria; streptococcus mutans in dental plaque. A regular consumption of probiotic products will not only have a beneficial effect on general health but also on the oral health and can be used as a preventive approach against dental caries. Thus, the time has come to shift the paradigm of the treatment from specific bacteria elimination to alteration of the bacterial ecology by using probiotics.

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Nil

CONFLICTS OF INTEREST

There are no conflicts of interest

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