Biotherapeutic Potential of *Lactobacillus* Probiotic Strains on *Streptococcus mutans* Biofilm in Dental Caries—Pathogenesis Revisited

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**ABSTRACT**

This review gives an insight on the anticariogenic role of each of the individual strains of *Lactobacillus* probiotics on *Streptococcus mutans* bacterium associated with dental caries and also attempts to emphasize the commercially approved oral probiotic products using *Lactobacillus* strains exclusively, against dental caries. Probiotics in bacteriotherapy have been an emerging strategy in the management of ‘Dental caries’. Probiotics, protect the tooth surfaces from the competing microbial pathogens in the mouth. *Lactobacillus* species despite its acidogenicity has taken a breakthrough in the intervention of dental caries due to its acid buffering capacity with dairy product administration and as late colonizers for dental caries. This article compiled the pathogenesis of individual *Lactobacillus* strains of probiotics in a well-illustrated manner on *S. mutans* and also the list of the commercial products of oral probiotics with exclusive *lactobacillus* strains on *S. mutans* and its effect on dentistry. Probiotic formulations and recent strategies with future perspectives are emphasized. Also, the role of individual *Lactobacillus* strains and the associated commercial products against dental caries have been compiled to give additional knowledge regarding the changing strategic approach towards dental caries from the laboratory and clinician point of view. Restorative treatment strategies for dental caries act against both disease causing microbial species and commensal of the oral cavity resulting in undesirable adverse effects. As there is a need to switch to probiotics as a natural therapeutic approach, this review has sheds light on proposed pathogenesis, commercial products and future perspective that will promote researchers to work on new *Lactobacillus* probiotic formulations with greater efficacy in overcoming the emerging multispecies generating with time in caries.

**Keywords:** Biofilm, Dental caries, *Lactobacillus*, Probiotics, *Streptococcus mutans*.

**BACKGROUND**

The oral cavity is a complex habitat of abundant diversity with multivariate microbiome species. According to the *Global Burden of Disease Study 2017*, dental caries of permanent dentition is considered to be the most common oral disease seen. Internationally, 2.3 billion people and more than 530 million children are affected by dental caries in primary teeth.1 Dental caries (Tooth decay) occurs as a complex disease resulting from an ecological imbalance of oral microbiome with interactions in the host tissues. Among 700-1000 microbial species (predominantly facultative and anaerobic bacteria), micro-organisms belonging to *Streptococcaceae* and *Lactobacillaceae* family; *Actinomyces* spp., *Atopobium* spp., *Bifidobacterium*, nonmutan *Streptococcus*, *Propionibacterium* spp., and *Veillonella* spp., have been primarily associated with dental caries.2 Dental caries occurs as an interaction between cariogenic micro-organisms, fermentable carbohydrates (quality and quantity), time and host factors (saliva and Gingival Crevicular Fluid [GCF]). Considerable efforts are being taken by dental practitioners to reduce the overall prevalence of dental caries by preventive (fluoride supplementation), restorative (fillings, crowns and root canal treatments) or surgical therapy (extractions). These modes of treatment target both disease producing and normal micro-flora of the oral cavity causing undesirable adverse effects. Considering this problem, it’s important to focus on oral healthcare products for selective inhibition of pathogenic microorganisms by restoring normal microbial ecological balance.
in the mouth. Management for oral diseases must be targeted either to attack microbial biofilm directly or the target molecules/ processes involved in the adhesion mechanism of microbes over the host surface.

In the last few years, intense research has shown the positive impact on introducing new oral healthcare products targeting the maintenance of the beneficial community of microbiome in the human body. Probiotics are live micro-organisms that when administered in adequate amounts; confer a health benefit on the host. To date, ongoing studies have evaluated the effects of the outgrowth of non-pathogenic strains of bacteria (termed Probiotics), as a novel concept in the gut flora. The term ‘Probiotic’, denotes ‘for life’, and is derived from the Greek language. In 1907, probiotics was first recorded by Ukrainian-born Nobel laureate, Russian bacteriologist, Dr. Metchnikoff who considered it as “fight microbe with a microbe”. This had shown marked improvement in the gastrointestinal mucosa by inhibiting the growth of pathogens. While in 1965, Probiotics was first introduced by Lilly and Stillwell as “Substances secreted by one microorganism that stimulates the growth of the other”. Although several studies revealed that probiotics have an important role in the prevention of dental caries. Selection of probiotics’ in oral health care products for dental caries that exhibit completely positive effects, should take into consideration the biotherapeutic effect; cost and physical and chemical properties of bacterial strains, which still remain a challenge to mankind.

Lactic Acid Bacteria (LAB) have been ‘Generally Regarded as Safe (GRAS)’ with no reported negative effects after long usage in fermented food items. Among the accepted groups of probiotics for oral health, Lactobacillus species despite its acidogenicity has taken a major breakthrough for therapeutic intervention for dental caries due to its acid buffering capacity with dairy product administration and as late colonizers for dental caries. However, research is still ongoing towards the discovery of new probiotics with a definite dosage and combination (probiotic formulations) that conferred maximum benefit in the host. This review sheds light on the possible mechanism of action and observed effects of individual strains of Lactobacillus probiotics on oral micro-organism Streptococcus mutans biofilm in dental caries. This article also emphasizes currently approved oral Lactobacillus stains exclusively, against dental caries.

‘Dental Plaque Biofilm’ Vs ‘Dental Caries’

‘Dental Plaque Biofilm’ is described as a microbial community with a complex plethora of microorganisms ranging from opportunistic pathogens to definitive pathogens conglomerating into a mass on a tooth or tissue surface. These microbial species exist as a sessile community on the oral surfaces of normal mucosa or pathology, within a matrix of polymeric substances of bacterial or salivary origin. This is formed via an ordered sequence of events, resulting in a structurally and functionally organized species-rich microbial biofilm. Based on the bacterial species, microbes contribute 10-25% while exo-polysaccharides form 75-90% of the oral biofilm.

The Human Microbiome Project by the National Institute of Health (NIH) has demonstrated at least nine biological niches of dental biofilm at specific sites with distinct characteristics in the oral cavity. They include mucosa of the mouth, hard palate, keratinized gingiva, palatine tonsils, supragingival and subgingival plaque, saliva, throat and dorsal surface of the tongue. Resident consortia on various sites in the oral cavity differ in their composition, establishment and metabolic activity due to constant interactions with environmental factors like saliva and GCF. Of importance, bacterial species in the oral biofilm are stated to be less sensitive to anti-microbial agents and host-defense mechanisms. Normally, these oral microbes adhere to the tooth immediately following mechanical cleansing via three critical steps including (i) initial attachment of the tooth surface by pioneer bacteria (bacterial adhesins and proline-rich polysaccharides), (ii) co-adhesion and co-aggregation of the microbes with different bacteria (polypeptides, carbohydrates and nucleic acids) and (iii) propagation of the bacteria via nutrients and genetic components. Biological disparity in this oral microbiome due to multiple host factors and environmental disturbances in the mouth can contribute to the development of dental caries.

‘Dental caries’ therefore refers to a multifactorial disease that occurs due to the imbalance between the mineralization of dental hard tissue and oral microbial biofilm surrounding tooth and tissue surfaces in the mouth. As time progresses, dental plaque biofilm matures and undergoes calcification due to the deposition of minerals from host saliva to form a yellowish substance termed ‘Calculus’ or Tartar’ formation around the teeth. Mature oral biofilm occurs after a series of step-wise processes from the adhesion of planktonic cells to single- or mixed bacterial communities. An increase in the thickness and complexity of this dental plaque biofilm results in damage to the teeth and surrounding tissues causing dental caries.

Role of S. mutans Biofilm in Dental Caries

Oral health shows positive effects as long as a symbiosis between microorganisms with oral ecosystem according to the ‘Ecological Plaque Hypothesis’ is followed. Slight disruption (dysbiosis) in the interactions between oral microbial species is sufficient to result in ‘Dental Caries’ pathology. According to ‘Chemo-parasitic theory’ by Miller, Dental Caries occur primarily due to the combination of key factors such as micro-organisms, host tissue, dietary sugars and time added later. In addition to these basic factors, depicted risk factors appear to play a major role in altering the progression of caries pathology (Figure 1).
Amongst the diversified population of the oral microbiome, *S. mutans* is a facultative, anaerobic and gram-positive bacteria that have gained widespread attention as they appear to be the most predominant and early colonizer associated with etiology of dental caries. *S. mutans* constitutes about 20% of all oral bacteria. The cariogenic potential of *S. mutans* is mainly attributed to (i) The capability to produce large quantities of extracellular polymers of glucan and mutans from sucrose that aid in the permanent colonization of hard surfaces and in the development of the extracellular polymeric matrix in situ; (ii) The ability to transportation and metabolize a wide range of carbohydrates into metabolic end product such as lactic acid (acidogenicity) and (iii) The ability to survive under conservational strain conditions (low pH). As the disease progresses, at the molecular level it has been observed that, these acidogenic and acidicuric *S. mutans* are known to exhibit a switch of genes that allows themselves to outgrow other bacterial species under favourable oral health conditions (Figure 2).

Over a decade ago, according to the 'Specific plaque hypothesis' by Loesche Dental caries was considered to be an infectious disease associated with specific micro-organisms that were eliminated using appropriate antimicrobial treatment. Recently, literature stated that dental caries supports the polymicrobial concept with multiple microbes involved in different stages of the disease pathology. Apart from *S. mutans*, a few strains of the genus *Lactobacillus* namely, *L. gasseri*, *L. fermentum* and *L. salivarius* considered commonly as LAB have been proven to be associated with advanced stages of dental caries. This chain reaction continues with an increase in acid production resulting in demineralization and dissolution of hard tissues subsequently causing dental caries.

**Probiotics – Does it stop Dental Caries?**

To date, several studies have focused on treating caries through various modes of treatment strategies and a few preventive methods. However, due to the biological properties of the bacteria and the multifactorial nature of dental caries, preventive methods have shown to be less promising. To overcome this, efforts have been ongoing in order to introduce methods that maintain adequate diversity in the bacterial population by preventing dysbiosis and pathology in the oral cavity.

The introduction of probiotics on *S. mutans* biofilm for symbiosis, reduces the negative effects of the pathogenic bacteria and helps in maintaining a strong positive balance in the oral microbiome essential for general oral health. Overall, probiotics used in therapeutic ranges reverse the imbalance in the oral microbial ecosystem, modulate the potential pathogenicity of biofilm and normalize the host oral microflora.

Among the broad genera of probiotics, strains of genera *Lactobacillus and Bifidobacterium* have been considered harmless and possess effective properties in resisting all the environmental factors of the oral cavity and produce antimicrobial properties stated in the caries pathology. Combinations of probiotic strains would aid in sustained long-term benefits in oral health. Probiotics have demonstrated significant success in the oral cavity due to their ease of delivery through the mouth, natural method of therapy with minimum or no adverse effects, immediate effects seen competing with oral pathogens, and effective reduction in oral tissue inflammation and infection. Probiotics can be administered as a probiotic culture concentrate, prebiotic fibres, and dairy and non-dairy products in the form of suitable powder, capsules and gelatine tablets. In general, it’s safe to use probiotics that have met the selection criteria (Table 1).

In the oral cavity, selected probiotics must show survival capacity, resist environmental conditions and have good adhesion properties either by co-adhesion or co-aggregation with existing oral microbes or competing with existing binding sites on saliva-coated tooth surfaces resulting in oral microbiome balance (shift towards non-pathogenic bacteria) These probiotics eventually colonize and outgrow other oral microbial pathogens by secretion of anti-microbial substances for a prolonged period of time. Possible anticariogenic action of probiotic bacteria in dental caries pathology have been illustrated (Figure 2). In addition to all the direct mechanisms of action of *Lactobacillus* probiotics, specific and non-specific immune mechanisms play a supportive role in the evasion of oral diseases.

### EFFECT OF LACTOBACILLUS PROBIOTICS ON S. MUTANS BIOFILM IN DENTAL CARIES

The genus *Lactobacillus* is a diverse heterogeneous bacterium present as normal commensals in the oral cavity, mucosa of the gastrointestinal tract and vagina of the human body. They make up only 1% of the human oral microflora. *Lactobacillus* species are facultative anaerobes, catalase-negative, non-spore forming rods and gram-positive bacteria that appear to grow better under microaerophilic conditions. Gram stain morphology of *Lactobacillus* appears as short, plump to long, slender rods, in

**Table 1: Probiotics Selection Criteria.**

<table>
<thead>
<tr>
<th>Criteria</th>
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<tbody>
<tr>
<td>Non-pathogenic and non-toxic.</td>
</tr>
<tr>
<td>Replace and resist the existing pathogenic micro-flora.</td>
</tr>
<tr>
<td>Capable of surviving and metabolizing in the surrounding environment.</td>
</tr>
<tr>
<td>Continue being viable under storage for duration.</td>
</tr>
<tr>
<td>Possess prolonged shelf life.</td>
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<tr>
<td>Possess good sensory properties.</td>
</tr>
<tr>
<td>Able to communicate and interact with immune cells able to bind to the tooth surface.</td>
</tr>
<tr>
<td>Secrete anti-microbial substances against pathogens altering host ecology.</td>
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</tbody>
</table>
Figure 1: Risk factors for dental caries.

Figure 2: Role of Streptococcus mutans in dental caries.
chains or palisaded manner. Commonly, Lactobacilli appear as small to medium grey colonies with alpha hemolysis on blood agar culture and white, mucoid colonies on MRS (Man, Rogosa, and Sharpe) agar medium.\textsuperscript{35} Other than the human microbiota, Lactobacillus can be predominantly isolated from dairy products (raw milk, fermented milk, cheese and yoghurt) and non-dairy products (fruits–apples, grapes and bananas; kefir grains, poultry, sweet potato, sauerkraut - fermented cabbage, sourdough bread).\textsuperscript{36}

Among the Genus Lactobacillus, few strains are considered to act as resident acidogenic or aciduric bacteria in the biofilm for the propagation of dental caries or as probiotics reducing the negative effects of resident dental caries-producing bacteria. On one hand, a few Lactobacillus bacteria (L. gasseri, L. fermentum and L. salivarius) as stated earlier, appear to be late colonizers of dental caries pathology due to their poor adhesion properties.\textsuperscript{37}

This interspecies variation associated with dental caries severity may be attributed to the difference in fermentation properties and ability to survive in altered pH conditions. On the other hand, Lactobacillus probiotic species namely L. rhamnosus, L. reuteri, L. casei, L. paracasei, L. acidophilus, L. salivarius, L. brevis, L. bifidum, L. fermentum, L. bulgaricus, L. plantarum, L. delbrueckii, L. sporogenes and L. thermophilus have shown to be useful to the host and hence used in the manufacture of fermented probiotic products.\textsuperscript{38}

The first ever used probiotic in dental research is credited to Lactobacillus species, ‘Lactobacillus rhamnosus Gorbach-Goldin (LGG)’ in the oral cavity resulting in alteration of the oral microbiome and long-term beneficial effect on host health. Bacteria must be capable of adhering to the tooth surface, and become an integral part of the biofilm; finally, it must compete with cariogenic bacteria reducing the level of colonization in dental caries.\textsuperscript{39-42} Overall, Lactobacillus species as probiotics have shown a positive impact on oral health as illustrated (Figure 4).

The immune system additionally performs a crucial role in host body for the prevention of metabolic disease and infectious diseases such as dental caries. In the oral cavity at the molecular level, probiotics can aid in the regulation of the immune response through modulation of specific cytokines and chemokines.\textsuperscript{43-45}

Table 2: Approved Lactobacillus Probiotics Products for oral health.

<table>
<thead>
<tr>
<th>Manufacturer</th>
<th>Probiotic Product (Name/Dosage)</th>
<th>Lactobacillus strain</th>
<th>Functions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Jarrow Formulas</td>
<td>Jarro Dophilus Oral Probiotic 1 billion cells/lozenge</td>
<td>L. brevis</td>
<td>Promotes the health of teeth.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>L. plantarum</td>
<td></td>
</tr>
<tr>
<td>Henry Blooms</td>
<td>Oral health Ice Fresh Probiotic Mints 3 billion CFU/mint</td>
<td>L. paracasei</td>
<td>Balances mouth pH; Balanced oral bacteria maintenance.</td>
</tr>
<tr>
<td></td>
<td>Kids Probiotic toothpaste 50 g</td>
<td>L. paracasei</td>
<td>Healthy mouth flora; Cavity preparation; Acid protection.</td>
</tr>
<tr>
<td></td>
<td>Probiotic toothpaste 100 g</td>
<td>L. paracasei</td>
<td>Healthy mouth flora; Maintains healthy teeth and gums; Promotes fresh breath.</td>
</tr>
<tr>
<td></td>
<td>Probiotic mouthwash 375 mL</td>
<td>L. paracasei</td>
<td>Healthy mouth flora; Maintains healthy teeth and gums; Promotes fresh breath.</td>
</tr>
<tr>
<td>Hyperbiotics</td>
<td>PRO-Dental, Natural mint flavor Chewable tablets 3 billion CFU/tablet</td>
<td>L. paracasei, L. reuteri, L. sakei and L. salivarius</td>
<td>Help freshen breadth; Support microbial balance.</td>
</tr>
<tr>
<td>Enzymedica</td>
<td>Digest Gold Smiles Oral Health mint 12 billion cells/mint</td>
<td>L. plantarum</td>
<td>Support for healthy teeth and peridontium.</td>
</tr>
<tr>
<td>Biogaia</td>
<td>Prodentis for teeth and gums Lozenges 200 million CFU/Lozenge</td>
<td>L. reuteri (ATCC PTA 5289 +DSM 17938)</td>
<td>Helps the good microorganisms keep a natural balance in the mouth.</td>
</tr>
</tbody>
</table>
Figure 3: Possible actions of probiotic bacteria in dental caries pathology.

level, *Lactobacillus* probiotics inhibit the action of cytokines and chemokines (Interleukin-8 [IL-8] and Prostaglandin [PGE2]) thereby regulating immune cells in the process of inflammation associated with sequelae to dental caries. Other mechanisms involve increased production of salivary Immunoglobulin A (IgA), inhibition of collagenases and reduction of cytokine-mediated apoptosis process. This alteration of the immune status locally and systemically results in reduced inflammation and destruction of oral tissues.\(^{43,44}\)

**TYPES OF LACTOBACILLUS PROBIOTICS PREPARATIONS**

Probiotics have been introduced in the oral cavity in the form of oral dosage forms such as capsules, tablets, chewing gum, lozenges, powder and nanoparticles.\(^{45,46}\)

**Specifically-Targeted Antimicrobial Peptides (STAMP) technology**

In STAMP technology, targeting the moiety (species-specific binding peptide) of antimicrobial fusion peptide enables the targeted carriage of an attached antimicrobial peptide towards the host surface. From this technology, *S. mutans* may be eliminated from dental infection.

**Designer Probiotics**

In this approach, probiotic bacteria are furnished with genetic components on their surface essential to express receptor mimic structures to prevent stress against pathogens. ‘Effector strain’ of *S. mutans* (zero pathogenic potential) replaces the cariogenic or ‘Wild strain’ of *S. mutans* to inhibit the initiation and further progression of tooth decay, thereby helping in the remineralization of hard tissues.\(^{47}\)

**APPROVED LACTOBACILLUS PROBIOTICS PRODUCTS FOR ORAL HEALTH**

Probiotic products have been approved for various clinical conditions and are readily available in the market. Probiotic strains range from individual strains of *Lactobacillus, Bifidobacterium, Streptococcus* and *Bacillus* to a probiotic blend (combination of strains) for more effective action.\(^{48}\) List of commercially available certified probiotics with exclusive *Lactobacilli* effect on dental caries has been presented (Table 2).

**Future Prospective**

The recent literature on dental caries suggests that it has been associated with a diversity of the microbial community and multifactorial occurrence from person to person. Although promising results are taking place with the effect of *Lactobacillus* probiotics on *S. mutans* biofilm in dental caries, oral microbial diversity still remains a major challenge. To note, even though scientific research has taken to the level of clinical trials to test the effect of probiotics on oral health, effects may not hold true as seen in one species or strain with the other probiotics always. An in-depth understanding of the probiotics (formulation,
**Figure 4:** Role of *Lactobacillus* probiotic strains in dental caries.

### Antimicrobial mechanism

- **Bactericidal/Bacteriostatic**
- **Bacteriocin & byproducts**
- **Oxidative and acidic stress**

#### Antimicrobial substances

<table>
<thead>
<tr>
<th>Species</th>
<th>Antimicrobial substance</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>L. reuteri</em></td>
<td>Reuterin &amp; Reutericyclin</td>
</tr>
<tr>
<td><em>L. gasseri</em></td>
<td>Gasserin</td>
</tr>
<tr>
<td><em>L. fermentum</em></td>
<td>Fermesins</td>
</tr>
<tr>
<td><em>L. paracasei</em></td>
<td>Paracins, Defensin - Human neutrophil peptide</td>
</tr>
<tr>
<td><em>L. plantarum</em></td>
<td>Lipoteichoic Acids</td>
</tr>
<tr>
<td><em>L. salivarius</em>, <em>L. rhamnosus</em></td>
<td>Salivaricin E, Streptin, Streptococcin</td>
</tr>
<tr>
<td><em>L. sanguinis</em>, <em>L. casei</em></td>
<td>Hydrogen peroxide &amp; organic acid</td>
</tr>
</tbody>
</table>

#### Competitive inhibition

- **Co-aggregation**
- **Modulation of adhesion mechanism**

<table>
<thead>
<tr>
<th>Species</th>
<th>Antiplaque substance</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>L. fermentum</em></td>
<td>Bio surfactants</td>
</tr>
<tr>
<td><em>L. acidophilus</em>, <em>L. rhamnosus</em>, <em>L. reuteri</em>, <em>L. casei</em>, <em>L. paracasei</em>, <em>L. lactis</em>, <em>L. salivarius</em></td>
<td>Adhesion to salivary pellicle coated hydroxyapatite</td>
</tr>
<tr>
<td><em>L. salivarius</em>, <em>L. rhamnosus</em></td>
<td>Directly inhibits glucan production</td>
</tr>
<tr>
<td><em>L. rhamnosus</em></td>
<td>Binds with salivary agglutinin gp340</td>
</tr>
<tr>
<td><em>L. plantarum</em>, <em>L. brevis</em>, <em>L. bulgaricus</em></td>
<td>Improves lactose intolerance</td>
</tr>
<tr>
<td><em>L. kefiranofaciens</em></td>
<td>Inhibits the gene expression for adhesion</td>
</tr>
<tr>
<td><em>L. paracasei</em>, <em>L. rhamnosus</em>, <em>L. plantarum</em></td>
<td>Direct interference</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Species</th>
<th>Antiplaque mechanism</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>L. rhamnosus</em>, <em>L. casei</em></td>
<td>Anti-inflammatory mechanism</td>
</tr>
<tr>
<td><em>L. rhamnosus</em>, <em>L. acidophilus</em>, <em>L. reuteri</em></td>
<td>Immune modulation</td>
</tr>
</tbody>
</table>

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*L. gasseri*, *L. salivarius*, *L. plantarum*, *L. brevis*, *L. bulgaricus*,
*L. casei*, *L. paracasei*, *L. sanguinis*, *L. rhamnosus*, *L. reuteri*.
*L. acidophilus*, *L. bifidum*, *L. thermophilus*.
safety, efficacy, dosage, duration of the treatment and vehicle of administration), multiple species of Lactobacillus probiotic strain and targeting of the metabolites produced by diverse oral microbiome associated with dental caries may show conclusive effects in future.

CONCLUSION

The oral cavity is composed of numerous communities of micro-organisms and exhibits enormous inter and intra-species variation. Administration of an appropriate probiotic formulation confers a strong ecological balance and positive outcomes in oral health. In this article, the role of S. mutans biofilm with emphasis on probiotic action on dental caries.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ABBREVIATIONS

ATCC: American Type Culture Collection; CFU: Colony forming unit; GCF: Gingival crevicular fluid; GRAS: Generally regarded as safe; Ig: Immunoglobulin; IL: Interleukin; L: Lactobacillus; LAB: Lactic acid bacteria; LGG: Lactobacillus rhamnosus Gorbach-Goldin ; NIH: National Institute of Health; PG: Prostaglandin; PGE: Prostaglandin E; S: Streptococcus; spp: Species; STAMPS: Specifically targeted antimicrobial peptides.

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