Consumption of Yogurt Containing Probiotic Bifidobacterium lactis Reduces Streptococcus mutans in Orthodontic Patients

Armelia Sari Widyarman1, Shirley Trisna Yunita1, Tjokro Prasetyadi2

1 Department of Microbiology, Division of Oral Biology, Faculty of Dentistry, Triakti University – Indonesia
2 Department of Orthodontic, Faculty of Dentistry, University of Prof. Dr. Moestopo – Indonesia

*Corresponding Author: Armelia Sari Widyarman, Faculty of Dentistry, Triakti University – Indonesia.
Email: armeliasari@trisakti.ac.id

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ABSTRACT

Background: Probiotic bacteria is commonly used as a food supplement intended to benefit the host by improving intestinal bacterial balance. Probiotics have also been investigated from the perspective of oral health. Objectives: The purpose of this study was to investigate the effect of daily intake of yogurt containing probiotic Bifidobacterium animalis subsp. lactis BB-12 (B. lactis) on salivary Streptococcus mutans (S. mutans) counts in patients undergoing fixed orthodontic treatment. Methods: Saliva samples were collected from each subject (n = 7; mean age, 21 years) using spitting method in centrifuge tubes at baseline and two weeks after daily probiotic yogurt consumption. B. lactis BB-12 and S. mutans ATCC 25175 were cultured in BHI-broth (37ºC, anaerobic conditions). After 48 hours incubation, the number of colonies on each dilution plate was used to extrapolate a standard curve. The total number of target DNA molecules were identified using Real-Time PCR followed by SYBR Green reagents and 16S rRNA gene specific primers S. mutans and B. lactis BB-12. Data were analyzed statistically using paired-sample t-tests. Results: Statistical evaluation indicated that there was a significant reduction in the presence of S. mutans before probiotic yogurt consumption, (4.73 ± 1.43) log10 CFU/mL and after two weeks of daily consumption of probiotic yogurt, (4.03 ± 0.77) log10 CFU/mL, p = 0.001. Moreover, no B. lactis was found in the saliva of any of the subjects before probiotic consumption, but after two weeks of consumption, B. lactis was found in the saliva of four subjects. Conclusions: Consuming probiotic yogurt containing B. lactis reduced the quantity of S. mutans in the saliva of subjects during fixed orthodontic treatment. Thus, the probiotic bacteria could be beneficial in improving oral health.

Keywords: Bifidobacterium lactis, orthodontic subject, probiotic, saliva, Streptococcus mutans

Background

Even though there have been advances in orthodontic appliances and treatment protocols, white spot lesions due to enamel demineralization are still serious concerns to orthodontists and patients. The prevalence rates of white spot lesions among orthodontic patients have been reported to be between 4.9% and 84%.
White spot lesions are caused by *Streptococcus mutans*, which are commonly found on teeth during and after orthodontic treatment.1 Fixed orthodontic appliances have been found to induce specific changes in the oral environment such as demineralization due to plaque accumulation.2 The complex design of orthodontic bands and brackets may create an ecological environment that facilitates the establishment and growth of cariogenic *Streptococcus mutans.*3 Plaque bacteria ferment carbohydrates to produce acid and reduce the plaque pH to 4.5-5.0 that caused demineralization.4 One recent strategy to prevent demineralization and dental caries is the ingestion of probiotic bacteria.5 Metchnikoff was the first to state that probiotics could provide health benefits.6 Probiotics have been associated with gut health, and most clinical interest has focused on the prevention or treatment of gastrointestinal infections and diseases. However, several investigators have also suggested the use of probiotics for oral health purposes.7 The World Health Organization has defined probiotics as “live microorganisms which, when administered in adequate amounts, confer a health benefit on the host.”8 Probiotics may also prove useful for the prevention of oral diseases, including caries and periodontal disease.9,10 The aim of the present study was to investigate the effect of yogurt consumption containing probiotic *Bifidobacterium animalis* subsp. *lactis* BB-12 (*B. lactis*) on salivary *Streptococcus mutans* (*S. mutans*) counts in patients undergoing orthodontic treatment with fixed appliances.

**Material and Methods**

This study was approved by the Faculty of Dentistry, Trisakti University Ethics Committee under number: 181/KE/FKG/2015. Salivary samples were collected from subjects (orthodontic patients) (*n* = 7; mean age, 21 years) before and after two weeks of daily probiotic yogurt consumption (100 mL/bottle, 1x10⁹ CFU/mL). Saliva were collected by spitting method into a 15 mL centrifuge tube until 2 mL saliva obtained in each tube. The collected saliva samples are stored in a cooler box and placed in laboratory refrigerator at -20°C.

**Bacterial Culture**

*B. lactis* BB-12 and *S. mutans* ATCC 25175 were cultured in brain heart infusion (BHI) broth (Thermo Scientific, USA) at 37°C for 48 hours under anaerobic conditions (10% CO₂, 10% H₂, 80% N₂). The standard plate count method was used to determine bacterial concentration by the serial dilution technique. The number of bacterial cells on each dilution was used to extrapolate the bacterium number from a standard curve of *S. mutans* and *B. lactis* BB-12.

**DNA Extraction**

DNA samples were extracted from saliva and cultured bacteria using the heat-shock method. The samples were centrifuged at 4500 x g for 15 minutes at 4°C and pellets were washed with phosphate buffer saline (PBS). An aliquot of 100 μL of cell suspension containing 10⁸ cells/mL of *B. lactis* BB-12 and *S. mutans* ATCC 25175 were transferred to microtubes and centrifuge at 10.000 x g for 10 minutes at 4°C. The suspension was incubated at 99°C in a boiling water-bath for 20 minutes and vigorously homogenized by vortex for 10 seconds and the tube was frozen on ice (0°C) for 10 minutes. Subsequently, centrifugation was done at 10.000 x g for 2 minutes and supernatant was carefully pipetted into new 1.5 mL microcentrifuge tubes. The suspension containing DNA sample was stored at -20°C.

**qPCR**

The total amount of target DNA was identified and quantified using Real-Time PCR with SYBR Green (Applied Biosystems, USA) and 16S rRNA gene specific primers for *B. lactis* and *S. mutans*. The sequences of forward and reverse primers for *B. lactis* in this study were 5’-CCT TAC CTG GGC TTG ACA TGT-3’ and 5’-GAC GTA AGG GGC ATG ATC-3’. The sequences of forward and reverse primers for *S. mutans* were 5’-ACT ACA CTT TCG GGT GGC TTG G-3’ and 5’-CAG TAT AAG CGC CAG CAG TTT CAT C-3’.11 The reaction mixture in 20 μL volume contained: 5 μL nuclease free water (NFW), 10 μL SYBR Green reagent, 1 μL of each primers, and 3 μL
template DNA. Amplifications were done with the following temperature profiles. Initial template denaturation step at 95°C for 10 minutes (1 cycle), followed by 40 cycles of 94°C for 15 seconds and annealing at 60°C for 1 min, 95°C for 15 seconds. The Real-Time PCR was performed in triplicate. Quantitation was done using standard curves made from known concentrations of DNA containing the respective amplicon for each set of primers.

Statistical Analysis

Differences between the experimental groups were analyzed using paired samples T-test. A $p$-value less than 0.05 ($p < 0.05$) was considered statistically significant. Shapiro Wilk test was further used to test for normality and Levene’s test was used to test for homogeneity of variance previously. Data were analyzed using SPSS Statistics for Windows software version 20 (IBM, USA).

Results

The numbers of salivary $S. mutans$ present in pre- and post-probiotic consumption samples in all subjects are shown in Table 1 and Fig. 1. After the test period (2 weeks) of probiotic yogurt consumption, the total number of $S. mutans$ counts in the saliva of five subjects showed a significant decrease compared to the baseline values ($p < 0.05$). The levels of $B. lactis$ BB-12 in pre- and post-probiotic consumption samples for all subjects are given in Fig. 2. Before consumption of probiotic yogurt, no subjects showed any $B. lactis$ colonies. However, after 2 weeks of consumption, the saliva samples of four subjects displayed a number of $B. lactis$ colonies. Statistical evaluation showed that there was a significant difference in the average number of $S. mutans$ before probiotic consumption (4.73 ± 1.43) log10 CFU/mL and after two weeks of daily probiotic yogurt consumption (4.03 ± 0.77) log10 CFU/mL, $p < 0.05$. The results showed that probiotic yogurt consumption brought about a statistically significant reduction in salivary $S. mutans$ in fixed orthodontic patients compared to the baseline.

Figure 1. Comparison scores of $S. mutans$ on each subject with a fixed orthodontic appliance, baseline and after two weeks of daily consumption of probiotic yogurt.
Table 1. Distribution of salivary *S. mutans* in adolescents with a fixed orthodontic appliance, baseline and after two weeks of daily consumption of probiotic yogurt.

<table>
<thead>
<tr>
<th>Subject</th>
<th><em>Streptococcus mutans</em> score, CFU (colony forming unit)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Baseline</td>
</tr>
<tr>
<td>1</td>
<td>$3.55 \times 10^4$</td>
</tr>
<tr>
<td>2</td>
<td>$1.08 \times 10^5$</td>
</tr>
<tr>
<td>3</td>
<td>$4.68 \times 10^4$</td>
</tr>
<tr>
<td>4</td>
<td>$1.06 \times 10^2$</td>
</tr>
<tr>
<td>5</td>
<td>$5.33 \times 10^6$</td>
</tr>
<tr>
<td>6</td>
<td>$3.11 \times 10^4$</td>
</tr>
<tr>
<td>7</td>
<td>$4.38 \times 10^5$</td>
</tr>
</tbody>
</table>

**Figure 2.** Comparison scores of *B. lactis* for each subject with a fixed orthodontic appliance, baseline and after 2 weeks of daily consumption of probiotic yogurt.
Discussion

Orthodontic patients wearing fixed appliances can experience higher risk of dental caries during orthodontic treatment. It is well established that the prevalence of S. mutans is increased in plaque and saliva during treatment with fixed orthodontic appliances, which may lead to white spot lesion formation. The intervention started 6 months after the insertion of the appliances to avoid the confounding effect of an immediate decrease in bacterial counts that may take place at appliance insertion. The counts of bacteria were estimated using Real-Time PCR in which have a high sensitivity and accuracy in amplification reaction. B. lactis have also the qualified presumption of safety (QPS) status of the European Food Safety Authority, and they are generally recognized as safe (GRAS) by the FDA in the US.

Our results showed that daily consumption of probiotic yogurt for two weeks decreased the S. mutans counts in saliva and reinforced previous findings regarding bifidobacteria-derived probiotics. In accordance with previous studies that have been performed to validate the survival and positive effects of the probiotic bacteria B. lactis BB-12, one study reported by Caglar et al. showed that short term consumption of yogurt containing B. lactis DN 173010 affected the salivary levels of S. mutans during orthodontic treatment with fixed appliances. A similar effect was found in a study carried out by Cildir et al., in which 24 healthy children undergoing orthodontic treatment showed a statistically significant reduction in salivary levels of S. mutans after received probiotic B. lactis DN 173010 (2 x 10^8 CFU/g).

Probiotic-containing foods such as yogurt drink or ice cream can be attractive vehicles for probiotic intake, delivering both health-promoting and mood-boosting effects. Another study using ice cream containing B. lactis BB-12, if eaten daily for 10 days, has been shown to lead to significant reductions in S. mutans. In some studies, it is conceivable that the means of administration might positively affect the effects observed as related to S. mutans reduction. There is agreement between the results of the studies mentioned above and our study in that probiotic B. lactis may reduce salivary levels of S. mutans. Our results showed that the saliva of five subjects had decreased levels of S. mutans. However, saliva samples from two subjects displayed increased levels of S. mutans, this may have occurred due to the subject habits such as sugar dietary consumption or lack of tooth brushing. With regard to B. lactis, only four subjects displayed B. lactis colonies after two weeks of daily yogurt consumption.

The discrepancies in these results indicate that the data should be interpreted with caution. First, the severity of caries may affect the role of probiotic bacteria such as B. lactis. In the oral cavity, bifidobacteria may play a role in deep dentine caries progression but not in enamel demineralization. Thus, resident oral bifidobacteria are able to colonize the carious dentine depending on the local environmental conditions, that is, poor oral hygiene and frequent intake of cariogenic foods. Second, there are no long-term studies available on the effect of probiotic bacteria on oral microflora, and third, the dosage of probiotic organisms for long term or synergetic effects on oral health still needs to be explored. In any case, the application of probiotic strains of bacteria to prevent or slow the progression of dental caries showed promising results, even though only a few studies have demonstrated clear clinical outcomes. Several studies in the literature have shown the effect of yogurt-based probiotics on S. mutans in the saliva of orthodontics patients, and these studies indicate that subjects with large amounts of S. mutans were reduced from 63% to 21% after 2 weeks of consumption. Further research on adolescent patients with fixed orthodontic appliances are still required to determine whether probiotic supplements can lower the risk of patients concerning enamel mineralization. If successful, probiotic home-care intervention may become an alternative treatment for enamel demineralization during orthodontic treatment. Such studies may provide evidence to clinicians for recommending to patients the consumption of probiotics in addition to “classical” oral hygiene practices and dietary counseling.
Conclusion

In conclusion, this study shows the potential of probiotic yogurt containing B. lactis BB-12 to reduce the levels of S. mutans as pathogenic bacteria in saliva during fixed orthodontic treatment. Therefore, consumption of probiotic yogurt containing B. lactis BB-12 may be useful to improve oral health. Further clinical studies with other oral bacterial strains are still needed to verify and explore this result.

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Conflict of Interest

The authors declare that there are no conflicts of interest.

References


