Influence of biosurfactants on microbial attachment to voice prostheses and teeth

Hoogmoed, Christianus Gerhardus van

This thesis deals with detrimental biofilm formation on the teeth in the oral cavity and voice prostheses, inserted in laryngectomized patients between the digestive tract and the trachea. In contrast to the mucosal surfaces, where a biofilm does not develop because of a constant renewal of the epithelial surfaces by desquamation of the colonized epithelial surface cells, teeth and voice prostheses support the formation of a thick biofilm. In **Chapter 1** biofilm formation was described in general and more specifically for teeth and voice prostheses. Diseases as caries and periodontitis are caused by factors, which shift the "healthy" biofim (plaque) to a pathogenic plaque. Dysfunctioning of voice prostheses is caused by yeast ingrowth in the silicone rubber of voice prostheses. In view of the continuing high prevalence of caries and the short lifetime of voice prostheses, alternative or supplemental methods are needed to reduce the causative microorganisms in the respective biofilms. One approach could be the use of biosurfactants releasing bacteria. Therefore, the aim of this thesis is to explore the potential of biosurfactants releasing bacteria in inhibiting the adhesion of harmful microorganisms to silicone rubber voice prostheses and teeth.

Biosurfactants were reviewed in **Chapter 2**. Biosurfactants are amphiphilic compounds released by several microbial strains and species. Whereas some strains release biosurfactants in readily detectable amounts, it is hypothesized that many strains and species may actually release biosurfactants in minute amounts, only detectable by axisymmetric drop shape analysis by profile (ADSA-P). Biosurfactants have a distinct tendency to adsorb to interfaces and adsorbed biosurfactants will affect the physico-chemical properties of the interface and in turn microbial adhesion to the interface through influencing the Lifshitz-Van der Waals-, electrostatic- and acid-base interactions. Therewith, biosurfactants released by microorganisms adhering in a biofilm will interfere with the adhesion of other organisms.

In **Chapter 3** adhesion of yeasts, two *Candida albicans* and two *Candida tropicalis* strains isolated from naturally colonized voice prostheses, to silicone rubber with and without a salivary pellicle film in the absence and presence of adhering *Streptococcus thermophilus* B, a biosurfactant releasing dairy isolate, was studied. Coverage of 1 to 4% of the surface of silicone rubber substrata with adhering *S. thermophilus* B gave significant reductions in initial yeast adhesion regardless of the presence of a conditioning film. Mechanistically, this interference in yeast adhesion by *S. thermophilus* B was not due to direct physical effects, but to biosurfactants released by the adhering bacteria, because experiments with *S. thermophilus* B cells that had released their biosurfactants prior to adhesion to silicone rubber and competition with yeasts did

not show interference with initial yeast adhesion. The amounts of biosurfactants released were highest for mid-exponential- and early-stationary phase bacteria (37 $mg.g^{-1}$ cell dry weight), but biosurfactants released by stationary phase bacteria (14 $mg.g^{-1}$ cell dry weight) were the most surface active. The crude biosurfactants released were mixtures of various components, with a glycolipid-like component being the most surface active. A lipid-enriched biosurfactants fraction reduced the surface tension of an aqueous solution to about 35 $mJ.m^{-2}$ at a concentration of only 0.5 $mg.ml^{-1}$. The amount of biosurfactants released per *S. thermophilus* B cell was estimated to be sufficient to cover approximately 12 times the area of the cross section of the bacterium, making biosurfactants release a powerful defense weapon in the post-adhesion competition of the bacterium with microorganisms such as yeasts. Pre-adsorption of biosurfactants to the silicone rubber prior to allowing yeasts to adhere, was as effective against *C. albicans* GB 1/2 adhesion as covering 1 to 2% of the silicone rubber surface with adhering *S. thermophilus* B, but a pre-adsorbed biosurfactants layer was less effective against *C. tropicalis* GB 9/9.

Both the character of *Streptococcus mitis* biosurfactants and their influence on the adhesion of *Streptococcus mutans* NS to glass were studied in **Chapter 4**. Two *S. mitis* strains (BA and BMS) released maximal amounts of biosurfactants when they were grown in the presence of sucrose and were harvested in the early-stationary phase. The *S. mitis* biosurfactants reduced the surface tension of aqueous solutions to about 30-40 $mJ.m^{-2}$. Biochemical and physico-chemical analyses revealed that the biosurfactants released were glycolipids. An acid-precipitated fraction was extremely surfactive and identified as a rhamnolipid-like component. In a parallel plate flow chamber, the number of *S. mutans* NS adhering to glass with and without a salivary pellicle in the presence of biosurfactants releasing *S. mitis* BA and BMS (surface coverage between 1 to 4%) was significantly reduced compared with the number of *S. mutans* NS adhering to glass in the absence of *S. mitis*. *S. mutans* NS adhesion in the presence of non-biosurfactants-releasing *S. mitis* BA and BMS was not reduced at all. In addition, pre-adsorption of isolated *S. mitis* biosurfactants to glass drastically reduced the adhesion *S. mutans* NS cells and their bond strength to glass, as shown by the increased percentage of *S. mutans* NS detached by the passage of air bubbles through the flow chamber. Pre-adsorption the acid-precipitated fraction inhibited *S. mutans* adhesion up to 80% in a dose-responsive manner. These observations indicate that *S. mitis* plays a protective role in the oral cavity and protects against colonization of surfaces with a salivary pellicle by cariogenic *S. mutans*.

In **Chapter 5** the influence of isolated biosurfactants released by *S. mitis* BMS on the adhesion of a variety of oral bacterial strains was examined. For a first screening, the effects of *S. mitis* BMS biosurfactants on the adhesion to pellicles of eight pioneer and four cariogenic strains was determined in a microtiter plate assay. The adhesion of three of the four cariogenic strains was inhibited with more than 70% by *S. mitis* BMS biosurfactants adsorbed to pellicle-coated wells. Only one of the pioneer species showed more than 70% reduction. The reduction for the other species did not exceed 50%. Subsequently, adhesion of the cariogenic strains, *Streptococcus mutans* ATCC 25175 and *Streptococcus sobrinus* HG 1025 and the pioneer species, *Actinomyces naeslundii* T14V-J1 and *Streptococcus oralis* J22, to biosurfactants-coated enamel with and without a salivary pellicle was studied in the parallel plate flow chamber. A biosurfactants coating to enamel chips with or without a pellicle in a parallel plate flow chamber did not cause a significant reduction in the number of adhering pioneer organisms. A biosurfactants coating on bare and pellicle-coated enamel showed a clear reduction in the number of adhering cariogenic organisms. In conclusion, these observations indicate that *S. mitis* biosurfactants could play a protective role in the oral cavity against adhesion of cariogenic bacteria.

Interaction forces between enamel with and without a salivary pellicle and *Streptococcus sobrinus* HG 1025 were measured by AFM in **Chapter 6**. Additionally, the influence of a *Streptococcus mitis* BMS biosurfactants coating, discouraging adhesion of *mutans Streptococci*, on these interaction forces was studied. Enamel particles with and without a salivary pellicle or biosurfactants coating were attached to AFM cantilevers and interaction forces with *Streptococci*, immobilized in a membrane filter, measured. Upon approach, a repulsive force was measured that ranged from 83 nm to 53 nm for enamel with and without a salivary pellicle, respectively, while the additional presence of a biosurfactants coating strongly increased the range of these repulsive forces to 283 and 253 nm, respectively. Upon retraction of enamel particles, a small adhesion force (-0.9 ± 0.9 nN) was measured for bare enamel, that disappeared after biosurfactants coating. Both the prevalence and magnitude of the adhesion forces decreased for enamel with a pellicle, with a minor effect of a biosurfactants coating. These results provide the first direct measurement of the interaction forces between an oral microorganism and enamel and advance our understanding of the working mechanism of bacterial surfactants in preventing adhesion of cariogenic organisms.

In the general discussion (**Chapter 7**) the physiological role and the dual applicability, namely as isolated biosurfactants adsorbed from solution or as biosurfactants released from

adhering bacterial cells, of the *S. mitis* and *S. thermophilus* biosurfactants are discussed. Within the scope of the AFM experiments the pitfalls, which are present during the attachment of the enamel particle to the AFM cantilever, as well as bond aging between enamel and the oral bacterium *S. sobrinus* HG 1025 are considered.

Summarizing, the *S. mitis* BMS and *S. thermophilus* biosurfactants offer possibilities in the prevention of harmful microbial adhesion to enamel and silicone rubber, respectively.