



## Signal peptide pheromones in oral streptococcal biofilms

Oral streptococci are known to modulate various physiological properties by responding to secreted signal peptide pheromones. These molecules accumulate in the environment and trigger a genetic response in the target cells at a threshold cell density, thereby synchronising a metabolic outcome with population density. In this review, we summarise how signal peptide pheromones of oral streptococci function. The possibility of targeting these peptide pheromones for the prevention of biofilm-associated infections such as dental caries is also discussed.

The oral cavity harbours over 700 different bacterial taxa, representing one of the most diverse and complex ecosystems in the human body<sup>1</sup>. Oral bacteria grow in physically and physiologically distinct niches found in the mouth in a multi-species biofilm community. Dental plaque is an example of a microbial biofilm with a very complex microbial composition. After a thorough cleaning, oral streptococci and *Actinomyces* spp. are the first to appear on the tooth surface and, as the biofilm

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matures, it becomes more diverse and ecologically stable. Aciduric and acidogenic streptococci, including *Streptococcus mutans*, can increase their numbers in plaque when provided with a continued supply of dietary carbohydrates. We are now beginning to understand the molecular mechanisms used by this bacterium to survive and predominate under these conditions that often result in dental caries. Bacteria living in biofilms display a phenotype that is distinct from that exhibited by their free-living planktonic counterparts, including an increased tolerance to antimicrobials and other physical and chemical assaults. Currently, there is a rethinking of strategies that will likely lead to novel control agents of biofilm-

associated infections, such as dental caries and periodontal disease.

Bacteria are able to sense and respond to changes in the cell population density by monitoring the accumulation of secreted signal peptide pheromones resulting in altered gene expression<sup>2</sup>. This phenomenon known as quorum-sensing (QS) enables cell-cell communication within and between bacterial populations. In this report, we will discuss the various phenotypes controlled by QS in oral streptococci, as well as the potential presented by these systems to control biofilm-associated infections.

### Quorum-sensing in oral streptococci

Oral streptococci constitute approximately 20% of the normal human oral flora. They colonise the mouth and upper respiratory tracts of humans as commensal bacteria. Gram-positive bacteria, including oral streptococci, use secreted peptides as signalling molecules in concert with a two-component signal transduction system comprised of a membrane-bound histidine kinase (HK)

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sensor protein and an intracellular response regulator (RR) to detect the accumulation of specific signal molecules that trigger an appropriate response by altering gene expression (Figure 1). In streptococci, a variety of QS signalling peptides have been discovered, which include antimicrobial peptides and the competence stimulating peptide (CSP). Interestingly, their allelic variation and receptor-specificity enable a specific species to determine the 'type' and density of its population. While communication elicited by streptococcal peptides was initially thought to be exclusively intra-species, evidence of cross-species communication induced by the *Streptococcus salivarius* signal peptide salivaricin A (SaIA) was found to stimulate lantibiotic SaIA1 production from different *Streptococcus pyogenes* strains, demonstrating cross-species communication<sup>3</sup>.

## Quorum-sensing in pneumococci

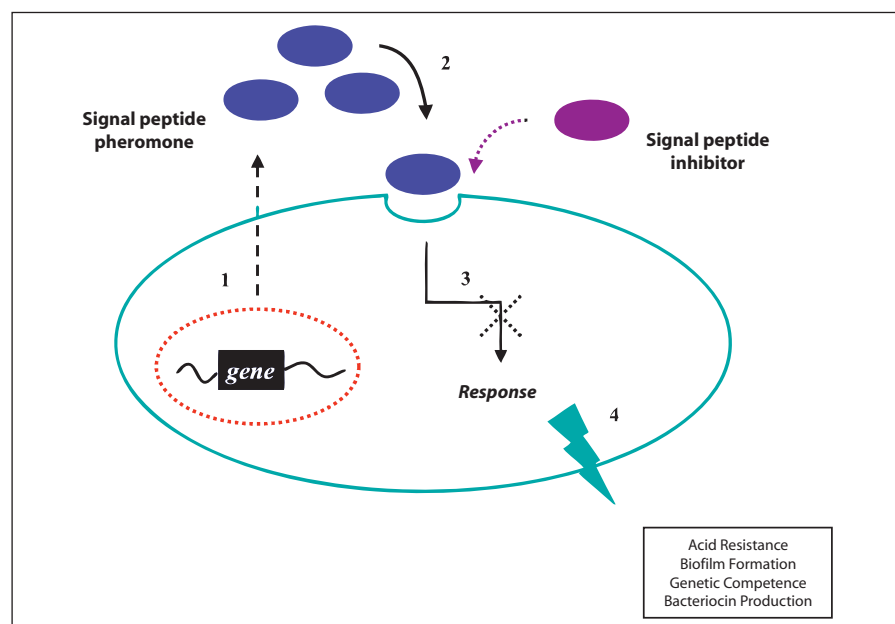
The mechanism of peptide-mediated QS is best characterised in *Streptococcus pneumoniae*, which uses this process to induce competence for natural genetic transformation and to produce several class II type bacteriocin-like peptides<sup>4</sup>. In *S. pneumoniae*, competence development and bacteriocin production are regulated by two independent QS processes consisting of the ComABCDE and the BlpABCSRH systems, respectively. Each QS system has its own auto-regulatory signal peptide consisting of CSP encoded by *comC* and the bacteriocin-inducing peptide (BIP) encoded by *blpC*, which are both post-translationally processed and exported extracellularly via the ComAB and BlpAB transporters, respectively. At a particular growth-phase, these peptides are

detected by their respective HK sensor protein (ComD/BlpH), which results in the autophosphorylation at a specific histidine residue. The phosphoryl group is then transferred to the cognate RR inducing a conformational change in the regulatory domain and the activation of the RR. The activated form of the RR binds to the promoters of several target genes and regulates their transcription. Interestingly, an *S. pneumoniae comD* null mutant showed reduced virulence in a murine infection model demonstrating the involvement of QS in pneumococcal pathogenicity as well<sup>5</sup>. Out of these two QS systems, the molecular genetics of the *S. pneumoniae* competence regulon has been extensively studied. DNA microarray studies of the global gene expression conducted by Peterson<sup>6</sup> revealed that following the induction with CSP, at least 188 genes are transiently affected during four temporally distinct expression profiles categorised as early, late, delayed gene induction and gene repression<sup>6</sup>. Notably, a large cluster of these genes belonged to the stationary growth phase and although many had a direct role in DNA uptake and homologous recombination, the variety of processes affected by the CSP suggested a broad role in the alteration of the bacterium's phenotype when living in close proximity to its neighbours.

## Quorum-sensing-mediated phenotypes in oral streptococci

The first connection between QS signaling peptides and biofilms was discovered in *Streptococcus gordonii*, where a biofilm defective transposon-generated mutant was found to have a mutation in the *comD* gene encoding the HK receptor involved in competence<sup>7</sup>. In *S. mutans*, the efficiency of genetic transformation was found to be 10 to 600 times greater when cells were grown as a biofilm as opposed to the planktonic state<sup>8</sup>. In addition to genetic competence, *S. mutans* regulates a number of other physiological processes via a 21 amino acid CSP suggesting that this peptide may be the 'master switch' activating the biofilm phenotype. Data to support this hypothesis include the involvement of

Figure 1. Schematic representation of oral streptococcal QS. 1) In the first step, the species-specific signal peptide pheromone is synthesised and exported from the cell. 2) The accumulation of the signal peptide pheromones, as a function of cell population density, is sensed by the HK resulting in autophosphorylation followed by phosphotransfer to a cognate (RR). 3) The phosphorylated RR activates and/or represses genes involved in the physiological response. 4) In oral streptococci, QS regulates many important virulence factors including bacteriocin production, biofilm formation, acid resistance, and the activation of natural genetic competence. Interference with the expression of virulence factors by specifically targeting the QS process is a promising strategy for the control of infectious diseases. This can be achieved by signal peptide inhibitors that are capable of competing and interfering with the native signal peptide pheromone for binding to the HK receptor. Attenuation of virulence by interfering with QS processes has special promise with infectious biofilms where signal molecules are often involved in switching to the biofilm phenotype.





CSP in biofilm architecture<sup>9</sup> and acid tolerance<sup>10</sup>. Optimisation of these systems via QS appears essential for survival of *S. mutans* in biofilms. Further work has shown that *Streptococcus intermedius* also has a peptide-mediated QS system that functions optimally in biofilms<sup>11</sup>, suggesting that many related bacteria also rely on this density-modulated phenotypic switch. By employing PCR to map the incidence of *comCDE* alleles in oral streptococci, it was demonstrated that a wide variety of streptococcal species belonging to the mitis, anginosus, mutans, and sanguinis groups possessed the *comCDE* genes<sup>12</sup> and are likely to use a CSP-mediated QS system during biofilm growth. A clue as to some of the functions of CSP in a mixed community emerged by the discovery that bacteriocin production by *S. mutans* is a QS-dependent mechanism. Interestingly, it has been demonstrated that interaction with other oral streptococci interfered with *S. mutans* bacteriocin production and that this inhibition was due to the inactivation of *S. mutans* CSP<sup>13</sup>. This inhibition was more significant during growth in biofilms.

## Concluding remarks and future perspectives

Many oral streptococci serve as primary colonisers of the tooth pellicle, thereby promoting the formation of a dental biofilm. While dental biofilm is implicated in causing infections, its formation and functional integrity is, at least partly, dependent on the QS systems of its constituent species. Hence, these systems provide potential targets to develop therapeutics against biofilm-associated infections. For example, research is being conducted on the design and development of *S. mutans* CSP analogs that could interfere with the CSP-dependent QS process leading to inhibition of activation of the biofilm phenotype<sup>14</sup>. Due to the ability to attenuate virulence traits without necessarily killing constituents in a mixed bacterial consortia, targeting QS provides a unique tool that is worth extensive investigation.

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