# Evaluating the Effect of Probiotic Containing Milk on Salivary *mutans streptococci* Levels

Juneja A * / Kakade A **

***Purpose:*** *To evaluate the changes in mutans streptococci counts in saliva after short term probiotic intervention and its delayed effects on salivary mutans streptococci count.* ***Methods:*** *40 children in the age group of 12-15 years with medium to high caries activity were randomly divided into Group I Control (plain milk group) and Group II Experimental (probiotic supplemented milk group). Duration of the study was 9 weeks; which was evenly divided into three phases: baseline, intervention and post-treatment period; each phase consisting of three weeks. After baseline period of 3 weeks, children in group I were given plain milk and in group II milk containing probiotic Lactobacillus rhamnosus hct 70 for 3 weeks; followed by a 3 weeks follow up period. After every phase saliva samples were collected to estimate salivary mutans streptococci counts.* ***Results:*** *The difference in the post follow up mutans streptococci count of group I and group II, was highly significant with p value < 0.001. In the control group, the difference in the mean salivary baseline, post treatment and post follow up mutans streptococci counts was not statistically significant (p > 0.001). In the experimental probiotic group, the difference in mean salivary baseline, post treatment and post follow up mutans streptococci counts was statistically highly significant ( p = 0.000, p ≤ 0.001).* ***Conclusions:*** *Statistically significant reduction in salivary mutans streptococci counts immediately after consumption of probiotic Lactobacillus rhamnosus hct 70 containing milk suggest a beneficial effect of probiotic Lactobacillus rhamnosus hct 70 in the prevention of dental caries.*

***Keywords:*** *probiotics, mutans streptococci, caries, Lactobacillus rhamnosus.*
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## INTRODUCTION

Dental caries is one of the most prevalent diseases in humans, second only to the common cold. It's non-life-threatening nature and ubiquitousness has minimized its significance in overall human health. The economic burden for the treatment of this dental infection is staggering.[1-3] Although a disease of multifactorial origin, it is considered a result of the interplay of three principal factors: host, microflora and diet, to which a fourth component time has been added. A group of phenotypically similar but genetically different streptococcal species, known as *mutans streptococci*, are considered the main etiological agents for dental caries in humans.[3-6] Based on DNA homology, *mutans streptococci* are divided into seven species: *Streptococcus mutans, S. sobrinus, S. ratti, S. riceti, S. downei, S. ferus, and S. macacae;* which can be subdivided into eight serotypes: a, b, c, d, e, f, g and h. Of these species, *S. mutans and S. sobrinus* have been implicated as the primary causative agents of dental caries in humans.[7] The changes in the homeostasis of the oral cavity with an overgrowth of *Streptococcus mutans* is recognized as the primary cause of the disease. *S. mutans* strongly adheres to tooth structure and releases acids by the fermentation of carbohydrates, leading to the demineralization of the tooth. This attachment is mediated mostly by the interaction of surface proteins and bacterial polysaccharides. *Streptococcus mutans* usually comprises less than 1% of the flora of children with negligible caries activity.[8,9]

The application of health-promoting bacteria for therapeutic purposes is one of the strongest emerging fields not only in medical but dental science. The growing research in herbal treatments has led to the discovery of various phytochemicals to limit the virulence of *S. mutans*.[10] Most treatments are now aimed at either elimination of this bacterium or suppression of its virulence. The term 'probiotic' as officially adopted by the International Scientific Association for Probiotics and Prebiotics can be defined as "Live microorganisms, which when administered in adequate amounts, confer a health benefit on the host."[11] There is considerable scientific evidence of their potential and real benefits *in vitro*

* Akanksha Juneja, MDS, Assistant Professor, Department of Pediatric and Preventive Dentistry, Jamia Millia Islamia.
** Adesh Kakade, MDS, Professor and Head, Department of Pediatric and Preventive Dentistry, Nair Hospital Dental College, Mumbai, Maharashtra,

Send all correspondence to: Dr Akanksha Juneja, Department of Pediatric & Preventive Dentistry, Faculty of Dentistry, Jamia Millia Islamia, New Delhi, India.

Phone: 09971404330, 09250819178

Email Add: aka_juneja@yahoo.com

and animal experiments and to a lesser extent in humans. The best known and studied probiotics are the lactic acid bacteria and bifidobacteria, which are widely used in yoghurts and other dairy products. They retain viability during storage and survive passage though the stomach and small bowel and are generally regarded as safe, i.e. non-pathogenic and non-toxic.[12]

Probiotic organisms are thought to act through a variety of mechanisms including the competition with potential pathogens for nutrients or enterocyte adhesion sites, including degradation of toxins, production of antimicrobial substances, and local and systemic immunomodulation.[13-15] *Lactobacillus acidophilus, Lactobacillus casei, Lactobacillus lactis, Lactobacillus helviticus, Lactobacillus salivarius, Lactobacillus plantrum, Lactobacillus bulgaricus, Lactobacillus rhamnosus, Lactobacillus johnsonii, Lactobacillus reuteri, Lactobacillus fermentum, Lactobacillus delbrueckii, Streptococcus thermophilus, Enterococcus faecium, Enterococcus aecalis, Bifidobacterium bifidum, Bifidobacterium breve, Bifidobacterium longum and Saccharomyces boulardii* are some of the commonly used bacterial probiotics.[16] Within dentistry, studies with *Lactobacillus rhamnosous* GG,[17-19] *Lactobacillus reuteri,*[20] and *Lactobacillus casei*[21] have defined their potential in interacting with *S. mutans* by reducing the number of this caries initiating pathogen, thus suggesting a role of probiotics in caries prophylaxis.[22] *Lactobacillus rhamnosus* belongs to the heterofermentative lactobacilli group, which cannot ferment either sucrose or lactose and has also shown to increase humoral immunity.[13,18] Yet, there is a paucity of information regarding the contributions of probiotics to oral health.

Keeping in mind the probable role of probiotics in preventing dental caries the present study was designed and carried out in the Department of Pediatric and Preventive Dentistry, Nair Hospital Dental College, Mumbai, Maharashtra, India in collaboration with the Department of Microbiology, Topiwala National Medical College and BYL Nair Charitable Hospital, Mumbai and Fourrts India Ltd. to evaluate the effect of probiotic *Lactobacillus rhamnosus hct 70* in milk on salivary mutans streptococci levels.

## MATERIALS AND METHOD

Before the commencement of the study, necessary approval was obtained from the local ethical committee (Ethical Committee of Nair Hospital, under Municipal Corporation of Greater Mumbai, Mumbai, India) and permission was sought from the concerned school authorities. A total of 85 children, 10-18 years of age were screened from Regina Pacis Boarding School, Byculla, Mumbai; of which 50 children were initially selected to carry out the caries activity test. Out of these, 40 children which satisfied the inclusion and exclusion criteria were selected.

Children in the age group of 12-15 years with medium to high caries activity (class 2 and 3 on the basis of Dentocult Strip mutans test), having permanent dentition and no associated oral pathological anomalies and periodontitis were selected. There was no history of intake of antibiotics for a minimum period of 4 weeks and no history of any preventive treatment like professional fluoride application for the past 6 months. Children with compromised immune status, medically compromised conditions and lactose intolerance were excluded from the study.

After eliciting detailed medical history and obtaining written consent from parents- or guardian the selected children were divided into 2 groups:

Group I (plain milk group): Children receiving plain milk

Group II (probiotic group): Children receiving *Lactobacillus rhamnosus hct 70* containing milk

Midmorning saliva samples were collected at least one hour after breakfast.[23] The children were seated comfortably and asked to chew paraffin wax (1gram) at a constant rate of 70 chews/ minute for about two minutes. The children were then told to expectorate every 30 seconds to 60 seconds. Paraffin stimulated salivary samples (1.0 ml) were collected from each child in an appropriately labeled sterile container.[24] The microbial load was estimated from a single saliva sample obtained from each of the children.

### Study Design

Duration of the study was 9 weeks; which was evenly divided into three phases: baseline, intervention and post-treatment period; each phase consisting of three weeks.

### 1. Baseline Period (Duration 3 weeks)

Forty children who had met the selection criteria were given the protocol instructions and then dietary modifications were explained to them and to their parents i.e.

- No use of probiotic containing products like curd, cheese etc.
- Use of non fluoridated toothpaste
- No commercial fluoride application or dental treatment

They were given non fluoridated tooth paste and a toothbrush to be used during the period and were asked to brush twice a day. The use of other fluoride products was not allowed. After 3 weeks, midmorning saliva samples were collected at least one hour after the breakfast from each child and were used for calculating mutans streptococci counts.

### 2. Intervention / Treatment Period (Duration 3 weeks)

During this period Group I children received plain milk and Group II children received milk containing probiotic *Lactobacillus rhamnosus hct 70* with daily consumption of $2.34 \times 10^9$ CFU/day in divided dose. They were given 150 ml of milk each time, two times a day for 3 weeks. They were asked to postpone brushing of teeth for at least one hour after drinking of milk. Instructions were given to drink milk slowly and to drink it unheated. At the end of 3 weeks salivary samples were collected at least 1 hour after breakfast and salivary mutans streptococci levels were determined.

### 3. Post treatment / Follow up Period (Duration 3 weeks)

The last phase of the study was the post treatment period consisting of 3 weeks. During this phase they were asked to follow regular instructions as given in the beginning of the study period. At the end of 3 weeks, midmorning saliva samples were collected at least one hour after the breakfast from each child and used for calculating mutans streptococci counts.

The within-subject changes in *Streptococcus mutans* counts from baseline to the intervention period were calculated. The results obtained were tabulated and analysed using Paired and Unpaired t-test and Chi Square test using software named Statistical Package for Social Sciences.

## RESULTS

The growth of *mutans streptococci* was assessed in all 18 children in group I and group II. The mean salivary baseline *mutans streptococci* count in group I (plain milk group) was 916.67 ± 205.798 x $10^5$ CFU/ml (Table I, Graph I) and in group II (probiotic group) was 1131.67 ± 273.738 x $10^5$ CFU/ml (Table II, Graph I). When the baseline colony counts of group I were compared with group II, the difference in the mutans streptococci was not significant with p value > 0.001 (Table III, Graph II). Similarly, the mean salivary post treatment mutans streptococci count in group I was 875 ± 201.648 x $10^5$ CFU/ml (Table I) and in group II was 672.22 ± 203.081 x $10^5$ CFU/ml (Table II). When the post treatment colony counts of group I were compared with group II, the difference in the mutans streptococci was non significant with p value > 0.001 (Table III, Graph I).

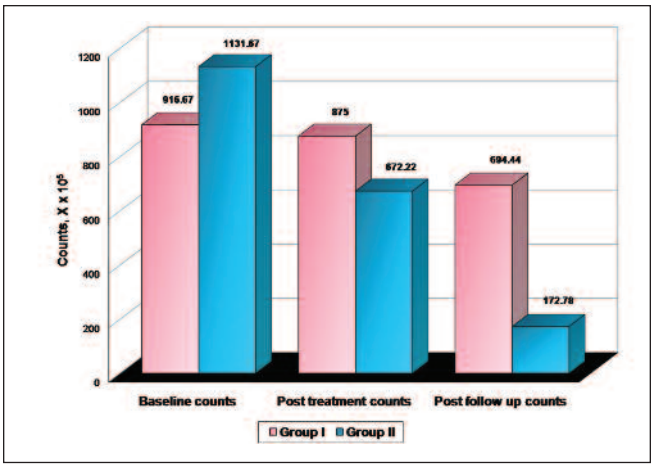
However, the mean salivary post follow up mutans streptococci count in group I was 694.44 ± 173.11 x $10^5$ CFU/ml (Table I) and in group II was 172.78 ± 133.452 x $10^5$

**Table I.** Comparison of mutans streptococci counts in Group I (control)

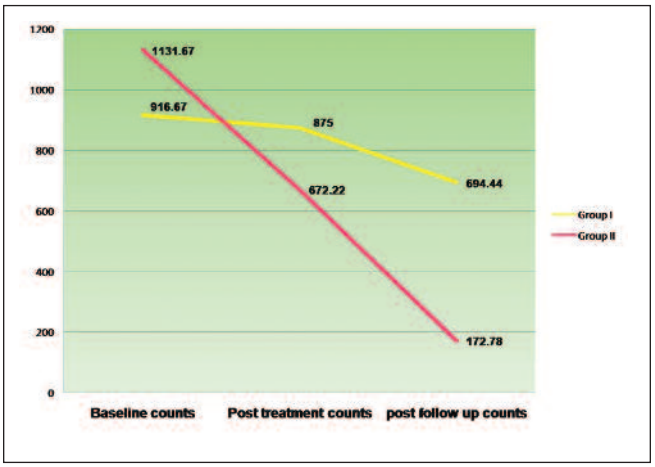
| No. | Baseline counts X x $10^5$ CFU/ml | Post treatment counts X x $10^5$ CFU/ml | Post follow up counts X x $10^5$ CFU/ml |
|---|---|---|---|
| 1. | 1000 | 900 | 800 |
| 2. | 1200 | 1200 | 1000 |
| 3. | 700 | 900 | 600 |
| 4. | 900 | 1200 | 1000 |
| 5. | 600 | 400 | 400 |
| 6. | 1000 | 900 | 600 |
| 7. | 800 | 900 | 700 |
| 8. | 700 | 900 | 700 |
| 9. | 700 | 1000 | 800 |
| 10. | 1100 | 900 | 900 |
| 11. | 1300 | 1050 | 500 |
| 12. | 1100 | 800 | 600 |
| 13. | 1200 | 1000 | 800 |
| 14. | 750 | 500 | 500 |
| 15. | 900 | 800 | 600 |
| 16. | 700 | 800 | 700 |
| 17. | 900 | 700 | 500 |
| 18. | 950 | 900 | 800 |
| **Mean** | 916.67 ± 205.798 | 875.00 ± 201.648 | 694.44 ± 173.111 |

**Table II.** Comparison of mutans streptococci count in Group II (Experiment)

| No. | Baseline counts X x $10^5$ CFU/ml | Post treatment counts X x $10^5$ CFU/ml | Post follow up counts X x $10^5$ CFU/ml |
|---|---|---|---|
| 1. | 1100 | 700 | 0 |
| 2. | 1100 | 500 | 150 |
| 3. | 1000 | 700 | 350 |
| 4. | 1600 | 800 | 100 |
| 5. | 1800 | 1100 | 50 |
| 6. | 1050 | 750 | 200 |
| 7. | 1400 | 900 | 400 |
| 8. | 1100 | 800 | 150 |
| 9. | 1350 | 800 | 200 |
| 10. | 1050 | 600 | 300 |
| 11. | 900 | 500 | 200 |
| 12. | 970 | 500 | 250 |
| 13. | 1150 | 450 | 250 |
| 14. | 1100 | 700 | 10 |
| 15. | 1050 | 700 | 50 |
| 16. | 1200 | 900 | 400 |
| 17. | 600 | 300 | 50 |
| 18. | 850 | 400 | 0 |
| **Mean** | 1131.67 ± 273.738 | 672.22 ± 203.081 | 172.78 ± 133.452 |




**Graph 1.** Comparison of Mutans Streptococci counts in Group I and Group II.




**Graph 2.** Change in Mutans Streptococci Counts in Group I and Group II.

CFU/ml (Table II). When the post follow up colony counts of group I were compared with group II, the difference in the mutans streptococci was highly significant with p value < 0.001 (Table III, Graph I).

In group I, the mean salivary baseline, post treatment and post follow up mutans streptococci counts were 916.67 ± 205.798 x$10^5$, 875.00 ± 201.648 x $10^5$, and 694.44 ± 173.111 x $10^5$ CFU/ml respectively (Table I). The difference in the baseline and post treatment counts was not found to be statistically significant (p=0.381, p>0.001) (Table IV, Graph II). However, the difference in the baseline and post follow up counts was found to be statistically significant (p=0.001, p≤0.001) (Table IV, Graph II); the difference in the post treatment and post follow up counts was not found to be statistically significant (p=0.003, p>0.001) (Table IV, Graph II).

In group II, the mean salivary baseline, post treatment and post follow up mutans streptococci counts were 1131.67 ± 273.738 x $10^5$, 672.22 ± 203.081 x $10^5$ and 172.78 ± 133.452 x $10^5$ CFU/ml respectively. The difference in the baseline and post treatment (Table IV, Graph II); baseline and post follow up (Table IV, Graph II) and; post treatment and post follow up counts was found to be statistically highly significant (p=0.000, p≤0.001) (Table IV, Graph II).

**Table III.** Comparison of mutans streptococci counts between plain milk and probiotic milk group

| Mutans count X x $10^5$ CFU/ml | Plain milk group | Probiotic group | p- value | Level of significance |
|---|---|---|---|---|
| **Baseline counts** | 916.67 ± 205.798 | 1131.67 ± 273.738 | 0.012 | NS |
| **Post treatment counts** | 875 ± 201.648 | 672.22 ± 203.081 | 0.005 | NS |
| **Post follow up counts** | 694.44 ± 173.11 | 172.78 ± 133.452 | 0.000 | HS |

**P value<0.001; HS=highly significant**

**Table IV.** Intragroup comparison of mutans streptococci counts in plain milk group and probiotic milk group

| Groups | Baseline counts | Post treatment counts | Post follow up counts | P value | Level of significance |
|---|---|---|---|---|---|
| **Plain milk group** | 916.67 ± 205.798 | 875.00 ± 201.648 | | 0.381 | NS |
| | 916.67 ± 205.798 | | 694.44 ± 173.111 | 0.001 | Significant |
| | | 875.00 ± 201.648 | 694.44 ± 173.111 | 0.003 | NS |
| **Probiotic group** | 1131.67 ± 273.738 | 672.22 ± 203.081 | | 0.000 | HS |
| | 1131.67 ± 273.738 | | 172.78 ± 133.452 | 0.000 | HS |
| | | 672.22 ± 203.081 | 172.78 ± 133.452 | 0.000 | HS |

**P value<0.001; HS=highly significant; X x $10^5$ CFU/ml**

## DISCUSSION

The fact that dental caries is a bacterially mediated process has been known for more than 115 years. Since then, research has refined the process of caries development to a multifaceted disease process. Currently, we know that the host, bacteria and nutrients are required to foment the production of organic acids and the subsequent demineralization activity.[25] Association of mutans streptococci with the initiation and prevalence of caries has been clearly established in several epidemiological studies.[26-36] The level of *Streptococcus mutans* in saliva has been shown to correlate with both past caries experience [26,37] and future caries activity.[38-40]

To combat the threat caused by mutans streptococci in the oral cavity, out of the many treatment modalities tried; bacteriotherapy can be considered an alternative and promising way which acts by displacing pathogenic micro organism.[41] Out of the many natural products available, milk and cheese are known to contain compounds that reduce the risk of dental caries.[18,42-45] Regarding milk and cheese, one should also recognize the large body of evidence relating to casein phosphopeptides and other milk-derived materials and their role in bio-mineralization and other processes. These dairy products have been shown to be anticariogenic in humans by increasing the calcium content of the plaque.[44,46,47] Hence, research combining the beneficial effects of probiotics with milk would have been beneficial.

Keeping this in mind, the present study was conducted in which, a total of 40 children of age group 12-15 years, were selected from Regina Pacis School of Byculla Area; Mumbai and were categorized into 2 groups i.e.; 20 children receiving plain milk in group I and 20 children receiving probiotic *Lactobacillus rhamnosus hct 70* containing milk in group II and, changes in the salivary levels of *Streptococcus mutans* was assessed. The 12-15 years age group was selected so that all permanent teeth have been exposed to the oral environment for a sufficient period. However during the course of the study 4 children fell ill, hence the sample size reduced from 20 to 18 in each group. In the present study, though there was reduction in the salivary counts of control group; it was statistically non significant except for difference in baseline and post follow up salivary counts, which was statistically significant; which was in accordance with Nase *et al*.[18] This could be attributed to instructions and motivation done in the beginning of the study, along with beneficial effect of milk without sucrose and increase in frequency of toothbrushing per day. However these aspects were common for both the groups. In contrast, Experiment group receiving probiotic *Lactobacillus rhamnosus hct 70* for 3 weeks[19,48-50] showed statistically highly significant difference in baseline and post treatment; post treatment and post follow up and baseline and post follow up salivary counts. All the individuals in this group showed reduction in salivary mutans

streptococci counts when compared with their baseline counts; though Ahola et al. (2002)[19] Nineteen had observed that mutans streptococci counts decreased in only 20% of individuals immediately after intervention, which further increased to 21% after the follow up period. This difference can clearly attributed to the beneficial effect of *Lactobacillus rhamnosus hct 70* on oral cavity.

*Lactobacillus rhamnosus* is one of the many probiotic strains which may be useful for the oral cavity.[18,19,51-54] LGG may compete with other oral microorganisms by producing antimicrobial substances,[13] such as pyroglutamic acid.[18] Mice that were infected with lactobacilli, but were free from streptococci and enterococci had a lower incidence of colonization by S. gordonii, according to Loach *et al*. [55] *Lactobacillus rhamnosus* has also shown to increase humoral immunity[56,57] and antibodies against it have been detected in saliva of experimental animals.[18] Consequently, the mechanism of action of a probiotic bacterium may be manifold also in the oral cavity. It belongs to the heterofermentative lactobacilli, which cannot ferment sucrose and lactose. It does not enhance caries and could therefore be safe for the teeth.[20,58]

Our Results indicate that *Lactobacillus rhamnosus hct 70* definitely has an inhibitory effect on oral mutans streptococci**.** To our knowledge, the present study is the first to examine the oral effects of *L. rhamnosus hct 70* on salivary mutans streptococci in the age group of 12–15 years old children. The explanation for the findings, and the mechanism of action, is not fully clear. While it might be possible that probiotics may competitively inhibit streptococci by replacement because of the direct contact with the oral tissues and biofilm due to slow ingestion of milk. However from the existing literature, it is questionable whether probiotic bacteria can permanently colonize in the mouth and whether or not they have any residual effect after discontinuation of intake. A systemic effect induced by the probiotic during periods of ingestion cannot be excluded, although this has not been convincingly evaluated in previous studies on oral ecology. Some additional information on this issue would possibly have been available if the salivary bacterial levels had been monitored more frequently during the intervention. Even though a significant effect of *Lactobacillus rhamnosus hct 70* was seen on mutans streptococci, further investigations should be carried out on a larger sample to further confirm the results and determine the mode of action of *Lactobacillis rhamnosus* in the oral cavity.

## CONCLUSIONS

The main findings of the study were:

- Statistically highly significant reduction in salivary mutans streptococci counts immediately after consumption of probiotic *Lactobacillus rhamnosus hct70* containing milk
- Further reduction in the salivary mutans streptococci counts during the post intervention period signifying the delayed effect of probiotic on mutans streptococci
- Milk seemed to have beneficial effects on oral cavity by reducing the salivary mutans streptococci counts
- Statistically significant reduction in salivary mutans streptococci counts for children of all selected age groups

The results of the present study suggest a protective and preventive role of probiotic *Lactobacillus rhamnosus hct 70* against dental caries and that short term consumption of probiotic *Lactobacillus rhamnosus* may have not only immediate but also delayed effect on salivary mutans streptococci counts. The results also suggest that probiotic intervention could be especially beneficial to those with the highest *Streptococcus mutans* counts. However, the influence of different probiotics on the risk factors of caries remains to be established. In the present study, as the sample size was small, more elaborate work on a larger sample is required to come to definite conclusions. Further research should focus on investigating the mechanism of action of probiotics and at the same time reporting of any harmful effect of consumption of probiotics which must of course be verified in prospective clinical trials. Nevertheless, the present observations merit further study in order to evaluate the effects of probiotics on the oral ecology, defining whether they are a problem or a possible solution.

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