

**Comparative Evaluation of Effects of Probiotics on Streptococcus Mutans and Sobrinus Count In Orthodontic Patients**

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**Abstract**

**Introduction:** Levels of pathogenic bacteria count have been found to be higher in patients undergoing orthodontic treatment. This increased levels of bacterial activity causes demineralization leading to white spot lesions. Probiotic bacteria have been found to be effective in limiting pathogenic organisms and also their reintroduction. Some studies have established that the level of S mutans and S sobrinus in saliva, which are the initiators of white spot

lesions was reduced after the use of probiotics which would be beneficial in orthodontic patients. However there are few studies on the effects of probiotics on S mutans and S sobrinus in orthodontic patients. This study was undertaken to evaluate the effect of probiotics on the streptococcus mutans and streptococcus sobrinus colonization levels which is known to cause white spot lesions on the teeth around the orthodontic brackets in young adults.

**Method:** A total of 50 patients undergoing fixed orthodontic treatment were included in the study. Test group received probiotic intervention (lactobacillus acidophilus tablets) for 15 days while control group received no intervention at all. Streptococcus mutans and sobrinus colonies were enumerated at baseline and after 15 days based on colony characteristics using stereomicroscope.

**Result:** A statistically significant reduction of the mutans streptococci levels was recorded after ingestion of the probiotic bacteria via the tablets, which was in contrast to the controls. A similar trend was seen for Streptococcus sobrinus.

**Conclusion:** A short-term daily ingestion of lactobacilli-derived probiotics delivered by tablets reduced the levels of Streptococcus mutans and Streptococcus sobrinus in young adults.

**Keywords:** Streptococcus Mutans, Streptococcus Sobrinus, Probiotics, Lactobacillus Acidophilus Strain

### Introduction

Enamel demineralization, consequent to orthodontic treatment is a major concern. They jeopardize the esthetic results of the treatment. Among the many orthodontic appliances, brackets can play a key role in enamel demineralization because their complex designs increase the retention of food particles and dental plaque by impeding access to the tooth surfaces for cleaning. (1)(2)

Enamel demineralization is caused by organic acids produced by cariogenic bacteria, Of these, Streptococcus mutans and Streptococcus sobrinus are the most commonly involved(3)(4)(5)(6). Levels of Streptococcus mutans were found to be higher in orthodontic patients than in nonorthodontic patients(7). Prevalence of S mutans was found to be about 2 times higher than that of S sobrinus on incisor bracket(2). In particular, S sobrinus

was closely associated with increases in smooth-surface caries(8)(9).

Overall prevalence of white spot lesions among orthodontic patients has been reported to be between 4.9% and 84%(10). White spot lesions occur as a result of imbalance between mineral loss and mineral gain(11).The lesions were greatest on the cervical and middle thirds of the crowns of the maxillary and mandibular first molars, maxillary lateral incisors, and mandibular lateral incisors and canines, and mainly on the vestibular surfaces(10).

It was shown some years ago that oral hygiene and topical fluoride regimens during treatment can reduce the prevalence of postorthodontic demineralized white spot lesions(12).Once formed, however, many of these early lesions appear to be surface demineralization rather than a subsurface lesion with an intact surface zone. Benson and coworkers, in a recent Cochrane systematic review, concluded that there is some evidence that the daily use of a sodium fluoride mouth rinse or glass ionomer cement to bond appliances may reduce the occurrence and severity of white spot lesions(13). However, Ogaard and coworkers warned against treating visible white lesions on labial surfaces with concentrated fluoride agents, since this arrests both demineralization and remineralization in the lesion by surface hypermineralization. Instead, these workers advocated allowing remineralization by saliva, as this results in greater repair and a less visible lesion(14).

In the 1980s, Reynolds drew attention to the fact that casein phosphopeptide amorphous calcium phosphate, which is a product derived from milk casein, was capable of absorbing through the enamel surface and interfere with the carious process(15). A regimen using a sorbitol-based chewing gum chewed for 20 minutes, 5 times daily for 3 weeks, showed significant remineralization of demineralized enamel when compared with controls without chewing gum(16).The technique of microabrasion

has also been advocated for the removal of postorthodontic demineralized white lesions.(17)(18)

Biological methods such as antibiotics, antimicrobial therapy with chlorhexidine, povidone iodine, fluoride, and penicillin have gained importance in recent years. The application of broad-spectrum antibiotics and antimicrobial therapy can suppress the caries infection but never totally eliminate it. None of these medicaments has been able to successfully preclude the regrowth of residual pathogens or reinfection from external sources; this means that antibiotic and antimicrobial therapies must be given at regular intervals for effective long-term results.(19)

At the turn of the 20th century, Elie Metchnikoff, a Nobel Prize-winning Russian, made the revolutionary discovery of probiotics. Probiotics are “live microbial food supplements which beneficially affect the host animal by improving its intestinal microbial balance.”(19). Lactic acid bacteria and bifidobacteria are the most common types of microbes used as probiotics, but certain yeasts and bacilli can also be helpful(19).They act by competitively inhibiting the pathogenic bacteria because they have greater adhesion to the tissues(19). They inhibit pathogens but do not inhibit friendly bacteria. Studies have shown that once the pathogenic organisms are replaced the reintroduction of the pathogen does not occur easily.(19)

Probiotics are commonly consumed as part of the diet in several cultures in the form of fermented foods such as yogurt and soy yogurt, or as dietary supplements with added active live cultures. They have proved to be beneficial in treating malnourishment, lactose intolerance, calcium availability, bowel problems such as constipation, urogenital infections, and atopic diseases such as antibiotic induced diarrhoea, and in improving the immune system, alleviating chronic intestinal

inflammatory diseases, and preventing and treating pathogen-induced diarrhoea.

A few studies have evaluated the effects of local administration of probiotic agents such as mouthwashes(20), lozenges(21), tablets, straws(22), milk(23),cheese(24),ice cream(25),chewing gums(26),yogurt(27)(28)(29)(30) and other supplements and have found that these have a beneficial effect on oral health. The benefits on oral health in preventing gingivitis, halitosis(31)(32) and caries (27)(28)have been recognized, and thus probiotics have been incorporated into mouthwashes and dentifrices for popular consumption. Some studies have established that the level of S mutans in saliva is reduced after the use of probiotics; this would be beneficial in orthodontic patients also(27). However, there are few studies in the literature on the effects of probiotics in orthodontic patients, since their use in our speciality is still in an infantile stage(27). S mutans concentration in plaque would be more representative of the caries-inducing potential in the anterior teeth where salivary clearance is less effective. Since the localized effect of probiotics on the plaque surrounding orthodontic brackets has not been studied, we conceived this study to evaluate whether probiotic systems are beneficial to orthodontic patients. It is desirable to establish which delivery system is more efficient, and thus this study was designed to compare the efficacy of systemic ingestion and topical applications.

### **Aims And Objectives**

The following are the aims and objectives of the study

1. To compare and evaluate effects of probiotic lactobacillus acidophilus on streptococcus mutans in the plaque surrounding brackets in orthodontic patients
2. To compare and evaluate effects of probiotic lactobacillus acidophilus on streptococcus sobrinus

in the plaque surrounding brackets in orthodontic patients

3. To draw clinical inferences from the same

### Methodology

The present in vivo study was conducted in the Department of Orthodontics and Dento facia lorthopaedics, Coorg Institute of Dental Sciences, Virajpet and Aavishkaar Research Center, Coorg Institute of Dental Sciences, Virajpet after obtaining ethical clearance from the Institutional Review Board.

### Materials used for patient examination

1. Patient record chart.
2. Examiners protection gears (Disposable gloves ,Disposable mouth mask)
3. Exploratory instruments (Mouth mirror and explorer, Kidney tray).

### Materials used for plaque collection

1. Universal Scaler
2. Ependorf tube
3. Labeling sticker and markers.

### Experimental samples

Nature's bounty Probiotic lactobacillus acidophilus tablets

### Materials and equipments used for microbiological assay

- Petri plate.
- Inoculating loops
- Laboratory glassware
- Sterile spreaders
- Electronic weighing balance
- SB-20M culture media
- Stereomicroscope
- Incubator and laminar air flow chamber
- Manual colony counter.

### Inclusion criteria

Study samples included in this study were:

1. Patients aged between 14 and 29 year

2. Patients undergoing orthodontic treatment with straight wire appliance
3. Patients undergoing orthodontic treatment with metallic brackets
4. Patients with permanent dentition
5. Patients with good oral hygiene

### Exclusion Criteria.

- Patients presenting allergies and idiosyncratic responses to product ingredients
- Patients with systemic conditions.
- Patients with poor oral hygiene
- Patients with no anti-inflammatory or antibiotics taken one month before the study

### Procedure

Selected Patients were divided into two groups.

group A- Control Group

group B – Probiotic Group

The proposed study was explained to the patients and his/her written consent was obtained prior to the study.

### Sample collection

The plaque-sampling was done using a standardized protocol. At the first sampling visit, plaque specimen was collected from the lateral incisor using a sterilized dental scaler with the same tip dimensions (#8/9 Orban DE hoe scaler, Hu-Friedy, Chicago, Ill). At the second sampling visit, the operator carefully removed or disengaged the ligation mechanism and removed the arch wires. Plaque specimens were collected from the labial surfaces immediately surrounding the orthodontic brackets of the maxillary lateral incisors using sterilized dental scaler with the same tip dimensions. Because the area of increased decalcification was generally immediately adjacent to the brackets, a 4-pass technique was used to move the instrument tip around the circumference of the bracket at the bracket tooth interface. Four passes, along the tooth at the bracket interface at the gingival, mesial,

distal, and occlusal aspects, were collected and transferred to screw cap vials containing 5000µl normal saline.<sup>(33)</sup>

#### **Administration of probiotic lactobacillus acidophilus.**

Selected patients were divided into group A and B (Control and Probiotic) by random sampling technique by using lot.

The pre plaque samples (S1) were collected in 5ml, sterile, collection cups from all the 50 selected patients before the commencement of the treatment regime and were immediately seeded on plates containing the SB-20M, in which sucrose will be replaced by coarse granular cane sugar and incubated in microaerophilia at 37°C for 72 hours. Patients in the experimental group were given probiotic lactobacillus acidophilus tablets (by the manufacturer Nature's bounty) and were instructed to take once daily half an hour after food consumption by slowly dissolving it in the mouth for 15 days. Patients in the control group were not given any tablets.

The post intervention plaque samples (S2) were collected after 15 days and were immediately seeded on plates containing the SB-20M media and incubated in microaerophilia at 37°C for 72 hours.

#### **Microbiological assay of plaque samples**

Preparation of the SB-20 culture medium.<sup>(34)</sup>

This culture medium has the following composition per liter:

bacto-casitone 15g; yeast extract 5g; L-cysteine 0.2g; sodium sulfite 0.1g; sodium acetate 20.0g; coarse granular cane sugar 200.0g; 15.0g agar; and distilled water. After autoclaving for 20 min at 120 degree Celsius and cooling to approximately 50 °C, bacitracin was added to a final concentration of 0.2 U per ml agar. The medium was poured in plates, stored at 4 °C and used within 7 days.

After 72 hours the colonies were counted using stereomicroscope based on colony characteristics.

#### **Identification of S. mutans and S. sobrinus**

Enumeration of colony forming units (cfu) on the SB-20M agar plates was performed by a single calibrated examiner under a dissecting microscope with the plates against a dark background in order to highlight the characteristics of the colonies. Colonies of S. sobrinus were circular and opaque milky white and were surrounded by a milky white halo, frequently exhibiting polysaccharide drops (Figure 9). Colonies of S. mutans showed a granular surface, similar to ground glass, with or without a scintillant polysaccharide drop on the surface (Figure 10). These colonies were sometimes star-shaped and appeared penetrating the surface of the agar.<sup>(34)</sup>

#### **Statistical Analysis**

The result obtained was tabulated and analyzed statistically.

#### **Results and Discussion**

The data obtained from this study, that is microbiological assay for streptococcus mutans and streptococcus sobrinus were tabulated and was statistically analyzed using MS Excel and SPSS (IBM Version 23). The level of significance was set at 0.05 at 95% confidence interval. The statistical methods used were descriptive statistics, students paired and independent t test.

The statistical results were then tabulated as:

Table 1 - Comparison of SM and SS at baseline and 15<sup>th</sup> day among control group

Table 2 - Comparison of SM and SS at baseline and 15<sup>th</sup> day among probiotic group

Table 3 – Comparison of mean differences of test and control group at baseline and 15<sup>th</sup> day among test and control group

Table 4 - Comparison of mean colony counts of SM and SS in control and probiotic group

Graph 1: Bar diagram showing comparison of SM and SS at baseline and 15<sup>th</sup> day among control group

Graph 2: Bar diagram showing comparison of SM and SS at baseline and 15<sup>th</sup> day among probiotic group

Graph 3: Bar diagram showing comparison of mean differences of test and control group at baseline and 15<sup>th</sup> day among test and control group.

Graph 4: Bar diagram showing comparison of mean colony counts of SM and SS in control and probiotic group

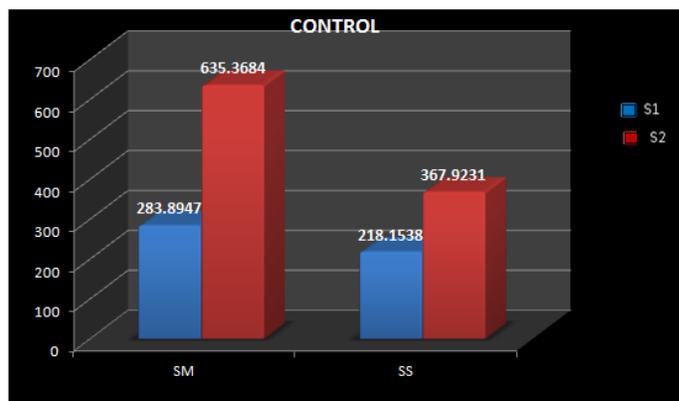
**Streptococcus mutans and sobrinus count evaluation in control group**

The mean value for the SM colony count in S1 was 283.8947(+/-441.41790) which increased to 635.3684(+/-720.96996) in S2 (table 1 and graph 1) with a T value -2.974 which is highly significant (table 3 and graph 3) and the difference was found to be 351.4737(+/-515.21445).

The mean value for the SS colony count in S1 was 218.1538(+/-480.06455) which increased to 367.9231(+/-635.50026) in S2 (table 1 and graph 1) with a T value -1.877 which is significant and the difference was found to be 149.7692(+/-287.70012) (table 3 and graph 3)

TABLE 1 : COMPARISON OF SM AND SS AT BASELINE AND 15TH DAY AMONG CONTROL GROUP

CONTROL Colony count		Mean	Standard deviation	T	Significance
SM	S1	283.8947	441.41790	-2.974	0.008(H.S)
	S2	635.3684	720.96996		
SS	S1	218.1538	480.06455	-1.877	0.049(S)
	S2	367.9231	635.50026		



Graph 1 : BAR DIAGRAM SHOWING COMPARISON OF SM AND SS AT BASELINE AND 15<sup>TH</sup> DAY AMONG CONTROL GROUP

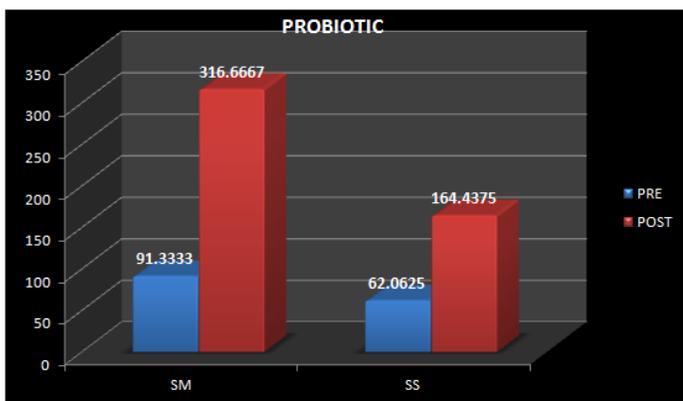
**Streptococcus mutans and sobrinus count evaluation in probiotic group**

The mean value for the SM colony count in S1 was 91.3333(+/-137.34028) , this increased to 316.6667 (+/-522.21091)in S2 (table 2 and graph 2) with a T value -1.780 which is significant and the difference was found to be 225.3333 (+/-537.06720) (table 3 and graph 3)

The mean value for the SS colony count in S1 was 62.0625 (+/-62.72955) and S2 was 164.4375 (+/-164.26968) (table 2 and graph 2) with a T value -2.819 which is significant and the difference was found to be 102.3750 (+/-145.25512) (table 3 and graph 3)

Table 2 : COMPARISON OF SM AND SS AT BASELINE AND 15<sup>TH</sup> DAY AMONG PROBIOTIC GROUP

PROBIOTICS Colony count		Mean	Standard deviation	T	Significance
SM	S1	91.3333	137.34028	-1.780	<b>0.049(S)</b>
	S2	316.6667	522.21091		
SS	S1	62.0625	62.72955	-2.819	<b>0.013(S)</b>
	S2	164.4375	164.26968		



Graph 2 : BAR DIAGRAM SHOWING COMPARISON OF SM AND SS AT BASELINE AND 15<sup>TH</sup> DAY AMONG PROBIOTIC GROUP

**Comparison of Streptococcus mutans in test and control group**

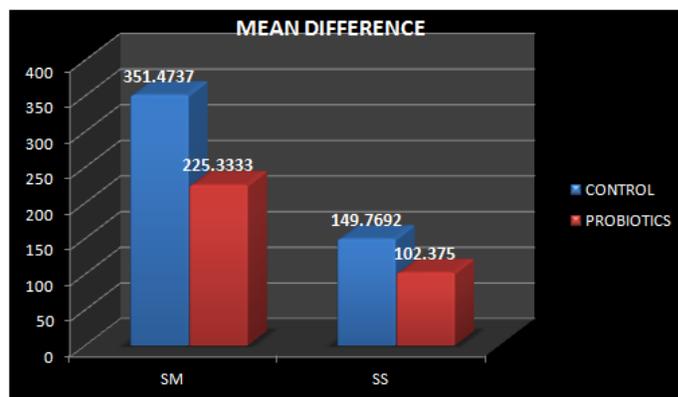
The mean difference of SM colony count in control and probiotic group was found to be 351.4737(+/-515.21445) and 225.3333(+/-537.06720) respectively with a T value - 1.729 which is significant

**Comparison of Streptococcus sobrinus in test and control group**

The mean difference of SS colony count in control and probiotic group was found to be 149.7692 (+/-287.70012) and 102.3750 (+/-145.25512) respectively with a T value - 2.576 which is significant

Table 3 : COMPARISON OF MEAN DIFFERENCES OF TEST AND CONTROL GROUP AT BASELINE AND 15<sup>TH</sup> DAY AMONG TEST AND CONTROL GROUP

Colony count	Mean	Standard deviation	T	Significance
SM	Control	351.4737	- 1.729	<b>0.047(S)</b>
	Probiotics	225.3333		
SS	Control	149.7692	- 2.576	<b>0.016(S)</b>
	Probiotics	102.3750		



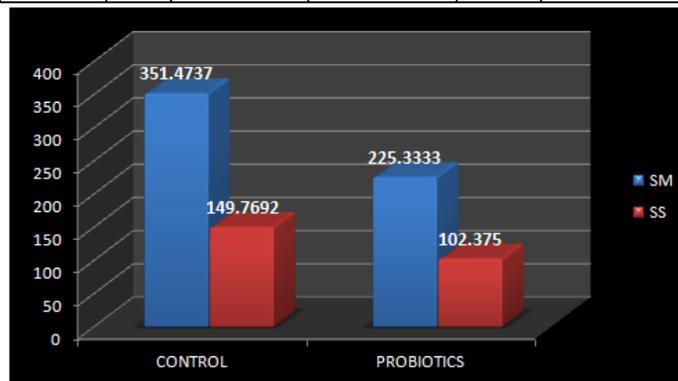
Graph 3: BAR DIAGRAM SHOWING COMPARISON OF MEAN DIFFERENCES OF TEST AND CONTROL GROUP AT BASELINE AND 15<sup>TH</sup> DAY AMONG TEST AND CONTROL GROUP.

**Comparison of mean colony counts of SM and SS in control and probiotic group**

Effect of probiotic was found to be more significant in SM count than in the SS count with a t value 0.886

Table 4 : COMPARISON OF MEAN COLONY COUNTS OF SM AND SS IN CONTROL AND PROBIOTIC GROUP

Colony count	Mean	Standard deviation	T	Significance
Control	SM	351.4737	1.278	0.211(N.S)
	SS	149.7692		
Probiotic	SM	225.3333	0.886	<b>0.011(S)</b>
	SS	102.3750		



Graph 4: BAR DIAGRAM SHOWING COMPARISON OF MEAN COLONY COUNTS OF SM AND SS IN CONTROL AND PROBIOTIC GROUP

## **Discussion**

White spots or areas of decalcification are carious lesions of varying extent. Prevalence of white spot lesions consequent to orthodontic treatment was found to be significantly high.<sup>10</sup> The plaque-retentive properties of the fixed appliance predispose the patient to an increased cariogenic risk. Furthermore, there is a rapid shift in the composition of the bacterial flora of the plaque following the introduction of orthodontic appliances. More specifically, the levels of acidogenic bacteria, such as *S. mutans*, become significantly elevated in orthodontic patients. If these bacteria have an adequate supply of fermentable carbohydrates, acid by-products will be produced, lowering the pH of the plaque. As the pH drops below the threshold for remineralization, carious decalcification occurs. The first clinical evidence of this demineralization is visualized as a WSL. Once formed, however, many of these early lesions appear to be surface demineralization rather than a subsurface lesion with an intact surface zone. Remineralization of these white lesions is a natural phenomenon resulting in only a partial reversal of what is an early caries lesion. However, the best approach during orthodontic treatment is to prevent these lesions from occurring.

Numerous studies have demonstrated that of all of the bacteria isolated from the oral cavity, *Streptococcus mutans* and *sobrinus* are the prime culprits of tooth demineralization. These same bacteria are also acid tolerant and therefore could survive the low pH observed in carious lesions. While the *mutans* streptococci are considered to be the prime suspects in the caries process, the *sobrinus* are associated with smooth surface caries. The consequent reduction in pH catalyzes the demineralization of enamel, dentin and cementum in teeth, resulting in caries lesions. Production of short-chain carboxylic acids by *Streptococci* – chiefly lactic acid

production by *Streptococcus mutans* – is the primary etiology of caries, however other bacteria with similar properties such as *Lactobacillus*, *Actinomyces*, *Bifidobacteria*, *Atopobium*, *Propionibacterium*, and *Veillonellac* contribute.

Probiotics have the potential to modify the oral microbiota and are being investigated to prevent or treat diseases of the oral cavity, such as dental caries and the periodontal diseases, which are associated with a shift in the microbial composition and activity of the biofilm, and the resulting reaction of the host, it can be due to direct interactions within dental plaque (colonization resistance). This mechanism could possibly include the disruption of plaque biofilm formation through competition for binding sites on host tissues and other bacteria, and through competition for nutrients. The production of antimicrobial compounds by probiotic species that inhibit other oral bacteria may also be a significant mechanism. It is known that cariogenic bacteria produce a range of antimicrobial agents including organic acids, hydrogen peroxide, peptides, bacteriocins and anti-adhesion molecules. It can be also due to indirect probiotic actions within the oral cavity, including the modulation of both innate and adaptive immune function. Within this context, it is possible that cariogenic bacteria can interact with immune competent cells, such as macrophages and T-cells, leading to an alteration in the production of cytokines and subsequent effects on overall immunity. Bacteria in the oral cavity are known to adhere to surfaces and to each other in the form of coaggregates and plaque biofilms. This presence in healthy adults, as well as its known early colonization of the oral cavity and the existence of various bacteriocins with potent activity against common Gram-positive oral pathogens, has made it a species of potential to recalibrate an aberrant oral microbiota, and thus suggests its use as a probiotic.

Lactobacillus acidophilus was preferred in this study as the probiotic bacterium because of its known probiotic potential and its acid resistance and bile salt's tolerance.<sup>35</sup>

This bacterium such as other lactic acid bacteria produces various compounds such as organic acids, diacetyl, hydrogen peroxide, and bacteriocins or bactericidal proteins. According to a study, it is cleared that the presence of Lactobacillus acidophilus can cause reduction in the adherence of Streptococcal strains that it is probably related to interaction between bacteria. This adherence reduction was found to be significantly stronger in the case of mutans streptococci.<sup>36</sup>

In the present clinical trial, the study interval was decided to be 15 days which was based on a study by cildir et al where daily consumption of probiotic yogurt for 2 weeks decreased the mutans streptococci counts in saliva.<sup>27</sup> The subjects were asked to move the probiotic tablet around the mouth while melting them. Compliance was excellent in all groups, with no drop-outs or reported side or adverse effects

Microbial counts increased in the S2 samples of both the control and probiotic group. This was in accordance with studies by Gwinnett et al where in it was demonstrated that orthodontic appliances increased the plaque levels in orthodontic patients.<sup>37</sup> This was due to their complex designs that increase the retention of food particles and dental plaque by impeding access to the tooth surfaces for cleaning. The increase in white spot lesions among orthodontic patients can be attributed to this finding. The results of this study confirmed findings of another study by ahn et al where in it was reported that the prevalence of S mutans on incisor brackets was higher than that of S.sobrinus.<sup>2</sup>

On comparing the mean differences of the control and probiotic group with respect to SM and SS count it was found that the increase in colony count was significantly

higher in the control group. Similar reduction in colony counts of SM was noted in other studies by Cagler et al, Ahola et al and Cildir et al. The reduction could be explained by a combination of local and systemic immune response as well as non-immunologic defence mechanisms. The principal health promoting effects are ascribed to enhancement of mucosal immune defence and macrophage activity as well as elevations of the numbers of killer cells, T-cells, and interferon. In the oral cavity, lactobacillus is quite acidogenic and may play a role in deep dentine caries progression rather than in the early enamel demineralization.<sup>38</sup> To be effective against oral infections, probiotic bacteria need to adhere to the oral mucosa and dental tissues as part of the biofilm and compete with the growth of dental pathogens. Hence it was stressed that the subjects should move the probiotic tablet around the mouth while melting them rather than simply swallowing them. Probiotics can create a biofilm, acting as a protective lining for oral tissues against oral diseases. Such a biofilm keeps bacterial pathogens off oral tissues by filling a space, pathogens would invade in the absence of the biofilm; and competing with cariogenic bacteria and periodontal pathogens growth.<sup>32</sup>

### Comparison with other studies

Our study is in agreement with the study conducted by Cagler et al which concluded that a short-term daily ingestion of lactobacilli-derived probiotics delivered by prepared straws or lozenges reduced the levels of salivary mutans streptococci in young adults.

Our study is in agreement with the study by cildir et al which concluded that Short-term daily consumption of fruit yogurt containing Bifido bacterium animalis subsp. lactisDN-

173010 may reduce the levels of mutans streptococci in saliva during orthodontic treatment with fixed appliances.

Our study was in agreement with a study performed by Jose je et al which concluded that The consumption of probiotic curd and the use of probiotic toothpaste cause a significant decrease in the S mutans levels in the plaque around brackets in orthodontic patients.

Our study was in disagreement with a study performed by Pinto GS et al which concluded that daily ingestion of yogurt with or without B. animalissubsp. Lactis for a period of 2 weeks was beneficial in reducing total microbial counts in dental plaque. No additional benefits were achieved by the use of the probiotic strain. However the control group was given yoghurt for 2 weeks unlike no intervention at all.

### Limitations of the study

However, the findings must, for a number of reasons be interpreted with caution. First, the sample size was limited and caries associated bacteria in saliva should be regarded as an intermediate end point for caries. It remains unclear whether or not this really is beneficial for patients. Furthermore, the semi-quantitative nature of the microbial estimations was a limitation. Second, there are no long-term studies available on the effect of probiotic bacteria on the oral microflora, and thirdly, the optimal daily dose is not yet established. Finally, head-to-head tests of different probiotic strains are still lacking and it is possible that a combination of probiotic strains could be even more effective. In any case, it seems obvious that adolescent patients with fixed orthodontic appliances constitute a very suitable group for further studies of risk patients concerning enamel mineralization and probiotic supplements. If successful, the probiotic homecare intervention may be a cost-effective alternative for white spot lesion prevention during orthodontic treatment.



Figure 1: Nature's bounty lactobacillus acidophilus probiotic tablets

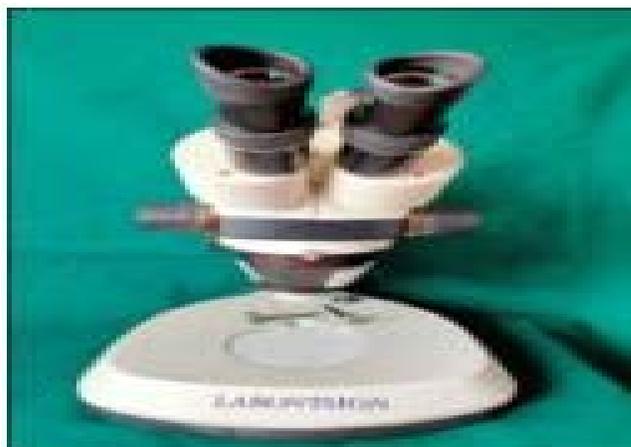


Figure 2: Stereomicroscope

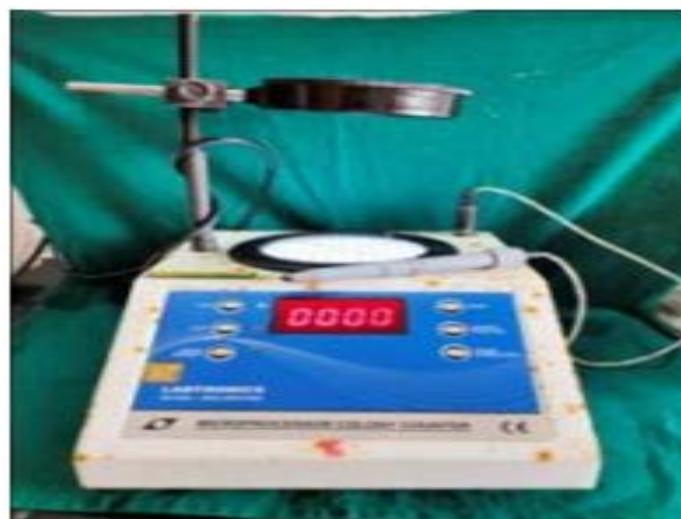


Figure 3: Colony counter



Figure 4: Eppendorf tube, Sterile swab, Petridish



Figure 7: Microbiological Incubator



Figure 5: Sodium acetate, L-cysteine, Sodium sulfite



Figure 8: Digital weighing apparatus



Figure 6: Colony formation after 2 days incubation



Figure 9: Streptococcus sobrinus (circular and opaque milky white and were surrounded by a milky white halo)



Figure 10: Streptococcus mutans (classical look of crushed frosted glass.)



Figure 11: Streptococcus mutans and sobrinus

### Conclusion

Within the limitations of this present study, the following conclusions can be drawn:

1. Probiotic Lactobacillus Acidophilus has significantly decreased the salivary Streptococcus mutans count.
2. Probiotic Lactobacillus Acidophilus has significantly decreased the salivary Streptococcus sobrinus count.
3. The total microbial count increased with the placement of fixed orthodontic appliances
4. SM count was more than that of the SS count on the lateral incisor bracket.

In conclusion, lactobacillus acidophilus can be considered in WSL management among orthodontic patients because

of its capacity to compete for binding with Streptococcus mutans and Streptococcus sobrinus, thereby reducing their counts in the oral micro flora.

However longer duration follow up after administration of lactobacillus acidophilus probiotic for its observable oral colonization, as well as microbiological assay of plaque biofilm after consumption of lactobacillus acidophilus in a larger sample size should be considered before extrapolating these findings in its clinical application of management of WSL.

### List of Abbreviations

% - Percentage

+/- - Plus or minus

$\mu$ L - microlitres

$^{\circ}$ C - degree celsius

et al - All others

MS- Mutan streptococci

SM - Streptococcus mutans

SS - Streptococcus sobrinus

WSL - White spot lesions

### References

1. Gorelick L, Geiger AM, John A. Incidence of white spot formation after bonding and banding. Am J Orthod. 1982;81:93–8.
2. Ahn SJ, Lim BS, Lee SJ. Prevalence of cariogenic streptococci on incisor brackets detected by polymerase chain reaction. Am J Orthod Dentofac Orthop. 2007;131(6):736–41.
3. Babaahmady KG, Challacombe SJ, Marsh PD, Newman HN. Ecological Study of Streptococcus mutans, Streptococcus sobrinus and Lactobacillus spp. at Sub-Sites from Approximal Dental Plaque from Children. Caries Res. 1998;32:51–8.
4. Hamada S, Slade HD. Biology, immunology, and cariogenicity of Streptococcus mutans. Microbiol Rev [Internet]. 1980;44(2):331–84. Available

- from:  
<http://www.ncbi.nlm.nih.gov/pubmed/6446023%0>  
[Ahttp://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=PMC373181](http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=PMC373181)
5. Fragkou S, Balasouli C, Tsuzukibashi O, Argyropoulou A, Menexes G, Kotsanos N, et al. Streptococcus mutans, Streptococcus sobrinus and Candida albicans in oral samples from caries-free and caries-active children. Eur Arch Paediatr Dent. 2016;17(5):367–75.
  6. Seki M, Karakama F, Terajima T, Ichikawa Y, Ozaki T, Yoshida S, et al. Evaluation of mutans streptococci in plaque and saliva: Correlation with caries development in preschool children. J Dent. 2003;31(4):283–90.
  7. Bishara SE, Ostby AW. White Spot Lesions: Formation, Prevention, and Treatment. Semin Orthod. 2008;14(3):174–82.
  8. Soet JJDE, Holbrookt WP, Magnusdottirt M, Graaff JDE. Longitudinal Study of Dental Caries. Microb Ecol Health Dis. 1993;6(1 993):237–43.
  9. Madison KM, Bowen WH, Pearson SK, Falany JL. Enhancing the Virulence of Streptococcus sobrinus in Rats. J Dent Res. 1991;70(1):38–43.
  10. Willmot D. White Spot Lesions After Orthodontic Treatment. Semin Orthod. 2008;14(3):209–19.
  11. Featherstone JDB. The science and practice of caries prevention. J Am Dent Assoc [Internet]. 2000;131(7):887–99. Available from: <http://dx.doi.org/10.14219/jada.archive.2000.0307>
  12. Shannon IL, West DC. Prevention of decalcification in orthodontic patients by daily self- treatment with 0.4% SnF2 gel. Pediatr Dent. 1979;1(2):101-2.
  13. Benson P, Shah A, Millett D, Dyer F, Parkin N, Vine R. Fluorides, orthodontics and demineralisation: a systematic review. Prim Dent Care. 2005;13(1):36–36.
  14. Øgaard B. White Spot Lesions During Orthodontic Treatment: Mechanisms and Fluoride Preventive Aspects. Semin Orthod. 2008;14(3):183–93.
  15. Reynolds EC. The Prevention of Sub-surface Demineralization of Bovine Enamel and Change in Plaque Composition by Casein in an Intra-oral Model. J Dent Res. 1987;66(6):1120–7.
  16. Leach S a, Lee G, Ldgjar WM. tin Human Enamel in sim byChewing Sorbitol Gum. 2015;
  17. Croll TP. Enamel microabrasion for removal of superficial dysmineralization and decalcification defects. J Am Dent Assoc. 1990;120(4):411–5.
  18. Welbury RR, Carter NE. The Hydrochloric Acid-pumice Microabrasion Technique in the Treatment of Post-orthodontic Decalcification. Br J Orthod. 2014;20(3):181–5.
  19. M.H. Anderson. A Probiotic Approach to Caries Management. Pediatr Dent. 2006;28(2):151–3.
  20. Hillman JD, McDonell E, Hillman CH, Zahradnik RT, Soni MG. Safety assessment of ProBiora 3 , a probiotic mouthwash: Subchronic toxicity study in rats. Int J Toxicol. 2009;28(5):357–67.
  21. Çaglar E, Kuscu OO, Cildir SK, Kuvvetli SS, Sandalli N. A probiotic lozenge administered medical device and its effect on salivary mutans streptococci and lactobacilli. Int J Paediatr Dent. 2008;18(1):35–9.
  22. Çaglar E, Cildir SK, Ergeneli S, Sandalli N, Twetman S. Salivary mutans streptococci and lactobacilli levels after ingestion of the probiotic bacterium Lactobacillus reuteri ATCC 55730 by straws or tablets. Acta Odontol Scand. 2006;64(5):314–8.
  23. Petersson LG, Magnusson K, Hakestam U, Baigi

- A, Twetman S. Reversal of primary root caries lesions after daily intake of milk supplemented with fluoride and probiotic lactobacilli in older adults. *Acta Odontol Scand.* 2011;69(6):321–7.
24. Ahola AJ, Yli-Knuutila H, Suomalainen T, Poussa T, Ahlström A, Meurman JH, et al. Short-term consumption of probiotic-containing cheese and its effect on dental caries risk factors. *Arch Oral Biol.* 2002;47(11):799–804.
25. Çaglar E, Onder Kusu O, 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.
26. Twetman S, Derawi B, Keller M, Ekstrand K, Yucel-Lindberg T, Stecksén-Blicks C. Short-term effect of chewing gums containing probiotic *Lactobacillus reuteri* on the levels of inflammatory mediators in gingival crevicular fluid. *Acta Odontol Scand.* 2009;67(1):19–24.
27. Cildir SK, Germec D, Sandalli N, Ozdemir FI, Arun T, Twetman S, et al. Reduction of salivary mutans streptococci in orthodontic patients during daily consumption of yoghurt containing probiotic bacteria. *Eur J Orthod.* 2009;31(4):407–11.
28. Çaglar E, Sandalli N, Twetman S, Kavaloglu S, Ergeneli S, Selvi S. Effect of yogurt with *Bifidobacterium* DN-173 010 on salivary mutans streptococci and lactobacilli in young adults. *Acta Odontol Scand.* 2005;63(6):317–20.
29. Jose JE, Padmanabhan S, Chitharanjan AB. Systemic consumption of probiotic curd and use of probiotic toothpaste to reduce *Streptococcus mutans* in plaque around orthodontic brackets. *Am J Orthod Dentofac Orthop* [Internet]. 2013;144(1):67–72. Available from: <http://dx.doi.org/10.1016/j.ajodo.2013.02.023>
30. Pinto GS, Cenci MS, Azevedo MS, Epifanio M, Jones MH. Effect of yogurt containing *bifidobacterium animalis* subsp. *lactis* DN-173010 probiotic on dental plaque and saliva in orthodontic patients. *Caries Res.* 2014;48(1):63–8.
31. Zhu Y, Xiao L, Shen D, Hao Y. Competition between yogurt probiotics and periodontal pathogens in vitro. *Acta Odontol Scand.* 2010;68(5):261–8.
32. Flichy-Fernández AJ, Alegre-Domingo T, Peñarrocha-Oltra D, Peñarrocha-Diago M. Probiotic treatment in the oral cavity: An update. *Med Oral Patol Oral Cir Bucal.* 2010;15(5):677–80.
33. Pellegrini M, Pouncett J, Jay M, Pearson MP, Richards MP. Tooth enamel oxygen “isoscapes” show a high degree of human mobility in prehistoric Britain. *Sci Rep* [Internet]. 2016;6:1–9. Available from: <http://dx.doi.org/10.1038/srep34986>
34. Saravia ME, Nelson-Filho P, Ito IY, da Silva LAB, Da Silva RAB, Emilson CG. Morphological differentiation between *S. mutans* and *S. sobrinus* on modified SB-20 culture medium. *Microbiol Res.* 2011;166(1):63–7.
35. Millette M, Luquet FM, Ruiz MT, Lacroix M. Characterization of probiotic properties of *Lactobacillus* strains. *Dairy Sci Technol.* 2008;88(6):695–705.
36. Tahmourespour A, Kermanshahi RK. The effect of a probiotic strain (*Lactobacillus acidophilus*) on the plaque formation of oral streptococci. *Bosn J Basic Med Sci.* 2011;11(1):37–40.
37. Gwinnett AJ, Ceen RF. Plaque distribution on

bonded brackets: A scanning microscope study.

Am J Orthod. 1979;75(6):667-77.

38. Caglar E, Kargul B, Tanboga I Article R. Bacteriotherapy and probiotics' role on 2005.pdf. 2005;131-7.