10.5005/jp-journals-10024-1080

REVIEW ARTICLE

Quorum Sensing in Plaque Biofilms: Challenges and Future Prospects

Baswaraj Biradar, Prapulla Devi

ABSTRACT

Aim: This review intends to provide a brief overview regarding quorum sensing among bacteria in biofilms and also attempts to throw light on the new research focusing on interference with the quorum sensing.

Background: Dental plaque is an example of microbial biofilm leading to periodontal disease and dental caries. Quorum sensing is widely employed by a variety of gram-positive and gram-negative bacterial species to coordinate various activities in biofilms. Quorum-sensing-interfering compounds have either a positive or a negative effect on the expression of bacterial phenotypes regulated by quorum sensing. These studies of bacterial quorum sensing have also suggested several ideal targets for drug design which can be promising in preventive and therapeutic aspects of periodontal diseases and dental caries.

Results: Studies have shown that periodontal disease and dental caries is caused by plaque biofilm bacteria. Quorum sensing is the means of communication between these bacteria to regulate a wide range of behavior patterns among them. The *in vitro* studies reviewed here have a vital role in opening up this field, because they reveal the basic machinery of cell—cell signaling in microbial communities. The signal machinery bacteria use to coordinate a variety of their activities is identified by these studies.

Further, this review aims to discuss several natural and synthetic methods which were used for manipulating bacterial quorum sensing.

Conclusion: The future challenge lies in the ability of the dental research to develop additional mechanisms for interfering with bacterial quorum sensing which can be used as preventive and therapeutic tools for combating oral polymicrobial diseases.

Clinical significance: This article aims at reviewing the literature and helping us to understand the ways of communication among bacteria in biofilms, which further open up the prospects in the treatment of diseases caused by biofilms.

Keywords: Quorum sensing, Biofilm, Acylated homoserine lactones, Autoinducers.

How to cite this article: Biradar B, Devi P. Quorum Sensing in Plaque Biofilms: Challenges and Future Prospects. J Contemp Dent Pract 2011;12(6):479-485.

Source of support: Nil

Conflict of interest: None declared

INTRODUCTION

Dental plaque is an example of microbial biofilm with a very complex microbial composition. As many as 500 different species of bacteria have been isolated from the oral cavity.[1] These bacteria exhibit coordinated group behaviors and are responsible for causing periodontal infections and dental caries.

Bacteria in biofilms encounter much higher local cell densities than free-floating, planktonic cell populations. An obvious consequence of this, is the elevated levels of metabolic by-products, secondary metabolites and other secreted or excreted microbial factors that biofilm cells encounter. Of particular interest are intercellular signaling molecules called the 'quorum-sensing molecules.' This ability, termed quorum sensing, functions through the secretion and detection of autoinducer (AI) molecules which accumulate in a cell density dependent manner. When the concentration of autoinducers reaches a threshold level, activation of the receptor leads to a signal transduction cascade to switch on specific genes in the bacterial cells, leading to a coordinated population response. As a group, bacteria behave in one way when there are few bacteria around them and in a different way when there are many bacteria present.

Quorum sensing is widely employed by a variety of gram-positive and gram-negative bacterial species to coordinate communal behavior.

RESULTS

Quorum sensing was first described for the luminous marine bacterium *Photobacterium fischeri* (*Vibrio fischeri*). In 1970, Kenneth et al observed that these bacteria do not

luminesce until they reach a high population density. Based on this observation, they hypothesized that bioluminescence in this organism was probably regulated by molecular messengers that traveled between cells. These messengers were called 'autoinducers' to refer to the fact that the autoinduction was in response to one's own culture supernatants, and they predicted that these molecules could enter target cells and activate the expression of the genes responsible for bioluminescence.[2-4]

Key Players in Quorum Sensing (Table 1)

Autoinducers (AI)

Autoinducers are usually small molecules that either diffuse freely across the cell membranes or are actively transported out of the cell.

Acyl homoserine lactones (AHLS): AHLs are the major group of autoinducer signals in gram-negative bacteria. They have a conserved homoserine lactone (HSL) ring with a variable acyl side chain. Based on the length of the acyl groups, AHLs can be broadly classified as short- or long chain molecules. Short-chain AHLs have 4 to 8 carbon atoms in the acyl moiety, while long-chain AHLs have 10 to 18 carbons. According to previous reports, a variety of different bacterial strains could make the same AHL, but this AHL may be involved in the regulation of different phenotypes in each strain. For example, 3-oxo-C6-HSL activates bioluminescence in *P. fischeri* but regulates exopolysaccharide production in *Erwinia stewartii*.[5] This cross talk has facilitated the creation of quorum-sensing indicator strains that can be used to detect the presence of AHLs in a given sample.

Autoinducer-2 (AI-2): AI-2 was first recognized as a quorum-sensing signal in *Vibrio harveyi* by Bassler et al.[6] Since then, this type of signaling has been discovered in many gram-negative bacteria. AI-2 is described as a global signal molecule for interspecies communication, as it is made by gram-positive as well as gram-negative bacteria.[7,8]

Table 1: Key players in quorum sensing

Autoinducers
• Acyl homoserine lactones
• Autoinducer 2
• Cyclic dipeptides
• Bradyoxetin
• Other types of autoinducers
Autoinducer Synthases
• AHL synthases
• AI-2 synthase
• Synthases for other types of autoinducers
Quorum Sensing Regulators
• LuxR-type regulators
• LuxP/Q-type regulators

Cyclic dipeptides: A new class of autoinducers was recently identified in strains of pseudomonas based on their ability to activate AHL biosensors. Structural analysis indicated that these new signal molecules were the diketopiperazines (DKPs) cyclo (L-Ala-L-Val) and cyclo (L-Pro-L-Tyr) respectively.

Other types of autoinducers: In addition to the above-mentioned autoinducers, additional signals have been identified in gram-negative bacteria, including autoinducer (AI-3) in *E. coli*.

Autoinducer Synthases

AHL synthases: Luxl is the enzyme responsible for the synthesis of AHLs in the quorum-sensing system of *V. fischeri*.[9]

The luxl synthase specifically catalyzes the amide bond formation between S-adenosylmethionine (SAM) and a fatty acyl carrier protein of a specific chain length. The specificity of the AHL synthase to a particular chain length varies depending on the bacterial strain.[5] This allows for the variability in the size of the AHLs made by different bacteria and accounts for the production of multiple AHL types by the same bacteria. Such bacterial strains usually have multiple synthases with each synthase being responsible for the synthesis of a limited range of AHLs.[10]

AI-2 synthase: LuxS is the synthase responsible for the production of the AI-2 signal molecule in *V. harveyi*.[11] LuxS is an S-ribosylhomocysteinase that catalyzes the cleavage of the thioether linkage of S-ribosylhomocysteine to produce L-homocysteine and 4,5-dihydroxy-2,3-pentanedione. This catalysis is common in both gram-positive and gram-negative bacterial pathways for AI-2 biosynthesis.[12]

Synthases for other types of autoinducers: In addition to the above-mentioned autoinducer synthases, other enzymes are expected to exist due to the discovery of new autoinducers, such as cyclic dipeptides, AI-3 and DSF.

Quorum sensing has been described in both gram-negative and gram-positive bacteria. In gram-positive bacteria, the signaling molecules are secreted peptides, whereas in gram-negative bacteria, two different systems of quorum sensing, which use different types of autoinducers, have so far been described.[7]

The first system was initially described in *V. fischeri* as the mechanism that controls the expression of bioluminescence in this microorganism.

In *V. harveyi*, this first system has been called system 1, and hence the autoinducer that controls it is called AI-1. In this case, the hydroxybutyryl homoserine lactone is the autoinducer. A second quorum-sensing system has been described in *V. harveyi*.[7] The structure of the autoinducer

for this system, which has been called AI-2, is still unknown, although it has been reported that its synthesis is dependent on the luxS gene.[7,11]

This second system seems to be more widespread among the microbial world than the one that uses AHLs as autoinducers and homologues for luxS have been identified in a large number of both gram-positive and gram-negative microorganisms.[7]

In 2001, different species of bacteria important in the composition of dental plaque were tested for production of extracellular autoinducer-like activities that stimulate the expression of the luminescence genes in *V. harveyi*. Several strains of *Prevotella intermedia, Fusobacterium nucleatum* and *Porphyromonas gingivalis* were found to produce such activities. Interestingly, these bacteria belong to the same phylogenetic group and they are periodontal pathogens important in the development of periodontal disease. They specifically produce extracellular signaling molecule related autoinducer-2 from *V. harveyi*. Nevertheless, they seem to be unable to produce homologues of acyl-homoserine lactones.[13]

Viridan streptococci, such as *S. gordonii*, *Streptococcus sanguinis* and *Streptococcus parasanguis*, make up a large portion (50-80%) of the commensals that colonize the tooth surface initially.[14] The importance of *Streptococci* in plaque development and periodontal disease has brought them to the forefront of biofilm research.

Ganesh kumar et al used microtiter plate system to determine the influence of various environmental factors on biofilm formation by the oral isolate *S. gordonii* and to search for genetic loci associated with biofilm development.[15] Among the genes identified in this study, were those that code functions required for competence (comD), peptidoglycan biosynthesis (PBP 2B, PBP 5, glmM, and bacA), oligopeptide transport (appC) and DNA replication/repair (mutT). Interestingly, comD encodes a sensor kinase that is required for the development of competence for genetic transformation in Streptococcus and the regulation of this two-component system is mediated by a cell-density-dependent (quorum-sensing) system, which depends on a competence-stimulating peptide signaling system.[16,17]

Like other quorum-sensing systems, the extracellular signaling molecule (in this case, the competence-stimulating peptide) is expressed continuously and as cell density increases, the concentration of competence-stimulating peptide increases and this, in turn, activates the transcription of a specific set of genes.

This process allows bacteria to distinguish between low and high cell density and to coordinately control gene expression in a population of cells. Genetic dissection of biofilm development in both gram-negative and gram-positive species has demonstrated that quorum sensing is required, indicating that the involvement of this process in biofilm development is widespread. Yet, the study on *S. gordonii* was the first report of a quorum-sensing two-component system being important in both genetic competence and biofilm formation. Furthermore, the connection between quorum sensing and competence during biofilm growth has only been demonstrated in gram-positive bacteria.

Biofilm formation and competence for genetic transformation also appear to be linked to the uptake of metals. Mutations in the adc operon, which shows high similarity to a metal uptake system of *Streptococcus pneumoniae*, resulted in a *S. gordonii* strain defective in biofilm formation and competence.[18,19] Since trace elements are essential for growth and survival, it is not surprising that sensing levels of available metals and biofilm development are linked. However, the authors propose an intriguing hypothesis that high levels of available metals, such as zinc or manganese, may be a signal for the cells to switch from surface-attached growth to planktonic growth and dissemination. In addition, these researchers discovered that another metal uptake system, a copper-transport operon, copYAZ, which is located immediately downstream of the adc locus, is also involved in biofilm development. Inactivation of this locus does not inhibit biofilm formation, however, mutation of either copZ or copY affects detachment from the biofilm.[20] These studies indicate that trace elements play an important role in the life cycle of this organism.

S. mutans, which is considered to be a principal etiological agent in human dental caries formation, is another Streptococcus commonly found to inhabit the oral cavity. This species has the ability to ferment an array of sugars producing acid end-products that result in demineralization of tooth enamel and caries formation. Hence, biofilm formation and the ability to adjust to unfavorable conditions, such as low pH, are key factors in the cariogenicity of this organism. Like the *Viridans streptococci*, *S. mutans* cells growing in a biofilm have been shown to incorporate foreign DNA much more efficiently (10 to 600-fold higher) than the same strain growing in liquid culture.[21] The development of this competence was shown to be dependent on growth rate, pH and the age of the biofilm. A proteomics study clearly demonstrated a role for the maintenance of transformation during biofilm growth.[22] Studies have shown that *S. mutans*, like *S. gordonii*, relies on the comCDE quorum-sensing system to form a biofilm, at least under certain growth conditions and for acid tolerance.[21] In addition, in *S. mutans* another quorum-sensing system (luxS) affects biofilm formation.[23] The auto-inducer

(AI-2) signaling molecule encoded by the gene luxS is a novel type of chemical signaling molecule. This molecule is a boron-containing furanone that is involved in both intra- and interspecies communication.[24,25] Unlike, the quorum-sensing signaling molecule discussed above that allows communication within a certain cell population, AI-2 is a nonspecific, universally recognized signal that has the potential to communicate cell-density to a mixed community of bacteria.

Tannerella forsythia is a key contributor to periodontitis, but little is known of its virulence mechanisms. It is shown that biofilm growth of *T. forsythia* is stimulated by sialic acid, glycolyl sialic acid and sialyllactose. The genome of *T. forsythia* contains a sialic acid utilization locus.This genomic locus also contains a putatively novel TonB-dependent outer membrane sialic acid transport system (TF0033-TF0034). In complementation, studies using an *Escherichia coli* strain devoid of its outer membrane sialic acid transporters, the cloning and expression of the TF0033-TF0034 genes enabled an *E. coli* nanR nanC ompR strain to utilize sialic acid as the sole carbon and energy source. Taken together, these data indicate that sialic acid is a key growth factor for this little-characterized oral pathogen and may be a key to its physiology *in vivo*.[26]

Interfering with Quorum Sensing

Countermeasures to cell-to-cell signaling have been explored in an attempt to reduce the ability of cells to form biofilms, attenuate virulence and modulate other processes influenced by quorum sensing. Inhibition of quorum sensing can be accomplished in several ways which include (1) enzymatic degradation of the signal molecule, (2) blocking signal generation and (3) blocking signal reception.[27-29]

Acylated homoserine lactones (AHLs) present in bacterial cultures are degraded nonenzymatically at pH values above 7. The degradation of AHLs at alkaline pH values is due to lactonolysis—that is, opening up of the lactone ring through hydrolysis of the ester bond of the ring to give an acylhomoserine. AHLs also can be degraded enzymatically. A number of bacteria produce lactonases that hydrolyze the ester bond of the homoserine lactone ring.[28]

AHL signaling is involved in biofilm formation by *P. aeruginosa* since a mutant of lasl, which is a gene that encodes synthesis of AHLs, does not produce a mature biofilm.[30] Hentzer et al[31] demonstrated that brominated furanones had an adverse effect on the architecture (i.e. interference with maturation) of *P. aeruginosa* biofilms and enhanced the detachment of bacteria from the biofilm.

AI-2 promotes biofilm and swimming motility in *E. coli*.[32] Ren et al[33,34] found that swarming activity and biofilm formation by *E. coli*, were inhibited by (5Z)-4-bromo-5-(bromomethylene)-3-butyl-2-(5H)-furanone at levels that had no effect on bacterial growth; however, swimming was not inhibited. The brominated furanone decreased the concentration of AI-2 but had no effect on luxS and pfs genes, which encode the proteins for AI-2 production.[34]

Rasmussen et al[35] constructed quorum sensing inhibitor selectors (QSIS) for screening for quorum sensing inhibitors and identified 4-nitro-pyridine-N-oxide and garlic extracts as the two most active inhibitors. When garlic extract, shown to have inhibited quorum sensing in *P. aeruginosa*, was added to a biofilm of *P. aeruginosa*, cells in the biofilm became more sensitive to the antibiotic tobramycin compared to nongarlic treated biofilms.[36]

NEGATIVE REGULATION OF QUORUM SENSING (TABLE 2)

Furanones: Structural Mimics

Halogenated furanones are naturally produced by the Australian red alga Delisea pulchra. Interestingly, the furanones have structural similarity to AHLs. Previous research has shown that furanones are capable of interfering with the quorum-sensing behavior of several bacterial strains.

Biochemical studies on the effect of specific halogenated furanones on LuxR protein overexpressed in *E. coli* indicate that the furanones are capable of interfering with AHL-LuxR interactions. Although furanones bind LuxR, the complex appears to be unstable. Binding of furanone to LuxR renders it highly unstable and accelerates its turnover rate. This results in the rapid disruption of the quorum-sensing-mediated gene regulation. Interestingly, addition of AHLs to the protein prior to the introduction of furanones results in protecting the LuxR protein against furanone-promoted

Table 2: Negative regulators of quorum sensing

Antiactivator proteins
Homologs of transcriptional regulators
AHL-degrading enzymes
RNA-dependent regulation
Eukaryotic Interference in Bacterial Quorum Sensing
Quorum-sensing cross talk between *A. tumefaciens* and Its host plant
Furanones: Structural mimics
L-canavanine as a quorum-sensing inhibitor
Human hormones interfere with bacterial quorum sensing
Other QSI compounds
Using Bacterial Components to Manipulate Quorum Sensing
Quorum quenchers
Transgenic plants
Synthetic analogs for quorum-sensing autoinducers

degradation. The mode of action of furanones and the nature of the interaction with the LuxR protein remain to be elucidated.[37,38]

Interestingly, recent work by Ren et al[33] shows that (5Z)-4-bromo-5-bromomethylene-3-butyl-2(5H)-furanone, naturally made by *D. pulchra*, inhibits AI-2-dependent quorum sensing in *E. coli*. They showed that the furanone completely inhibits swarming motility in *E. coli* and greatly inhibits biofilm formation in this strain.

L-Canavanine as a Quorum-Sensing Inhibitor

L-canavanine is an arginine analog found exclusively in the seeds of legumes.[39] L-canavanine is known to serve as an allelopathic substance by inhibiting the growth of certain bacteria.[40] Handelsman et al showed that canavanine has the potential to affect the population biology of *Bacillus cereus*.[41]

Human Hormones Interfere with Bacterial Quorum Sensing

A recent study on enterohemorrhagic *Escherichia coli* showed that human hormones cross communicated with the bacterial quorum-sensing system.[42]

Using Bacterial Components to manipulate Quorum Sensing

Quorum Quenchers

Dong et al initially identified AiiA from Bacillus species and showed that this enzyme inactivates the AHL signal and attenuates virulence when expressed in *Erwinia carotovora*.[43] Another study on quorum quenching isolated more than 20 bacteria belonging to the *Bacillus cereus* group which were capable of enzymatic inactivation of AHLs. Further genetic analyses revealed that the enzymes responsible for AHL inactivation were homologs of AiiA from Bacillus species strain 240B1. This enzyme is an AHL lactonase, known to act by hydrolyzing the lactone bond in the AHL.[44]

A second class of quorum-quenching enzymes was identified in Ralstonia strain XJ12B. The acylase AiiD isolated from this strain is capable of hydrolyzing the AHL amide. Expression of AiiD in *P. aeruginosa* PAO1 decreased its ability to swarm, produce elastase and pyocyanin and paralyze nematodes, all of which are quorum-sensing regulated phenotypes in this bacterium.[45]

DISCUSSION

It is shown that quorum sensing enhances the ability of bacteria to increase bacterial defenses against eukaryotic hosts, competing bacteria and environmental stresses. It is also shown that gene expression of some bacteria differs in biofilms formed on different dental surfaces and stressful circumstances of adjustment to the surface may persist enhancing intercellular signaling between bacteria.[46] The physiological and clinical aspects of quorum sensing have received considerable attention and have begun to be studied at the molecular level. However, little is known about whether quorum sensing plays an important role in biofilm formation, or on growth and/or toxin production of pathogens that cause periodontal disease and dental caries. Clearly, various genes and pathways are involved in biofilm formation in different bacteria; furthermore, various quorum sensing systems are present in different bacteria. Use of proteomic and genomic techniques should help to elucidate the phenotypes associated with quorum sensing and the mechanisms by which these pathways work in causing periodontal diseases and dental caries. Compounds that antagonize quorum sensors may potentially be useful in inhibiting growth, virulence mechanisms and/or biofilm formation of bacteria.

CONCLUSION

Biofilms have a major impact on human health. The oral cavity demonstrates significant interspecies cooperation between microbes in a biofilm mode of growth. Understanding bacterial community behavior will probably put new emphasis on alternative therapeutic strategies to treat diseases caused by plaque biofilm. The study of bacterial quorum sensing has suggested several ideal targets for drug design. Further investigations on the quorum-sensing systems could reveal additional mechanisms for interfering with bacterial quorum sensing.

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ABOUT THE AUTHORS

Baswaraj Biradar (Corresponding Author)

Professor, Department of Prosthodontics, Indira Gandhi Institute of Dental Sciences, Puducherry, India, e-mail: impressionscare@gmail.com

Prapulla Devi

Reader, Department of Periodontics, Indira Gandhi Institute of Dental Sciences, Puducherry, India