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ORIGINAL ARTICLE

Bacterial and Host Interactions of Oral Streptococci

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The oral microbial flora comprises one of the most diverse human-associated biofilms. Its development is heavily influenced by oral streptococci, which are considered the main group of early colonizers. Their initial attachment determines the composition of later colonizers in the oral biofilm and impacts the health or disease status of the host. Thus, the role of streptococci in the development of oral diseases is best described in the context of bacterial ecology, which itself is further influenced by interactions with host epithelial cells, the immune system, and salivary components. The tractability of the oral biofilm makes it an excellent model system for studies of complex, biofilm-associated polymicrobial diseases. Using this system, numerous cooperative and antagonistic bacterial interactions have been demonstrated to occur within the community and with the host. In this review, several recent identified interactions are presented.

Introduction

The oral microbial community is diverse. Previous studies utilizing culture-dependent and culture-independent molecular techniques have estimated the diversity within the oral cavity to consist of over 700 species or phylotypes (Kulik *et al.*, 2001; Aas *et al.*, 2005). However, a recent report using pyrosequencing analysis estimated the number of phylotypes to be greater than 19,000 (Keijser *et al.*, 2008). Not surprisingly, the oral cavity also contains the greatest biodiversity of any known human-associated biofilm. While the diversity in any particular individual is much lower, recent data suggest that there still may be over 70 species present (Aas *et al.*, 2005). Most likely, this number will increase as more-sensitive detection methods such as pyrosequencing are employed to sample individual biodiversity.

Oral biofilm formation is characterized by the initial adhesion of the early colonizers. A saliva film containing albumin, glycoproteins, acidic proline–rich proteins, mucins, sialic acids, and other compounds covers the tooth surface. This film, called acquired pellicle, provides receptors for the initial colonizers. After initial contact via specific surface adhesins, oral streptococci attach irreversibly to the pellicle components. This can be further promoted by excretion of polymeric substances like polysaccharides and DNA. Surface-attached bacteria are believed to respond to their new physical state, while their metabolic status and gene expression changes result in altered surface properties (Jenkinson, 1994a, 1994b, 1995; Davey and Costerton, 2006). This priming process provides further attachment sites for later colonizers, both Gram-positive and Gram-negative bacteria. The succession of biofilm development involves coaggregation and coadherence of oral bacteria, and if undisturbed develops into a stratified, complex biofilm. For a recent review describing the mechanisms and the ecological role of coadhesion and coaggregation, see Kolenbrander *et al.* (2006).

The initial colonization process is dominated by oral streptococci, which make up over 80% of the early biofilm constituents (Rosan and Lamont, 2000). Oral streptococci are in general referred to as viridans streptococci, but this is not an exclusive classification because the viridans streptococci contain as well members not isolated from the oral cavity. Oral streptococci are divided into five different groups: (1) Mutans group (prominent members are *Streptococcus mutans* and *Streptococcus sobrinus*), (2) Salivarius group (*Streptococcus salivarius*), (3) Anginosus group (*Streptococcus anginosus* and *Streptococcus intermedius*), (4) Sanguinis group (*Streptococcus sanguinis* and *Streptococcus gordonii*), and (5) Mitis group (*Streptococcus mitis* and *Streptococcus oralis*) (Whiley and Beighton, 1998; Facklam, 2002). Most of the oral streptococci are commensal, nonperiodonthopathogenic bacteria. Some are known to cause infective endocarditis when disseminated through the blood stream, like the early colonizer *S. gordonii*, *S. sanguinis*, and the recently identified *Streptococcus oligofermentans* (Herzberg, 1996; Herzberg *et al.*, 1997; Matta *et al.*, 2008). Although not considered an early colonizer, the best-studied oral *Streptococcus* is the opportunistic pathogen *S. mutans* (Loesche, 1986; Lemos and Burne, 2008). Its role in caries development is well established (Hamada and Slade, 1980; Russell, 2008).

This manuscript is dedicated to the memory of Professor Charles F. Schachtele.

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The oral cavity is a challenging environment for the long-term persistence of bacteria. Fluctuations in nutrient supply, temperature, pH, and the shear force of saliva flow have selected for a biofilm community adapted to high cell density, species diversity, and dynamic growth conditions. Further, different anatomical sites in the oral cavity have their own unique microenvironments, which shaped the evolution of a diverse bacterial flora. The buccal epithelium, subgingival crevice, maxillary anterior vestibule, tongue, soft and hard palate, tonsils, and the tooth surface are all colonized by different combinations of bacterial species or phylotypes (Aas *et al.*, 2005). The high diversity and density of the oral microbial community, different anatomical niches, and the limited resources of the oral environment have each shaped the interspecies interactions that might be required for the persistence of oral bacteria. The microbial composition is drastically affected by interspecies interactions, which are vastly influenced by the environment, which in turn, impact the health or disease status of the host. In this review, we summarize the recent advancement of our knowledge in the interspecies interactions among oral streptococci, between streptococci and other oral bacteria, and between streptococci and the human host.

Interactions Among Commensal Streptococci

A freshly cleaned tooth surface is colonized by pioneer oral streptococci. However, even this early event is not immune to interspecies competition. *S. sanguinis* and *S. gordonii* are among the first species to colonize the tooth surface and can be isolated from the same intraoral sites (Rosan and Lamont, 2000). Both organisms express specific cell surface adhesin molecules with similar binding capacities. Hence, they compete for binding to the same receptors provided by the host (Nobbs *et al.*, 2007b). Nobbs and colleagues demonstrated an interspecies antagonism for host-derived binding sites between *S. sanguinis* and *S. gordonii*. *S. sanguinis* has a greater natural prevalence in plaque and saliva, but is surprisingly unable to eliminate *S. gordonii*. Further, *S. gordonii* competes more effectively with *S. sanguinis* than any other tested oral streptococci for adherence to saliva-coated hydroxyapatite, a model system to simulate the natural tooth environment. Mutational analysis revealed that a specific surface adhesin, the sialic acid–binding protein Hsa, was responsible for conferring a competitive advantage to *S. gordonii* (Nobbs *et al.*, 2007b). Therefore, competition is influenced not only by sheer abundance but also by the efficiency of adherence to certain salivary components. This enables *S. gordonii* to establish itself in a competitive environment, even with the genetically similar competitor *S. sanguinis*. Because the early colonizers provide the attachment sides for the later colonizers, this competition has direct implications for the spatial and temporal composition of the developing dental biofilm.

Interactions Between Commensal and Pathogenic Streptococci

Clinical observations

The development of dental caries has been an important topic in oral microbiology research for multiple decades. Although often associated with a higher abundance of *S. mutans* (Hamada and Slade, 1980; Loesche, 1986), caries seems to be more polymicrobial in nature and thus a problem of bacterial ecology (Liljemark and Bloomquist, 1996; Fejerskov, 2004). Caries development is clearly associated with the overgrowth of a few bacterial species, including *S. mutans* and certain lactobacilli, and correlated with a reduction in overall species diversity (Caufield *et al.*, 2007; Li *et al.*, 2007). Interspecies interactions of oral streptococci play an important role in this shift. When present in the oral biofilm in high numbers, certain beneficial streptococci might be able to antagonize more cariogenic streptococci as suggested by clinical studies. Becker *et al.* (2002) determined the levels of several oral bacteria collected from healthy subjects and subjects with early childhood caries, a particularly virulent form of caries (Berkowitz, 2003). They found a significant increase in the amount of *S. mutans*, collected from subjects with caries compared to healthy subjects, as expected, but they also observed an inverse relationship with *S. sanguinis*. Healthy subjects had significantly higher numbers of *S. sanguinis* (*S. gordonii* was higher, but not significant), whereas subjects with caries possessed almost no detectable levels of *S. sanguinis* (Becker *et al.*, 2002). Likewise, high levels of *S. sanguinis* correlated with delayed acquisition of *S. mutans* (Caufield *et al.*, 2000). Mikx *et al.* (1972) reported a similar observation with sequential inoculation experiments in germfree rats over 30 years ago.

In vitro *studies*

Recently, our group was able to demonstrate a potential molecular basis of this clinically relevant antagonism: *S. sanguinis* and *S. gordonii* engage in chemical warfare with *S. mutans* for dominance over a given niche. The outcome is determined by the sequence of establishment, nutritional availability, and environmental pressures (Kreth *et al.*, 2005b, 2008). The oral streptococci utilize different weapons to face one another, but it seems that *S. mutans* has the better choice as described later. All three streptococci are able to produce small chromosomally encoded antimicrobial peptides called bacteriocins; several of them have been described in the literature. Bacteriocins of *S. mutans* have been very well characterized, especially the bacteriocins mutacin I and mutacin IV (Qi *et al.*, 2000, 2001). *S. gordonii* produces bacteriocins called streptocins and *S. sanguinis*, the bacteriocin sanguicin (Schlegel and Slade, 1972, 1973; Deng *et al.*, 2004). It has been experimentally confirmed for *S. mutans* and *S. gordonii* that the production of certain bacteriocins is controlled in a cell-density-dependent manner, optimized for expression in the high cell density environment found in the dental biofilm (Kreth *et al.*, 2005a; Heng *et al.*, 2007). One of the distinctive features of bacteriocins is the target range of susceptible bacteria. In the direct competition between the commensal *S. sanguinis* or *S. gordonii* versus *S. mutans*, only the bacteriocins produced by *S. mutans* are able to inhibit the growth of the competitor (Kreth *et al.*, 2005b). The bacteriocins produced by *S. gordonii* and *S. sanguinis* do not target *S. mutans*. Nonetheless, *S. gordonii* and *S. sanguinis* are able to inhibit the growth of *S. mutans* by generating an alternate weapon: hydrogen peroxide (H_2O_2) (Kreth *et al.*, 2005b). The production of H_2O_2 might provide an ecological advantage over bacteriocin production during the initial colonization, when cell density is not high enough to trigger bacteriocin

gene expression. H_2O_2 production is also oxygen dependent (Kreth *et al.*, 2008), and the oxygen tension in saliva is sufficient to allow for aerobic respiration and H_2O_2 production during initial colonization (Marquis, 1995). The oxygen tension declines once the biofilm reaches a certain thickness and cell density due to diffusion limitations. This could conceivably result in a decrease of H_2O_2 production to a non-inhibiting level. Under those biofilm conditions, *S. mutans* has the advantage by using bacteriocins to inhibit *S. sanguinis* and *S. gordonii*. Bacteriocin production is not influenced by oxygen availability (Kreth *et al.*, 2008), and expression is optimized for growth under the high cell density conditions of a mature biofilm, making *S. mutans* an aggressive competitor with *S. sanguinis* and *S. gordonii*. This may be one of the reasons *S. mutans* is able to initiate a shift in population composition. One has to keep in mind that these studies are *in vitro* experiments. Salivary peroxidase, for example, catalyzes the H_2O_2-dependent oxidation of thiocynante (SCN^-) to hypothiocyanite ($OSCN^-$) *in vivo*, leaving the described effect of H_2O_2 to limited microenvironments in the oral biofilm (Ashby, 2008). Further, no *in vivo* data are available about the effects of mutacins on the community structure of oral biofilms. Nonetheless, a study showed that about 88% of clinical *S. mutans* isolates produce some kind of mutacins, suggesting an ecological pressure to keep these antimicrobial compounds functional in the genome (Gronroos *et al.*, 1998).

The other advantage of bacteriocins produced by *S. mutans* is their mode of action, targeting the membrane of the susceptible species and causing leakage of cell contents (Oppegard *et al.*, 2007). *S. mutans* takes full advantage of this mode of action and coordinates bacteriocin production with another important cell function, competence development, to be most efficient in the competition with *S. sanguinis* and *S. gordonii* (Kreth *et al.*, 2005a, van der Ploeg, 2005). Competence is a physiological state that enables bacteria to take up free DNA from the environment (Cvitkovitch, 2001). The competence system ComCDE of *S. mutans* controls the production of mutacin IV before inducing the expression of genes required for DNA uptake. In this way, *S. mutans* can ensure that mutacin IV exerts its lethal effect first to cause the release of cellular contents from its target organism. Subsequently, *S. mutans* cells will become competent and take up the released DNA (Kreth *et al.*, 2005a). This interspecies interaction, although not beneficial for the lysed bacterial species, is important in the context of evolution. It ensures genetic exchange. *S. gordonii* is not just a simple victim of *S. mutans* and has developed a simple, but effective counter measure against *S. mutans* bacteriocin production. As mentioned above bacteriocin production is controlled by the competence system ComCDE (Kreth *et al.*, 2006). This two-component system relies on the extracellular signal competence–stimulating peptide (CSP) encoded by *comC* (Petersen and Scheie, 2000; Li *et al.*, 2001). CSP is the Achilles' heel of the cell-density-controlled expression of mutacin IV. To be effective in the control of mutacin production and competence development, CSP needs to be excreted. Once outside the cell, CSP eventually accumulates under high cell density and binds to its specific cell surface receptor, ComD (Cvitkovitch, 2001). This triggers a phosphorylation cascade and induces mutacin production followed by competence development (Kreth *et al.*, 2005a). *S. gordonii* is able to interfere with this regulatory cascade. Wang and Kuramitsu (2005) reported that supernatants from *S. gordonii* are able to inhibit the production of bacteriocins, but they did not affect the bacteriocins directly. They identified a protease, named challisin, which is able to degrade CSP, thus interfering with the bacteriocin/competence regulation cascade. This ability might not be unique to *S. gordonii*, since a search of the recently finished *S. sanguinis* genome (www.oralgen.lanl.gov) revealed a homolog of challisin. This demonstrates the complexity of oral streptococcal interactions shaped by co-evolution, competition, and a challenging growth environment. Interspecies inhibition strategies seem to be a major mechanism to cope with competitors. H_2O_2 is the weapon of choice for some streptococci, while others favor bacteriocin production.

Another countermeasure strategy was recently identified for *S. oligofermentans*, another oral streptococci (Tong *et al.*, 2003) and competitor of *S. mutans*. Surprisingly, *S. oligofermentans* is able to utilize the lactic acid produced by cariogenic species, such as *S. mutans*, to generate H_2O_2, leading to growth inhibition of the lactic acid producer (Tong *et al.*, 2007). This also protects *S. oligofermentans*, as it and many other nonpathogenic oral streptococci are inhibited by the low pH environment created by acid-tolerant cariogenic species. These interactions illustrate how the depth and elegance of interspecies interactions could be easily overlooked using the reductionist approaches typically employed in microbial genetics. Interspecies interactions only begin to reveal themselves when the host, environment, and the metabolic capacity of the biofilm (metabolome) are considered.

Interactions Between Streptococci and Other Early Colonizers

Streptococcus–Veillonella *interactions*

Metabolic interactions are found between oral streptococci and other members of the oral biofilm. A particularly close association exists between the oral streptococci and members of the genus *Veillonella*, of which *V. atypica* and *V. parvula* are the most prominent members in the oral biofilm. Veillonellae are Gram-negative early colonizers of the oral biofilm (Delwiche *et al.*, 1985) and are not able to use carbohydrates as carbon sources, but can use organic acids, like lactic acid, for growth (Distler and Kroncke, 1981). This has led to an symbiotic relationship between *V. atypica* and *S. mutans*, which heavily favors lactic acid production as a metabolic end product of carbohydrate fermentation (Harper and Loesche, 1983). However, recent studies suggest that we are only just beginning to understand this relationship. It was initially thought that the function of this symbiosis was simply to provide lactic acid as a carbon source for *Veillonella* and to detoxify the environment for *S. mutans* (Mikx and Van der Hoeven, 1975; van der Hoeven *et al.*, 1978), but recent studies suggest that the interactions between these two organisms are likely to be much more complex. For example, dual-species biofilms of *S. mutans* and *V. parvula* are less susceptible to antimicrobial treatment, suggesting a more intricate metabolic complementation (Kara *et al.*, 2006). Likewise, the transcriptional profile of *S. mutans* changed significantly in biofilms with *V. parvula* when compared to *S. mutans*'s single-species biofilms (Luppens *et al.*, 2008).

What causes this change is yet to be determined, but this interaction could involve interspecies metabolic signaling as suggested by another study with *V. atypica* and coaggregation partner *S. gordonii*. Juxtaposition of *V. atypica* and *S. gordonii* in a saliva-conditioned flow-cell increased the expression of *S. gordonii*'s amylase gene (Egland *et al.*, 2004). The authors argued that this induction of amylase gene expression in *S. gordonii* is most likely beneficial for *V. atypica*. Amylase degrades starch to its monomer glucose, which can be further used by *S. gordonii* for fermentation to lactic acid, promoting *V. atypica*'s growth. The data further suggested that whatever signal is involved in this interaction is only effective over a short distance (Egland *et al.*, 2004) and thus likely to be diffusible.

Streptococcus–Actinomyces *interactions*

Further, not only have diffusible signals been found to mediate interspecies interactions with *Veillonella*, but they are also required to mediate dual-species biofilm formation between *S. oralis* and *Actinomyces naeslundii*, respectively (McNab *et al.*, 2003; Rickard *et al.*, 2006). In this case the signal has been identified as autoinducer 2 (AI-2). AI-2 is a cell-density-dependent signaling molecule produced by several bacterial species, and many species can sense AI-2 and respond to it (Waters and Bassler, 2005). Therefore, AI-2 is considered as a nonspecific signaling molecule for interbacterial communication (McNab and Lamont, 2003). Accordingly, an *S. oralis* AI-2 mutant was not able to form a dual species biofilm with *A. naeslundii* (McNab *et al.*, 2003). It is conceivable that the metabolic signaling of AI-2 in the natural setting of the oral biofilm is likely to influence the response of multiple species and model oral biofilm formation.

Interspecies interactions seemed to be not limited to diffusible signals and can be influenced by additional factors that may require receptor/ligand-type interactions. For example, interspecies surface interactions have been implicated in regulating gene expression in *S. gordonii* (Jakubovics *et al.*, 2008). Jakubovics and colleagues (2008) demonstrated that *S. gordonii* is unable to grow at low arginine concentrations. The presence of *A. naeslundii* in coaggregates, however, increased the expression of arginine biosynthetic genes in *S. gordonii*, thus complementing the growth defect at low arginine. Surprisingly, this was not observed when both species were grown in a dual-species suspension without visible aggregation, excluding a regulation dependent upon a diffusible signal.

Interactions Between Streptococci and Later Colonizers

Streptococcus–Fusobacterium *interactions*

Coaggregation likely mediates a variety of cooperative functions beyond metabolic complementation. An excellent example is the coaggregation between *Streptococcus cristatus* and *Fusobacterium nucleatum* (Edwards *et al.*, 2006). *F. nucleatum*, a Gram-negative fusiform anaerobe, is known for its ability to adhere and invade human gingival epithelial cells. Conversely, *S. cristatus* is not considered as an invasive species, but it has been shown that large numbers of *S. cristatus* are able to adhere to *F. nucleatum*, resulting in formations known as corncobs, due to their visual appearance under the microscope (Lancy *et al.*, 1983). The coaggregation is mediated by an arginine-sensitive interaction and actually enables *S. cristatus* to passively invade human epithelial cells (Edwards *et al.*, 2006, 2007). This mixed-species invasion has a significant effect upon the host response as described later.

Streptococcus–Porphyromonas *interactions*

In contrast to the corporative relationship with *F. nucleatum*, *S. cristatus* is able to sabotage colonization by another late colonizer. *S. cristatus* has been demonstrated to down regulate the expression of the *Porphyromonas gingivalis fimA* gene, which encodes the surface protein responsible for the attachment to other bacteria (Xie *et al.*, 2000). As a consequence, *P. gingivalis* fails to bind to *S. cristatus*. The down-regulation only occurs after the initial contact of the two organisms and seems to require the presence, but not activity, of the arginine deiminase protein of *S. cristatus* (Lin *et al.*, 2008). Hence, *S. cristatus* may have the ability to shape its own neighborhood by selecting who is allowed to bind. It is not hard to imagine how this ability could influence the composition of later colonizers into the developing biofilm. A more friendly relationship exists between *P. gingivalis* and *S. gordonii*. *S. gordonii* is able to recruit *P. gingivalis* into a mixed-species biofilm community. This depends on several gene-products and mechanisms, including cell signaling through AI-2 (Kuboniwa *et al.*, 2006).

Streptococcus–Aggregatibacter *interactions*

A similar lactic acid–based cross-feeding described for *S. mutans* and *Veillonella* seems to exist between *S. gordonii* and the late colonizer and periodontal pathogen *Aggregatibacter actinomycetemcomitans* (Brown and Whiteley, 2007). However, this dual-species interaction has an additional benefit for *A. actinomycetemcomitans*. The H_2O_2 produced by *S. gordonii* during dual-species coculture stimulates the expression of the complement resistance protein ApiA in *A. actinomycetemcomitans*, significantly increasing its resistance toward host-innate immunity (Ramsey and Whiteley, 2009). This example demonstrates that the interspecies interactions impact as well the interactions with the host, as described in the next section.

Interactions Between Streptococci and the Host

Interference with immune response

Not only are oral streptococci able to adhere to the hard tissue of the tooth, but they can also closely associate with epithelial cells. In fact, certain streptococci are able to invade oral buccal epithelial cells. Therefore, oral streptococci likely have a close interaction with the host immune system. This also raises a question: how can the host immune system distinguish commensal or beneficial species from pathogens? *F. nucleatum* clearly has an advantage to associate itself with *S. cristatus*. Zhang and colleagues (2008) demonstrated that after invasion into several oral epithelial cell lines, *F. nucleatum* elicits an immediate host response with increased interleukin-8 (IL-8) expression. Association with *S. cristatus* attenuates the induction. A similar observation was reported by Hasegawa and colleagues (2007). They found that *F. nucleatum* is able to increase the production of

the cytokines IL-6 and IL-8, but *S. gordonii* failed to trigger an immune response. This implies the existence of a specific discrimination mechanism in the oral immune system for commensal bacteria and pathogens. Alternatively, the commensal bacteria may have developed a mechanism to suppress an immune response. This was demonstrated for *S. sobrinus*, an oral streptococcus with cariogenic potential. Surprisingly, it was found that the immune suppression was due to the presence of the cytoplasmic glycolytic enzyme enolase localized on the bacterial cell surface. Enolase triggers the release of the antiinflammatory cytokine IL-10 (Veiga-Malta *et al.*, 2004). It would be interesting to determine whether other oral streptococci employ the same mechanism, as the *S. sobrinus* enolase has high homology with over 90% on the amino acids level to enolases from other oral streptococci, including *S. gordonii*, *S. sanguinis*, and *S. mutans*.

Adherence

The initial adherence of oral streptococci to epithelial cells is likely to involve multiple surface adhesins. The adhesion process has been investigated in detail for *S. gordonii*. The surface proteins of the antigen I/II adhesin family, SspA and SspB, are crucial for adhesion to epithelial cells. Both surface adhesins can bind directly to β1 integrin followed by internalization (Nobbs *et al.*, 2007a). The antigen I/II family is widely conserved among oral streptococci and might serve as cell adhesin in other oral streptococci following a similar mechanism (Jenkinson and Demuth, 1997; Nobbs *et al.*, 2007a). Additionally, the *S. gordonii* antigen I/II family mediates the binding to other oral bacteria, like *A. naeslundii* and salivary components (Egland *et al.*, 2001; Jakubovics *et al.*, 2005). These promiscuous binding capacities to diverse components of the oral cavity demonstrate a coevolution of the oral streptococci with host cells, salivary components, and other members of the oral biofilm.

Outlook

The interactions of oral streptococci are multifaceted. They include intra- and interspecies interactions as well as interactions with the host. Certainly numerous interactions have yet to be identified. These will likely reveal themselves, as greater efforts are devoted to include more environmental and host factors into our experimental model systems. For example, retainers for removable enamel chips have been developed that can be kept in a human mouth to follow the natural development of an *in vivo* biofilm. Although many questions cannot be addressed via this approach, improvements in many existing techniques may provide additional avenues to do so. The unprecedented wealth of information about individual members of the oral biofilm as well as the forthcoming metagenome and microbiome data of the oral biofilm provide us with the opportunity to develop one of the most sophisticated models of polymicrobial human-associated biofilms. With these data we may be able to achieve what was only dreamed of in the past: to manipulate the ecology of the biofilm to specifically favor the development of a healthy oral flora.

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No competing financial interests exist.

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