Evaluation of salivary levels of *Streptococcus mutans* pre- and post-probiotics use

Priya Nimish Deo, Revati Deshmukh

Department of Oral and Maxillofacial Pathology, Bharati Vidyapeeth Dental College & Hospital, Pune, Maharashtra, India

**Abstract**

**Background:** Probiotics are “live microorganisms which when administered in adequate amounts confer a health benefit to the host.” They are thought to play a role in the maintenance of oral health.

**Aim:** The aim was to evaluate the effect of probiotics on the salivary levels of *Streptococcus mutans*.

**Materials and Methods:** Fifty subjects within the age group of 18-30 years were selected for study. 2 ml of unstimulated saliva was collected before and after 7 days of administration of probiotic (65 ml once daily) to the participant. Mitis salivarius bacitracin agar was used as culture media. Yakult probiotic, fermented milk containing over 6.5 billion *Lactobacilli casei* strain was used. The colony forming unit of *S. mutans* was calculated by surface count method, and the total colony count was calculated by consideration of the dilution factor. The mean salivary levels of *S. mutans* pre- and post-probiotics use were calculated. Paired sample t-test was used for statistical analysis.

**Results:** The study revealed a statistically significant reduction in the salivary levels of *S. mutans* after the use of probiotics.

**Conclusion:** This bacterio-therapy, which is a novel concept, is a non-invasive method for combating infection and dental caries. More research is needed to evaluate the effect of Probiotics on oral health.

**Keywords:** Dental caries, probiotics, *Streptococcus mutans*
To evaluate salivary levels of *S. mutans* after use of probiotics.
To compare the levels of *S. mutans* before and after the use of probiotics.

**Materials and Methods**

This study was carried out in the Department of Oral Pathology & Microbiology, Bharati Vidyapeeth Deemed University, Dental College & Hospital Pune. Ethical clearance was obtained from the ethical committee, Bharati Vidyapeeth Deemed University, Dental College & Hospital, Institutional Ethical Committee Pune. Written informed consent was obtained from the participants prior to the study. Fifty subjects within the age group of 18-30 years were selected for study.

**Materials used for saliva collection**

1. Sterile bottles with markings to collect the required amount (2 ml) of saliva.
2. Phosphate buffer saline (PBS) as the collecting medium for saliva.

**Materials used for the laboratory procedure**

1. *Mitis salivarius* bacitracin agar with potassium tellurite and bacitracin.
2. Agar plates
3. Calibrated loop
4. Anaerobic jar

**Saliva sampling and Microbiological study**

Before collection of the salivary sample, 2 ml of PBS (which is the collecting media for saliva) was autoclaved. 2 ml of unstimulated saliva from each participant was collected in a sterile container as soon as the participant reported to the department and the same quantity of salivary sample was collected after 7 days of administration of probiotic (65 ml once daily) to the participant. The salivary samples of the participants were identified by giving a code number which was written on the collecting bottle during sample collection and processing [Figure 1a]. The same code was used for that particular subject during the subsequent sample collection (after 7 days of probiotic administration). The collected salivary sample was then transported to the laboratory immediately and processed within 6 h. The sample was vortexed for 15 s and then inoculated using *mitis salivarius* bacitracin agar which is specific for *S. mutans* [Figure 1b]. Incubation was carried out in an anaerobic jar (5% CO₂) at 37°C for 48 h.

On culture, *S. mutans* appears as raised, convex, opaque, undulate, pale blue colonies showing granular appearance with a glistening bubble on the surface of the colony. This is thought to be due to increased production of glucan from sucrose [Figure 2].

After 48 h, colony forming units (CFUs) on the plate were counted, and the final count for the sample was calculated by considering the dilution. *S. mutans* count for the same participant was calculated after the administration of probiotics for 7 days and difference between the two was recorded using paired sample t-test.

**Results**

The CFU of *S. mutans* was recorded by surface count method, and the total colony count of *S. mutans* in the salivary sample was calculated by taking into consideration the dilution factor using the formula

**Total colony count calculation** – Number of colonies counted by surface plate method on the petri dish × dilution factor × 200

(for 2 ml volume of saliva)

The mean salivary levels of *S. mutans* before and after the administration of probiotics are given in Table 1. After 48 h, colony characteristics were studied, and the number of CFU of *S. mutans* (CFU/ml) of saliva was determined.

**Figure 1:** (a) Sample collection. (b) Culture media used (*mitis salivarius* bacitracin agar, potassium tellurite and bacitracin)

**Figure 2:** Streptococcus *mutans* count before and after administration of probiotics
The mean salivary levels of *S. mutans* at baseline were 10782 ± 1791.890. When compared after 7 days mean salivary levels of *S. mutans* was 10156 ± 1615.909 [Graph 1]. This study revealed a statistically significant reduction (P ≤ 0.03) in the salivary levels of *S. mutans* after 7 days administration of probiotics [Table 2].

This bacteriotherapy is a novel and promising concept for combating infection and preventing dental caries.

**Discussion**

Dental caries is a complex disease of bacterial origin that causes demineralization of enamel. It appears following changes in the homeostasis of the oral ecosystem leading to the proliferation of the bacterial biofilm, composed notably of streptococci from the mutans group. To have a useful effect in limiting caries, a probiotic should be able to stick to the tooth surface and become a part of the bacterial colonies which make up the biofilm. Finally, metabolism of food-grade sugars by the probiotic should result in low acid production. The benefits of adding probiotics into dairy products are in their ability to neutralize acidic conditions. It has been reported that cheese prevents the demineralization of enamel and promotes its remineralization.[6]

*S. mutans* are Gram-positive cocci, facultative anaerobes and are found in the oral cavity, which are proved to be initiating tooth decay. They are mesophilic and grow between 18 and 40°C. They break down sugar for energy and produce an acidic environment, which demineralizes tooth structure. *S. mutans* has three virulence factors; water-insoluble glycans, acid tolerance, and production of lactic acid.[7]

*S. mutans* is one of the major and most virulent of the caries causing microorganisms. It accumulates on the surface of the tooth and forms a biofilm by the ability to produce extracellular polysaccharides from sucrose, mainly water-insoluble glucan, with the help of enzyme glucosyltranferase. Inhibiting the colonization of *S. mutans* on the tooth surface can help to prevent biofilm formation and development of dental caries.[8]

As *S. mutans* is the main etiological factor for causing dental caries, the reduction of salivary levels of *S. mutans* can help reduce the incidence of dental caries.

Earlier, probiotics were associated with only gut health but recently several investigators have suggested their potential applicability in the improvement of oral health. Probiotics have amazingly come up with potential for not only preventing the attack of oral pathogens but also the ability to treat various oral diseases thus, assuring healthy living and increased longevity (Meurman, 2005). Probiotics reminds of the very old and forgotten concept of “Bacterio-therapy” which stated that beneficial bacteria occurring naturally in the human body can be administered in the patient’s body to restore health and well-being (Meurman, 2005). Similar organisms capable of adhering to and colonizing the surface of the oral cavity constitute oral probiotics.[9]

The mechanism of action of Probiotics in the oral cavity may be production of various anti-microbial substances, organic acids, hydrogen peroxide, carbon peroxide, biosurfactants. They also compete with pathogens for adhesion sites and are involved in the metabolism of substrates. These probiotics play a role in immunomodulation by stimulating non-specific immunity and by modulating humoral and cellular immune response. They also modify oral conditions by modification of oxidation-reduction potential and by modulating pH[8] [Table 1].

Probiotic bacterial adhesion to oral soft tissues is another aspect that promotes their health effect to the host. Cell adhesion is a complex process involving contact between the bacterial cell and interaction with surfaces. The epithelial lining of the oral cavity despite its function as a physical barrier, actively participates in immune response. It has been shown that probiotic bacteria can stimulate local immunity and modulate the inflammatory response. Lactobacilli as well as other Gram-positive bacteria express ligands for toll-like receptors which initiate immune responses enabling detection of both pathogens and indigenous microbiota by epithelial cells. Recognition of commensal bacteria

---

**Table 1**: The possible mechanisms of action of probiotics in the oral cavity

<table>
<thead>
<tr>
<th>Production of various substances</th>
<th>Binding of microorganisms in the oral cavity</th>
<th>Immuno-modulation</th>
<th>Modification of oral conditions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Antimicrobial substances</td>
<td>Compete with pathogens for metabolic end products</td>
<td>Stimulate immunity</td>
<td>Modification of oxidation conditions</td>
</tr>
<tr>
<td>Organic acids</td>
<td>Involvement in metabolism</td>
<td>Modulate potential</td>
<td>Modulating humoral and cellular immune response pH</td>
</tr>
<tr>
<td>Hydrogen peroxide</td>
<td>(competing with oral micro-organisms)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Carbon peroxide</td>
<td>of substrates</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diacetyl</td>
<td>(competing with oral substrates available)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Biosurfactants</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bacteriocins</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Table 2**: Comparison of mean salivary levels of *Streptococcus mutans* pre- and post-probiotic use

<table>
<thead>
<tr>
<th></th>
<th>Mean</th>
<th>Standard error mean</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pre-probiotic</td>
<td>10782.00</td>
<td>1791.890</td>
</tr>
<tr>
<td>Post-probiotic</td>
<td>10156.00</td>
<td>1615.909</td>
</tr>
</tbody>
</table>

P≤0.03=Statistically significant

Graph 1: Comparison of mean salivary levels of *Streptococcus mutans* pre- and post-probiotic use
by these receptors is necessary for homeostasis, epithelial cells protection from injury and repair stimulation.\(^{[11]}\)

A good probiotic should be a strain, which is capable of exerting a beneficial effect on the host animal, e.g., increased growth or resistance to disease. It should be non-pathogenic and non-toxic. It should be present as viable cells, preferably in large numbers. It should be capable of surviving and metabolizing in the gut environment. It should be stable and capable of remaining viable for periods under storage and field conditions.\(^{[12]}\)

Lactobacilli are the most common probiotic bacteria associated with the human gastrointestinal tract; therefore, it may also play an important role in the ecophysiology of the oral microbiota.\(^{[13]}\)

It has come to be accepted that Probiotic products should have a minimum concentration of $10^6$ CFU/ml or gram. A total of $10^8$ to $10^9$ probiotic microorganisms should be consumed daily for the beneficial probiotic effect.\(^{[14]}\)

Sohn et al. found that there was a link between the consumption of carbonated drinks and incidence of dental caries in children.\(^{[15]}\) Considering the increase in the consumption of carbonated drinks, it is the need of time to find more noninvasive and preventative measures for controlling dental caries.

The treatment of disease with the use of bacteria – Bacteriotherapy is a unique concept and can be used to improve oral flora and prevent dental caries.

The findings of our study revealed a significant reduction in the salivary levels of $S. mutans$ after 7 days of probiotic administration.

**Conclusion**

The use of probiotics for reducing dental caries may be non-invasive and can act as a preventive measure. Previously these Probiotics have been shown to improve gut health, and now their effect on the oral health has also been studied extensively. It is necessary to create more awareness among the dentists and the general population so that people can utilize this bacteriotherapy which is a novel concept and can help to reduce dental caries. More research and development are needed in this area to have a better understanding of these micro-organisms so that we human beings get their potential benefits and can act as an adjunct to control oral pathoses.

**Acknowledgment**

The authors thank Dr. Mrs. Rama Bhadekar, Associate Professor, HOD, Rajiv Gandhi Institute of Information Technology and Biotechnology, Pune for her cooperation and guidance.

**References**