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**Review Article**

# Non-Antibiotic Enzymes and Peptides for the Disruption of Mature Streptococcus Mutans Biofilms

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**Abstract:** Streptococcus mutans is the leading one of the oral microbiota causing tooth caries. It forms biofilms on teeth providing protection against the host defenses by making extracellular polysaccharides and carbohydrates. These biofilms increase bacterial persistence and enhances the pathogenicity of S. mutans. Such emergence of antibiotic resistance has significantly reduced the long-term usage of both synthetic and conventional antimicrobials (β lactams and chlorhexidine). The need to find and develop novel alternatives is owing to this growing resistance. Natural antimicrobials, like enzyme-based medications and other antimicrobial peptides (AMPs), provide good treatments. AMPs also minimize the chance of developing resistance and disrupts bacterial membranes preventing the formation of biofilms. By directly breaking down biofilm matrix and virulence factors, enzyme-based antimicrobials such glucosidases and proteases improve bacterial clearance. Both strategies are viable substitutes for conventional antibiotics since they are biocompatible, environmentally benign, and have several targets of action. The relevance of switching to natural antimicrobial drugs is shown by the collective research on *S. mutans* prevalence, biofilm biology, and resistance mechanisms. Incorporating AMPs and enzyme-based treatments into oral healthcare may offer safe, efficient, and resistance-reducing methods for managing and preventing dental caries.

**Keywords:** *Streptococcus Mutans*, Biofilm Formation, Enzyme Based Antimicrobials, Anti Microbial Peptides.

## 1.1 ORAL MICROBIOME

Dental caries and periodontal disorders are directly impacted by oral microorganisms. Through two-way communication between the oral cavity and the systemic organs, the dynamic oral microbiota collaborates with the host to reflect the information and status of immunity and metabolism [Peng, X., 2022]. The human oral cavity is home to more than 700 different types of bacteria.10 The human microbiome project (HMP) has five study objectives, including the oral cavity, nasal cavity, vagina, gut, and skin. The oral microbiome is one of the most significant and intricate microbial communities in the human body [Hathaway-Schrader, J. D. 2021].

### *1.2 Streptococcus Mutans*

Streptococcus mutans (S. mutans), the most common bacteria associated with ECC, contributes significantly to the development of caries because of its high acid production and tolerance capacity [Eriksson L, 2018]. On the other hand, S. mutans was found in the caries-free (CF) group and in some caries patients, either at a low level or not at all. This implies that caries may be caused by other species, especially bacteria that are closely related to *S. mutans*. Streptococcus mutans

(S. mutans) is thought to be the most prevalent bacteria linked to ECC and plays a significant part in the development of caries due to its exceptional capacity to produce and tolerate acid [Zhang L, 2020].

### 1.3 S. *Mutans* Biofilm Formation

The biofilms are composed of proteins, nucleic acids and an extracellular matrix called exopolysaccharide (EPS). Bacteria once wrapped with this microbial community, gets essential nutrients and fights against host invasion and antimicrobial chemicals. Biofilms can survive extremely acidic conditions, damaging tooth enamel and decay (Park, 2022). The three primary stages of the biofilm formation.

#### 1.3.1 Sucrose Dependant Biofilm Formation

*Streptococcus mutans* can cause dental caries in humans by using both sucrose-dependent and sucrose-independent methods to form dental plaque biofilms. Salivary pellicles can initially colonize through separate pathways, but strong microcolony structural changes, 3D maturation, and matrix accumulation are driven by sucrose-dependent mechanisms.

#### 1.3.2 Sucrose-Independent Biofilm Formation

Initial surface colonization depends on direct interactions between salivary components coating the tooth (acquired enamel pellicle) and bacterial surface adhesins in the absence of dietary sucrose [Lemos, J. A., 2019]. SpaP (also called P1 or PAc), which binds to salivary agglutinins and glycoproteins, is the most notable surface protein involved. This baseline adherence is also influenced by other cell surface-associated proteins including WapA.

### 1.5 Antibiotic Resistance

Due to the possibility of upsetting normal flora and increasing multi-drug resistance, systemic antibiotics are rarely recommended exclusively for *Streptococcus mutans*, the main bacteria causing tooth caries. Clinical isolates exhibit concerning multidrug resistance to fluoroquinolones, macrolides, and β-lactams. To prevent broad resistance, research into targeted oral therapies, like chlorhexidine or specific enzyme inhibitors, is typically preferred over systemic antibiotics [Alkarimi HA, 2012].

The capacity of bacteria to withstand the effects of a certain antibiotic because of innate structural or functional characteristics is known as intrinsic resistance (Blair *et al.,* 2015). It is believed that biofilm formation, efflux pumps, target site mutations, and resistance plasmids cause *Streptococcus mutans* to become resistant to common antibiotics (such as β-lactams, macrolides, and fluoroquinolones), leading to an increase in multidrug-resistant profiles. [Karikalan S., 2016]

### 1.6 Search for Alternative Anti Biofilm Agents

Facilitating biofilm dispersion, preventing the growth of antibiotic-resistant bacteria, inhibiting pathogen reproduction and metabolism, and maintaining the oral microbiome's homeostasis are all suitable anti-biofilm strategies (Simon *et al*., 2019). Antimicrobial peptides (AMPs) have been regarded as possible therapeutic candidates, however increasing AMPs' specificity against S. mutans is difficult.

### 1.7 Enzymes in Dispersing the Biofilms

Teeth caries is the result of bacterial acid production in biofilms and is made of matrix of extracellular polymeric materials, like proteins, glucans and extracellular DNA (eDNA) [Nyvad B., 2020]. Matrix polysaccharides, which are important for bacterial attachment to tooth surfaces, nutrition, and the mechanical stability of dental biofilms, are known to be produced by caries-related bacteria, particularly *Streptococcus mutans*.

#### 1.7.1 Glucanohydrolases

The enzymatic dispersion of biofilms catalysts known as glucanohydrolases (Mutanase and Dextranase) specially hydrolyze the α-(1→3) and α-(1→6) glycosidic bonds. The S. mutans matrix's sticky core is made up of these glucans [Klein, M. I., 2015]. By selectively breaking down the structural extracellular polysaccharides (EPS) matrix, such as α-1,3-glucans and α-1,6-glucans, glucanohydrolases (such mutanase and dextranase) prevent S. mutans biofilms. The built biofilm architecture is dispersed by this enzymatic breakdown, yet the beneficial oral commensal bacteria are not eliminated. These enzymes break down the EPS matrix, which is necessary for S. mutans adhesion and the formation of three-dimensional biofilms. Additionally, structural pathogenicity is specifically neutralized by these enzymes. To improve total plaque elimination, some papers have proposed combining them with antimicrobial peptides or novel natural inhibitors (such polyphenols or Gtf-inhibitors) [Zhang, Q., 2021].

#### 1.7.3 Dextranases

A group of enzymes known as dextranase [E.C. 3.2.1.11] catalyze the hydrolysis of the (α-1→6) glycosidic bond present in dextran to create glucose, isomaltose, and a number of additional linear or branched oligosaccharides. By lessening the cariogenic effect of sucrose in the oral cavity, the generated isomalto-oligosaccharides have a prebiotic effect.

Sugar cane molasses is a rich source of microbial dextranases, which are enzymes that can break down the polysaccharide dextran into low molecular weight fractions with a variety of medical and industrial uses [Del Rey, 2024].

The α-1→6 glycosidic linkages in dextran are hydrolyzed by the enzyme dextranase (E.C. 3.2.1.11). By dissolving the Extracellular Polymeric Substance (EPS) matrix of dental plaque, it acts as a potent antibiofilm agent by stopping *Streptococcus mutans* from sticking to tooth surfaces and breaking down preexisting biofilms.

**1.8 Nucleases (e.g., DNase I, DeoC)**

These enzymes break down extracellular DNA to help bacteria evade the immune system and maintain the 3D biofilm structure. By breaking down extracellular DNA (eDNA), a crucial structural scaffold needed for dental plaque construction and early cell-to-cell attachment, nucleases prevent *Streptococcus mutans* biofilms. Biofilm dispersion results from the disruption of its structural integrity by enzymes such exogenous DNase I or bacterial self-produced nucleases like DeoC (Liu J. 2017).

**1.9 Proteases (e.g., Ficin)**

These proteases interrupt cell-to-surface attachment by cleaving extracellular proteins in the EPS [Sun, Y., 2021]. Proteases mainly break down important virulence and adhesion proteins or interfere with quorum-sensing signaling pathways to prevent *Streptococcus mutans* biofilms. Without necessarily eliminating the bacteria, exogenous proteases (such as ficin) and particular protease inhibitors can dramatically reduce biofilm biomass and extracellular polysaccharide (EPS) synthesis.

**1.9.1 Ficin**

Fig tree latex is the source of ficin, a potent, non-specific sulfhydryl (cysteine) protease. The direct enzymatic degradation of structural proteins that bind bacterial cells and preserve the sticky extracellular polymeric substance (EPS) matrix is part of its action against microbial biofilms [Yu, J., 2022]. Ficin is thought to break down the structural protein backbone that shields implanted pathogens like Candida albicans, *Streptococcus mutans*, and Staphylococcus aureus by cleaving the peptide bonds at the carboxyl side of many amino acids.

**1.10 Chimeric Enzymes:**

Some engineered fused mutanase-dextranases enhance broad-spectrum degradation, preventing early dental plaque formation [Wang, S., 2023]. Chimeric enzymes are modified to target and break the EPS matrix of biofilms by combining domains from parental enzymes. When compared to natural enzymes, these customized constructions significantly increase antibiofilm efficacy by combining different catalytic or binding modules.

These enzymes target several structural polysaccharides at once by combining various functions (such as glucanase, dextranase, or mutanase) into a single chimeric molecule. In fact, powerful chimeric endolysins (enzybiotics) that quickly lyse biofilm-embedded bacteria like MRSA are produced by combining a muralytic catalytic domain with a cell wall-binding domain. These powerful designed enzymes enable deep disintegration and dissemination by breaking down particular biofilm components, such as extracellular DNA (eDNA) scaffolds or polysaccharide matrices [Al-Madboly, L.A., 2024].

**1.11 Antimicrobial Peptides (Amps)**

By rupturing bacterial membranes, preventing initial cell attachment, and inhibiting the formation of extracellular polysaccharides (EPS), antimicrobial peptides (AMPs) fight *Streptococcus mutans* biofilms. These cationic, amphipathic compounds are promising templates for sophisticated anti-caries oral care products because they also downregulate virulence-associated genes (such glucosyltransferases). [S. Jiang, 2023].

There are two classes of novel Peptide Candidates; natural and synthetic. Natural peptides include Nisin, polylysine, while synthetic ones are LL-37 and P-113.

Natural Generally Recognized as Safe (GRAS) antimicrobial peptides called nisin and poly-ε-lysine (ε-PL) work together to inhibit and dissolve *Streptococcus mutans* biofilms, which are the main cause of dental caries. Nisin creates holes in the bacterial membrane by attaching itself to cell-wall precursor lipid II. On the other hand, cationic poly-ε-lysine causes membrane degradation and increases membrane permeability by electrostatically attaching to the negatively charged bacterial cell surface.

Human cathelicidin host defense peptide LL-37 prevents *Streptococcus mutans* biofilm production and works in concert with other substances (such as EGCG) to break up dental plaques that have already formed. It interferes with initial bacterial adhesion and downregulates important virulence and development genes at sub-lethal concentrations [Guo YJ, 2016]. At larger concentrations, LL-37's +6 cationic charge draws it to negatively charged bacterial membranes, resulting

in cell leakage and death. LL-37 decreases cell attachment and inhibits microcolony growth at sub-inhibitory concentrations well below the minimum inhibitory concentration (MIC).

a) **P-113:** Without interfering with the normal oral flora, antimicrobial peptide P-113 derivatives (like P-113-DPS or modified constructions like 8DSS-C8-P-113) efficiently prevent *Streptococcus mutans* biofilm development, exopolysaccharide (EPS) synthesis, and bacterial adherence. These help in downregulating genes linked to virulence (gtfB, gtfC, gtfD and spaP) [Liu, Q., 2025]. When treated, *S. mutans* showed reduced acid production and biofilm formation claiming its anticaries agent.

b) **CLP-4:** CLP-4 is a synthetic AMP that targets *Streptococcus mutans*, inhibiting its functions and preventing the dental biofilms.

c) **Temporin Derivatives:** By permeabilizing cell membranes, downregulating adhesion genes, and decreasing surface hydrophobicity, temporin derivatives—ultra-short, α-helical antimicrobial peptides (AMPs) derived from frog skin—effectively inhibit and eliminate microbial biofilms, including MRSA and *Streptococcus mutans*. Temporin G are analogues targeting bacterial cell walls causing leakage.

## CONCLUSION

The increasing resistance of *Streptococcus mutans* to both synthetic and conventional antibiotics is posing a serious threat in oral healthcare. Agents that were once considered (macrolides, chlorhexidine) are now being stopped due to reduced efficacy and adverse side effects. This complicates the treatment of caries and antibiotic resistance in oral bacteria. Compounds from plants processes multi-target mechanisms like inhibiting glucosyltransferase breaking biofilm structure.

Because of its anti-inflammatory and antioxidant properties, these AMPs are safer alternatives to synthetic drugs. Further research is needed in examining and confirming natural antimicrobials as for maintaining oral health. Conversely, enzyme-based antimicrobials and antimicrobial peptides (AMPs) are alternatives with speicifc advantages. Enzyme-based antimicrobials (glucosidases, proteases, or DNases) degrade the biofilm thus preventing the dissolution of extracellular polymers. As such bacteria have a hard time in developing resistance. These enzymes are biodegradable and do not accumulate in the environment as like synthetic antibiotics. AMPs target both gram-positive and negative bacterial and fungal strains by breaking membranes and modulating host immune response. They also reduce virulence through inhibition of attachment. In conclusion, next-generation treatments in overcoming the hurdles of traditional antibiotics are antimicrobial peptides and enzyme-based antimicrobials. These promise a sustainable antimicrobial solution in clinical and agricultural settings. These aid in breaking the biofilms, thus preventing the resistance and generating environmentally favoured substitutes.

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