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REVIEW

Talk of the town: interspecies communication in oral biofilms

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Keywords: autoinducer-2; competence-stimulating peptides; dental plaque; mutualistic interactions; oral streptococci; saliva
Accepted 23 November 2009

SUMMARY

Mature dental biofilms consist of towering microcolonies in which the resident bacterial cells interact with one another and exchange messages in the form of signalling molecules and metabolites. These structures have been compared with the bustling office blocks and apartment buildings of busy cities. Social and communication networks are the lifeblood of large communities, and there is mounting evidence that mutually beneficial interactions between microbial cells are essential to the development of biofilms in the oral cavity. This review discusses the mutualistic partnerships that form between oral bacteria, and the contribution of interspecies communication to the formation of mixed microbial communities.

INTRODUCTION

Biofilms have been likened to miniature cities, with channels and voids permeating densely packed microcolonies, like roads and alleys running between tall buildings (Coghlan, 1996; Watnick & Kolter, 2000). Building a city is a complex process that requires input from a wide variety of specialists, from town planners and architects to engineers and labourers. Effective communication between workers is essential for the smooth running of the operation. In the same way, bacteria building oral biofilms adopt specialized roles and communicate with one another. The foundations of dental plaque are laid by the primary colonizers, predominantly streptococci, actinomyces and a few other genera. These organisms provide binding sites for coadhesion with other bacteria. As the biofilm grows in complexity, different microenvironments are formed within, and provide new niches for later colonizers, including periodontal pathogens such as *Porphyromonas gingivalis*, *Tannerella forsythia* and *Treponema denticola*. Throughout the development of dental plaque, adherent bacteria sense their neighbours and make appropriate responses. Some of these interactions involve signalling molecules that appear to have evolved specifically to elicit responses in neighbouring bacteria. In other cases, bacteria sense changes in their environment that are brought about by microbial metabolism in the oral biofilm. Understanding the adaptations that bacteria undergo to prosper in mixed species communities such as dental plaque promises to open new leads for controlling microbial biofilms. This review will discuss the evidence that oral bacteria form mutualistic associations and the role that interspecies communication plays in the development of oral microbial communities.

MUTUALISTIC INTERACTIONS IN MICROBIAL CONSORTIA

Saliva provides the major source of nutrients for bacteria in oral biofilms. Dental caries is dependent on the conversion of dietary sugars to acid. However,

evidence suggests that the host diet has little influence on the microflora during the early stages of dental plaque formation (Bowden & Li, 1997). The formation of microbial consortia has a major impact on the ability of oral bacteria to grow in biofilms supplied with saliva as the sole source of nutrients. For example, coculture is essential for biofilm formation under flowing saliva by *Streptococcus oralis* and *Actinomyces oris* (formerly *A. naeslundii* genospecies 2). *S. oralis* and *A. oris* are two common early colonizers, found in nascent dental plaque shortly after tooth cleaning. In a saliva-fed flow-cell biofilm model, neither *S. oralis* nor *A. oris* was able to grow as a monoculture. However, when these organisms were co-inoculated they flourished and produced a profuse biofilm (Palmer *et al.*, 2001). The mutualistic partnership between these organisms is dependent upon interspecies communication involving the signalling molecule autoinducer-2, and is discussed in more detail below.

Early colonizers may promote the establishment of other species that become more dominant in dental plaque as it develops. *Fusobacterium nucleatum* is thought to play a central role in the maturation of dental plaque because it coaggregates with almost all oral bacteria, including early colonizers and later colonizers (Kolenbrander *et al.*, 2006). Hence, *F. nucleatum* forms a coaggregation bridge between oral bacteria that do not naturally coaggregate with each other. Using *in vitro* models for biofilm formation, Periasamy *et al.* (2009) demonstrated that *F. nucleatum* was able to grow in biofilms with saliva as the sole source of nutrients when it was co-inoculated with *A. oris* and *S. oralis*. By contrast, *F. nucleatum* was unable to grow in monoculture. Similar community dynamics were observed in a static biofilm model, in which biofilms were formed on polystyrene pegs immersed in saliva, and transferred to fresh saliva every 12 h. Interestingly, in this model *F. nucleatum* grew between 12 h and 24 h when *A. oris* and *S. oralis* were present, but *F. nucleatum* cell numbers (measured by quantitative polymerase chain reaction) declined by 36 h. Therefore, although interactions between the three species were initially synergistic, competitive factors appeared to take over once the biofilm became established (Periasamy *et al.*, 2009).

In vivo, an individual's dental plaque may contain 100 or more different species of bacteria. Consequently, the scope for different interactions between oral bacteria is enormous. A relatively small number of *in vivo* studies in animal models have investigated the role of co-operation between bacteria in establishing biofilms. Interspecies co-operation appears to be important for the colonization of teeth by *Streptococcus mutans* and *Veilonella alcalescens* (McBride & Van der Hoeven, 1981). Colonization by *V. alcalescens* was dramatically enhanced when teeth were first colonized by *S. mutans*, and this effect depended upon the ability of the *V. alcalescens* strain to coaggregate with *S. mutans*. *In vitro* studies, such as those described above, are beginning to reveal the extent of synergism between oral bacteria in biofilms. The growth of *F. nucleatum*, for example, can be enhanced by both early colonizers and later colonizers. Hence, in flowcell biofilms supplied with saliva as the sole nutrient source, both *Veillonella* sp. and *Aggregatibacter actinomycetemcomitans* promoted the growth of *F. nucleatum* (Periasamy & Kolenbrander, 2009b; Fig. 1). In static biofilms, growth of *F. nucleatum* was not observed in the presence of *Veillonella* sp. and/or *A. actinomycetemcomitans*. Hence, the observed mutualistic interactions were apparently highly dependent on the prevailing flow of saliva. *In vivo*, there are significant differences in the flow of saliva over, for example, biofilms located on smooth surfaces close to the salivary glands compared with those in cracks and fissures or at the gingival margins, which are exposed to relatively stagnant saliva. Saliva flow may be an important factor in modulating the growth of microbial consortia in the oral cavity.

Using sensitive molecular techniques for the detection and identification of bacteria, it has been demonstrated that periodontal pathogens such as *P. gingivalis*, *A. actinomycetemcomitans* and *T. forsythia* are sometimes present at low abundance in supragingival dental plaque within 6–8 h after it starts to form on clean enamel surfaces (Li *et al.*, 2004; Diaz *et al.*, 2006). Recent evidence suggests that periodontal pathogens may be able to proliferate in saliva-fed biofilms as a result of mutualistic interactions with other bacteria. Thus, *A. actinomycetemcomitans* was able to grow in the presence of *Veillonella* sp. and/or *F. nucleatum*, but not in monoculture biofilms (Periasamy & Kolenbrander, 2009b; Fig. 1). *P. gingivalis* benefits from a variety of interactions with different bacteria. For example, under oxygenated conditions, the relatively aerotolerant

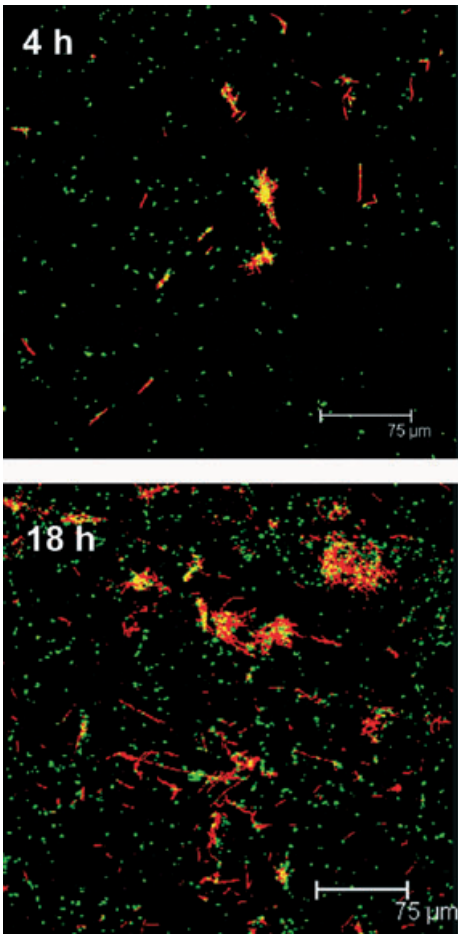

Figure 1 Mutualistic interactions between *Aggregatibacter actinomycetemcomitans* and *Fusobacterium nucleatum* promote the formation of mixed-species biofilms. Biofilms were formed on the surface of glass coverslips in flow cells using saliva as the sole nutrient source. Bacteria were labelled with fluorescent antibodies (*A. actinomycetemcomitans* labelled green, *F. nucleatum* red; co-localized cells appear yellow). Initial attachment (4 h after inoculation) is shown in the upper panel; the lower panel shows the biofilm after 18 h. By this time, both species had proliferated. By contrast, neither species was able to grow in monoculture (Periasamy & Kolenbrander, 2009b). Images were kindly provided by Dr S. Periasamy and Dr P. Kolenbrander.

F. nucleatum enables growth of the strict anaerobe *P. gingivalis* by scavenging O_2 (Diaz *et al.*, 2002). Furthermore, succinic acid produced by *T. denticola* serves as a nutrient source for *P. gingivalis* (Grenier, 1992). In turn, isobutyric acid from *P. gingivalis* stimulates the growth of *T. denticola*. *P. gingivalis* and *T. denticola* are frequently found together in dental plaque (Byrne *et al.*, 2009) and these organisms act synergistically to form biofilms (Kuramitsu *et al.*, 2005). Five other oral bacteria, *S. gordonii*, *A. oris*, *F. nucleatum*, *Veillonella* sp. and *A. actinomycetemcomitans*, were also shown to promote the growth of *P. gingivalis* in biofilms (Periasamy & Kolenbrander, 2009a). In each case, the interactions were mutualistic: growth of the partner organism was also enhanced. In this study, only one species tested (*S. oralis*) was incompatible with *P. gingivalis*. *S. oralis* inhibited mutualism between *P. gingivalis* and *A. actinomycetemcomitans* or *F. nucleatum*, but not between *P. gingivalis* and *S. gordonii*. Therefore the ability of periodontal pathogens to successfully colonize dental plaque may be strongly influenced by both synergistic and competitive interactions with other members of the oral microflora. Competition is clearly an important driving force in the dynamics of caries-associated oral biofilm communities. Advancing caries lesions are associated with decreased pH in the oral biofilm as a result of the activity of acidogenic and aciduric bacteria such as *S. mutans* and lactobacilli. This in turn reduces the overall microbial diversity in the vicinity because many oral bacteria are ill-equipped to survive prolonged periods of low pH (Li *et al.*, 2005). At moderately low pH, around pH 5.5, oral streptococci invoke adaptive responses to tolerate the acidic conditions (Quivey *et al.*, 2001). Therefore, acid produced by oral bacteria may be considered a chemical cue that induces changes in the surrounding microorganisms. However, as these adaptive changes are primarily defensive mechanisms, they will not be discussed in detail here. Instead, this review will focus on the role of communication in promoting the building of microbial networks.

METABOLIC CO-OPERATION

Saliva is a mixture of complex proteins and glycoproteins that are not easily digested by bacteria. The efficient degradation of saliva requires the concerted activities of many different enzymes, and microbial consortia are better equipped to carry out this role than individual species in isolation. This was clearly demonstrated for the growth of oral bacteria on hog gastric mucin, a model for human salivary mucins (Bradshaw *et al.*, 1994). A consortium of five different bacteria (*S. mutans*, *S. gordonii*, *Neisseria subflava*, *Veillonella dispar* and *F. nucleatum*) grew reasonably well on this substrate in a chemostat. However, when

additional species, *S. oralis*, *A. naeslundii*, *Lactobacillus casei*, *P. gingivalis* and *Prevotella nigrescens*, were introduced into the system, the total microbial load supported in the chemostat increased. The new species contained novel proteolytic and glycolytic enzyme activities, and the observed biomass increase in the system indicated that the expanded consortium released more energy from the substrate (Bradshaw *et al.*, 1994). Similarly, freshly isolated dental plaque was able to use terminal carbohydrates from purified human salivary mucin MUC5B, whereas single species isolated from the consortium could not (Wickström & Svensäter, 2008). Dental plaque can also break down the peptide backbone of MUC5B, and this activity was attributed to a four-species community consisting of three streptococci (*S. mitis* biovar 2, *S. gordonii*, *S. cristatus*) and *A. naeslundii* (Wickström *et al.*, 2009). Interestingly, proteolytic activity was strongly induced in the consortium during growth on MUC5B, but was not induced in single-species cultures. An intriguing question is whether the members of the consortium sense that they are in a mixed-species environment and upregulate proteases in preparation for syntrophic metabolism, or whether the stimulus for induction of proteases originates from the degraded MUC5B products. The former case would represent a clear instance where interspecies communication would benefit the participating organisms by maximizing their potential to extract energy from a substrate.

MECHANISMS OF COMMUNICATION

From an ecological standpoint, communication is the process by which organisms pass information in the form of signals from a sender to a receiver, and induce responses such as behavioural change or altered gene expression in the receiver (Keller & Surette, 2006). In this context, a signal is a substance that has specifically evolved to bring about responses in the receiving organism. Bacteria also sense changes in the local environment (cues) that are caused by neighbouring cells, and in this way receive information about the surrounding microbial population. In theory, signals that have evolved over many generations specifically for the purpose of conveying information from one cell to another should be robust and finely tuned because the costs of producing the signal must have been outweighed by the benefits of communication. Otherwise, the signal would not have succeeded through natural selection. There is a high chance that strategies designed to interfere with these signals will decrease the ability of the communicating bacteria to form mutualistic associations. On the other hand, chemical cues are often the products of bacterial metabolism, and as such have no production costs. The organism producing the cue may not be deliberately sending a message. Nevertheless, information about the sender is communicated to the receiver and results in altered gene expression and phenotype. There are many examples of secondary metabolites that are produced by some members of microbial communities and sensed by others, and this type of metabolic communication plays important roles in the development of varied biofilm communities (Monds & O'Toole, 2008). Some of the major signals and cues involved in interspecies metabolic communication between oral bacteria are discussed below.

Signalling molecules

Two classes of molecules produced by oral bacteria have been implicated as true signals, produced specifically for the purposes of cell-to-cell communication. These are competence-stimulating peptides (CSPs), synthesized by gram-positive bacteria such as streptococci, and autoinducer-2 (AI-2). There is some debate regarding the status of AI-2 as a true signal because this molecule appears to have a primary role in metabolism in at least some bacteria (Holmes *et al.*, 2009). Nevertheless, the widespread changes in gene expression induced by AI-2, combined with its apparent activity at extremely low concentrations (in the order of tens of nM or less) are consistent with a role in signalling. AI-2 and CSPs appear to be involved in intraspecies quorum sensing. However, there is accumulating evidence that they are also involved in a variety of interspecies interactions between bacteria (Fig. 2 and discussion below).

CSPs are short peptides, approximately 17–21 amino acids, produced by many streptococci from proteolytic digestion of the *comC* gene product. Historically, CSPs have been considered species-specific or even strain-specific signals. In *S. pneumoniae*, for example, strains that produce chemically distinct CSP molecules do not respond to each

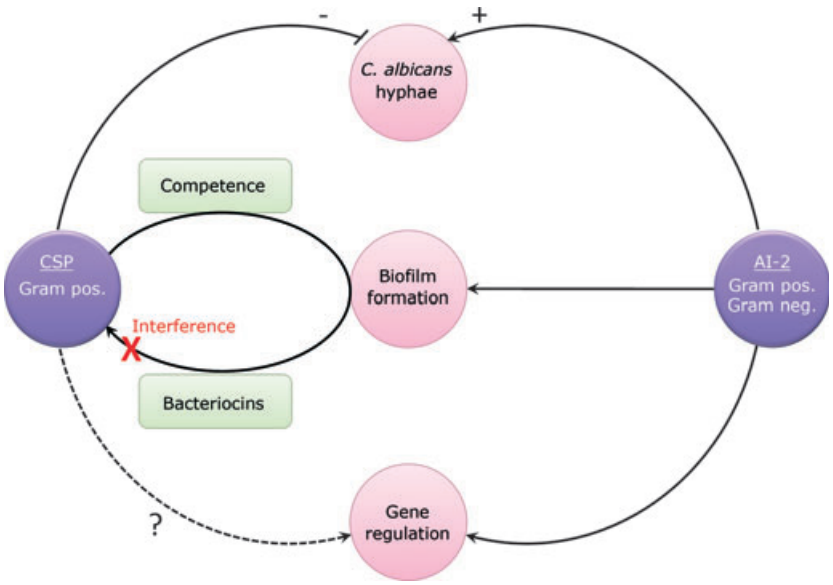

Figure 2 Comparison of interspecies signalling pathways involving competence stimulating peptides (CSPs) and autoinducer-2 (AI-2). AI-2 can be sensed by bacteria other than the organism producing the signal, resulting in marked changes in gene expression. It seems likely that CSPs of oral streptococci may be sensed by species that are distinct from those producing the CSP, but this has yet to be empirically demonstrated (indicated by a dashed line and question mark). CSP and AI-2 appear to have opposing effects on *Candida albicans* hyphal formation: CSP from *Streptococcus mutans* inhibits the formation of hyphae (–), whereas *Streptococcus gordonii* AI-2 is involved in promoting the initiation of hyphae (+). AI-2 is required for single-species or mixed-species biofilm formation by several oral bacteria, and the species that respond to the signal are not necessarily those that produce it. Sensing of CSP by the cells that produce it stimulates biofilm formation, competence for genetic transformation or the production of bacteriocins. Interference with the *S. mutans* CSP signal by different species of oral streptococci has been demonstrated, and enables these organisms to reduce the ability of *S. mutans* to compete effectively. Details of these pathways are discussed in the text.

other's signals. However, the existence of such specific pherotypes is not so clear in oral bacteria. CSPs from 36 *S. mutans* strains, isolated from several geographically separated locations, were virtually identical (Allan *et al.*, 2007). Three slightly different *S. mutans* CSPs, when added exogenously to *S. mutans* cultures, were able to induce competence not only in their cognate hosts (i.e. the strains that produced the specific CSP variant tested), but also in other strains. Oral streptococci are highly prevalent in dental plaque biofilms, and different species of streptococci interact with one another. At present virtually nothing is known about whether CSP signals from one species of oral streptococcus can cross the species barrier and induce competence or other responses in a different streptococcal species. However, it has recently been reported that *S. mutans* CSP inhibits the formation of hyphae in *Candida albicans* (Jarosz *et al.*, 2009), indicating that the ability to respond to CSP may not be confined to streptococci, or even to the domain Bacteria. Based on current sequence information, CSPs appear to be highly divergent: CSP variants are not conserved in different species of streptococci. However, sequence information is currently limited. The human microbiome project (Peterson *et al.*, 2009), which includes the sequencing of 1000 novel oral bacterial genomes along with extensive metagenomic analyses of the oral microflora, promises to shed more light on the extent of variation in *comC* gene sequences between different species and strains of oral streptococci.

CSPs have diverse effects on oral streptococci, including promoting competence, biofilm formation and DNA release (Perry *et al.*, 2009). In addition, the CSP sensing pathway in *S. mutans* is linked to the production of mutacins, bacteriocins with antimicrobial activity against a range of oral bacteria (Wang & Kuramitsu, 2005). There is evidence that oral microorganisms may interfere with the *S. mutans* CSP signal and consequently inhibit mutacin release. *S. gordonii*, *S. sanguinis*, *S. mitis* and *S. oralis* were shown to inhibit mutacin production by degrading

S. mutans CSP (Wang & Kuramitsu, 2005). In each case, addition of exogenous CSP fully restored mutacin production. The CSP-degrading factor of *S. gordonii* was identified as an extracellular protease termed challisin. *S. salivarius* was also shown to degrade *S. mutans* CSP and to inhibit biofilm formation (Tamura *et al.*, 2009). *S. mutans* and *S. salivarius* are not usually found in close proximity in the oral cavity because *S. mutans* colonizes tooth surfaces whereas *S. salivarius* is almost exclusively localized to soft tissues. However, it is possible that interactions between these organisms occur at a distance. In view of the multifunctional roles of CSP in oral bacteria, further investigations into the effects of mixed populations on CSP-mediated signalling are urgently required.

The molecule AI-2 has received a great deal of attention in recent years because it is so far the only signalling molecule found to be widespread among both gram-positive and gram-negative bacteria (Federle & Bassler, 2003). AI-2 is a product of the activated methyl cycle, generated by LuxS-mediated cleavage of the intermediate *S*-ribosylhomocysteine to homocysteine and 4,5-dihydroxy-2,3-pentanedione (DPD) (Chen *et al.*, 2002). In solution, DPD dissociates into several forms that are collectively known as AI-2. The *luxS* gene, encoding *S*-adenosylhomocysteinase (LuxS) is present in the genome sequences of many oral bacteria. Hence, a BLAST search with the translated amino acid sequence of *Escherichia coli* LuxS identified significant homology (*E* value $< 1 \times 10^{-10}$) with sequences in 25 different oral bacterial genomes from a total of 62 genome sequences currently annotated at the Human Oral Microbiome Database (Dewhirst *et al.*, 2008; Table 1). At least six different genera of oral bacteria have been shown to produce AI-2 at sufficient levels for detection using a *Vibrio harveyi* luminescence-based bioassay (Table 1). Detection of AI-2 by bacteria leads to profound changes in gene expression: for example, using DNA microarrays, 59 genes were found to be regulated in response to AI-2 in *S. mutans* (Sztajer *et al.*, 2008).

Many bacteria that respond to AI-2 also produce it, and therefore AI-2 may function as an intraspecies signal. However, there is evidence that AI-2 is also important for interspecies interactions in microbial communities. A screen of oral bacteria for AI-2 production found that the highest levels of AI-2 were produced by periodontal pathogens such as *P. gingivalis*, *Prevotella intermedia* and *F. nucleatum* (Frias *et al.*, 2001). In addition, *A. actinomycetemcomitans* was shown to possess a *luxS* gene and produce AI-2 (Fong *et al.*, 2001). The *luxS* gene from *A. actinomycetemcomitans*, when expressed in *E. coli*, was able to complement a *P. gingivalis luxS* mutant by restoring the expression of two AI-2 responsive genes, *uvrB* and *hasF*, to wild-type levels (Fong *et al.*, 2001). This study demonstrated that *P. gingivalis* can detect the AI-2 signal from *A. actinomycetemcomitans*, and provided the first indication that interspecies communication using AI-2 may occur in oral biofilms.

Clearer evidence for AI-2-mediated interspecies communication by *P. gingivalis* came from coculture studies with *S. gordonii* (McNab *et al.*, 2003). In these investigations, the *luxS* gene was knocked out in both *P. gingivalis* and *S. gordonii*. Biofilms formed

Table 1 Presence of the *luxS* gene in the genomes of oral bacteria, annotated at the Human Oral Microbiome Database (Dewhirst *et al.*, 2008)

Species with *luxS* gene	Reference demonstrating AI-2 production
Actinomyces odontolyticus	
Actinomyces oris (*A. naeslundii* MG1)	(Rickard *et al.*, 2008)
Aggregatibacter actinomycetemcomitans	(Fong *et al.*, 2001)
Atopobium rimae	
Bifidobacterium dentium	
Campylobacter rectus	
Capnocytophaga sputigena	
Eikenella corrodens	
Enterobacter cancerogenus	
Fusobacterium nucleatum	(Frias *et al.*, 2001)
Gemella haemolysans	
Lactobacillus paracasei	
Neisseria flavescens	
Neisseria lactamica	
Neisseria mucosa	
Neisseria subflava	
Porphyromonas gingivalis[1]	(Frias *et al.*, 2001)
Prevotella intermedia	(Frias *et al.*, 2001)
Propionibacterium acnes	
Streptococcus gordonii	(Blehert *et al.*, 2003)
Streptococcus mitis	
Streptococcus mutans	(Sztajer *et al.*, 2008)[2]
Streptococcus sanguinis	
Treponema lecithinolyticum	

[1]Two strains of *P. gingivalis* are in the database; both contain *luxS*.
[2]Reference demonstrates responses to autoinducer-2 (AI-2).

by a pair of *luxS* mutants lacked the structural characteristics, in particular the presence of microcolonies, compared with biofilms formed from wild-type *P. gingivalis* and wild-type *S. gordonii*. Wild-type *P. gingivalis* formed biofilm microcolonies with a *luxS* mutant of *S. gordonii*, and a *luxS* mutant of *P. gingivalis* formed structured biofilms with wild-type *S. gordonii*. These data indicate that *S. gordonii* and *P. gingivalis* sense AI-2 produced by either organism, and translate the signal into a biofilm phenotype. Sensing *S. gordonii* leads to changes in the expression of approximately 1–2% of *P. gingivalis* genes including *mfa1*, encoding minor fimbriae, and at least two genes that are required for normal mixed-species biofilm formation (Park *et al.*, 2006; Simionato *et al.*, 2006). However, the role of AI-2 in these responses was not explored.

An important function for AI-2 in mutualistic associations was demonstrated in studies of dual-species biofilms formed with *A. oris* and *S. oralis* (Rickard *et al.*, 2006, 2008). *In vitro*, *S. oralis* and *A. oris* monocultures formed poor biofilms in saliva-fed flowcells. By contrast, coculture of *S. oralis* and *A. oris* produced good growth of both partner organisms, and the formation of a relatively thick biofilm (Palmer *et al.*, 2001). Disruption of the *luxS* gene in *S. oralis* abrogated its ability to form luxuriant biofilms in partnership with *A. oris* (Rickard *et al.*, 2006). Complementation, either by restoring *luxS* expression in *S. oralis* or by adding synthetic DPD, restored the mutualistic partnership, indicating that AI-2 is an important signal in this interspecies interaction.

Recently, a function for AI-2 in communication between *S. gordonii* and *C. albicans* was reported (Bamford *et al.*, 2009). The *S. gordonii* cells, or spent culture supernatants, induced extensive hyphal formation by *C. albicans*. However, culture supernatants from an *S. gordonii luxS* mutant did not induce *C. albicans* hyphal formation, indicating that *C. albicans* can sense AI-2 from *S. gordonii*. Nevertheless, the addition of synthetic AI-2 to *C. albicans* cultures did not induce the production of hyphae, and it is likely, therefore, that communication between *S. gordonii* and *C. albicans* involves signals or cues in addition to AI-2. The transition from yeast to hyphal forms in *C. albicans* is controlled by a complex regulatory pathway, and is influenced by a variety of extracellular stimuli, one of which is hydrogen peroxide (H_2O_2). Concentrations of H_2O_2 between 0.5 and 10 mm stimulate hyphal outgrowth. *S. gordonii* secretes H_2O_2 and it is possible that this molecule contributes, at least in part, to the increased hyphal production by *C. albicans*.

Metabolic cues

Streptococcal H_2O_2 is an important molecule in competition and communication between oral bacteria. H_2O_2 is secreted by viridans streptococci, including *S. sanguinis*, *S. oralis*, *S. mitis*, *S. gordonii*, *S. parasanguinis* and some strains of *S. mutans*, and is responsible for the greenish tinge (α-haemolysis) produced when these organisms are cultured on blood agar. *In vitro*, in closed batch culture, H_2O_2 can reach concentrations sufficient to kill many other oral bacteria. However, the mouth is an open system in which small molecules such as H_2O_2 are continually washed away from the biofilm. At sublethal concentrations, H_2O_2 has been shown to induce a variety of responses in oral bacteria. In the viridans streptococci *S. sanguinis* and *S. gordonii*, H_2O_2 triggers the release of DNA by a mechanism that does not involve cell lysis (Kreth *et al.*, 2009). Extracellular DNA is an important component of bacterial biofilms. Treating *S. mutans* biofilms with DNase I, for example, reduces the surface-associated biomass by approximately 20% (Perry *et al.*, 2009). The release of DNA by streptococci may therefore help to stabilize the biofilm structure. H_2O_2 oxidizes macromolecules, and *S. gordonii* proteins are extensively oxidized during growth in aerobic batch culture (Jakubovics *et al.*, 2008b). Some strains of *A. oris* produce catalase which degrades H_2O_2. In a coculture containing *S. gordonii* and a catalase-producing *A. oris* strain, the concentration of H_2O_2 was two-fold to three-fold lower than in *S. gordonii* monocultures. Levels of protein oxidation in *S. gordonii* were dramatically reduced in cocultures with *A. oris* compared with monocultures. These data support the intriguing hypothesis that *S. gordonii* may use H_2O_2 not only as a competitive antibacterial compound, but also as a sensing molecule to obtain information about the surrounding microbial population.

Viridans streptococci produce lactate, which is used as a substrate for energy production by *A. actinomycetemcomitans* and by *Veillonella atypica*. Interestingly, both of these organisms have been shown to communicate with oral streptococci. In the

case of *A. actinomycetemcomitans*, sensing occurs through detection of H_2O_2 (Ramsey & Whiteley, 2009). *A. actinomycetemcomitans* produces a very restricted response to H_2O_2: just two genes were significantly upregulated by H_2O_2 in a microarray analysis (Ramsey & Whiteley, 2009). These were *katA*, encoding catalase, and *apiA*, encoding a 33-kDa outer membrane protein that binds to the human serum protein factor H and provides resistance to serum killing. In agreement with the function of ApiA in serum resistance, coculture with *S. gordonii* reduced the sensitivity of *A. actinomycetemcomitans* to human serum. It is not clear why *A. actinomycetemcomitans* responds to streptococcal H_2O_2 in this way. One possibility is that H_2O_2 signals an increased likelihood of inflammation, and *A. actinomycetemcomitans* benefits by preparing for the inflammatory onslaught.

Interspecies communication between oral streptococci and veillonellae appears to be driven by metabolic requirements. A number of studies have demonstrated that *Veillonella* spp. benefit from the presence of lactic acid-producing bacteria such as *Lactobacillus* spp., *S. mutans* and other oral streptococci. The interaction between *S. gordonii* and *V. atypica* involves metabolic signalling between the two organisms. *V. atypica* produces a soluble signal that induces expression of the *S. gordonii amyB* gene, encoding α-amylase, in juxtaposed *S. gordonii* cells (Egland *et al.*, 2004). *S. gordonii* stores intracellular polysaccharides during growth on rich media, and α-amylase is involved in the utilization of these stores when nutrients become scarce. By tricking *S. gordonii* into mobilizing its energy reserves, *V. atypica* causes *S. gordonii* to increase production of lactate, the primary energy source for *V. atypica*. The *S. gordonii* carbon catabolite protein CcpA is required for induction of *amyB* (Johnson *et al.*, 2009), indicating that the *V. atypica* signal may be a simple carbohydrate such as maltose, that is present in *V. atypica* lipopolysaccharide. Indeed, addition of maltose to *S. gordonii* resulted in induction of amylase (Johnson *et al.*, 2009). Cell–cell contact is not required for communication between *V. atypica* and *S. gordonii*, because separation of these species by a dialysis membrane did not prevent the upregulation of *S. gordonii* amylase in response to *V. atypica* (Egland *et al.*, 2004). Therefore, the signal must be released from *V. atypica* cells. If the signal is part of the lipopolysaccharide molecule, it may be released during cell wall turnover or following cell lysis. Clearly, there is plenty of scope for further investigations to elucidate the nature of this interesting signalling interaction.

The role of cell wall contact in initiating gene regulation has been studied using a coaggregating pair of oral microorganisms, *S. gordonii* and *A. oris*. A DNA microarray for *S. gordonii* was used to search for gene regulation in response to the formation of extensive mixed-species communities containing *A. oris* (Jakubovics *et al.*, 2008a). *S. gordonii* and *A. oris* are common early colonizers in dental plaque and these bacteria can be found in close proximity on tooth enamel. Many strains of *S. gordonii* and *A. oris* coaggregate, and the large aggregates formed are in many ways similar to biofilm communities. Comparison of *S. gordonii* gene expression in coaggregates with that in monocultures revealed a set of 23 genes that were significantly regulated following the formation of mixed-species communities. Of these, nine genes were involved in arginine biosynthesis. *S. gordonii* was shown to benefit from the presence of *A. oris* in arginine-restricted conditions. In cocultures, *S. gordonii* grew aerobically in low arginine, whereas in equivalent monocultures no growth was observed. Interestingly, coaggregation was essential for the change in arginine biosynthesis gene expression and for the ability of *A. oris* to enable *S. gordonii* growth in low arginine. These effects were not observed in cocultures that did not exhibit coaggregation (Jakubovics *et al.*, 2008a). Therefore, cell–cell contact apparently plays a fundamental role in communication between *S. gordonii* and *A. oris*.

FUTURE PERSPECTIVES

As in a city, where vibrant communities are formed by the mixing of people from different cultural and ethnic backgrounds, oral bacteria in biofilms sense their microbial neighbours and build productive mixed-species consortia. Signalling between bacteria may have important implications for the virulence of oral pathogens. For example, *Streptococcus cristatus* downregulates the expression of the *P. gingivalis* major fimbria gene *fimA* and inhibits biofilm formation by *P. gingivalis* (Xie *et al.*, 2007). Recent *in vivo* observations showing a negative correlation between *S. cristatus* and *P. gingivalis* support an important

role for this interaction in subgingival dental plaque (Wang *et al*., 2009). *S. cristatus* also modulates the ability of oral epithelial cells to respond to *F. nucleatum* (Zhang *et al*., 2008). Therefore, when assessing the ability of oral bacteria to cause disease it is essential to consider the community in its entirety rather than relying solely on observations of individual components. The ongoing development of high-throughput techniques such as DNA microarrays and massively parallel sequencing is already greatly enhancing studies of gene expression in mixed-species communities. It remains to be seen whether these approaches will lead to new interventions that can change the course of oral microbial diseases.

ACKNOWLEDGEMENTS

I am grateful to Dr S. Periasamy and Dr P. Kolenbrander for supplying images, and to Dr S. Hogg for helpful comments on the manuscript.

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