



REVIEW ARTICLE

# Quorum quenching: Signal jamming in dental plaque biofilms



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**Abstract** Quorum sensing helps bacteria to communicate with each other and in coordinating their behavior. Many diseases of humans, plants, and animals are mediated by communication called quorum sensing. Various approaches are being investigated to inhibit this communication to control the diseases caused by bacteria. Periodontal pathogens also communicate through quorum sensing and new approaches to treat periodontal disease using quorum sensing inhibition need to be explored.

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## Introduction

Dental plaque is an example of microbial biofilm with a complex microbial composition containing as many as 500 different species of bacteria that have been identified from the oral cavity. These exhibit coordinated group behavior that causes periodontal diseases and dental caries.<sup>1</sup> The dental biofilm is a dynamic microbial community that forms high cell density on the tooth and tissue surfaces in the oral cavity. The community adheres tightly to the acquired salivary pellicle and is thought to develop by the

coordinated and successive colonization of different microbial species.

These characteristics of biofilm growth and development suggest that oral organisms may express complicated intraspecies and/or interspecies communication mechanisms that facilitate a coordinated response by members of the microbial community in environmental flux.<sup>2</sup>

The process by which microorganisms monitor and regulate their population density through chemical signaling is termed quorum sensing.<sup>3</sup> The mechanism by which quorum sensing can be inhibited is called quorum quenching. Work over the past few years has confirmed that quorum-quenching mechanisms are widely preserved in many prokaryotic and eukaryotic organisms. These naturally occurring quorum-quenching mechanisms appear to play important roles in microbe–microbe and

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pathogen–host interactions. The discovery of quorum sensing signal-degradation enzymes in mammalian species represents a new milestone in quorum sensing and quorum quenching research.<sup>4</sup>

## Quorum sensing

Quorum sensing is a process of chemical communication among bacteria, and is defined as gene regulation in response to cell density, which influences various functions, such as virulence, acid tolerance, and biofilm formation. The first indication that bacteria communicate through small chemical signals arose from studies of the marine organism *Vibrio fischeri*.<sup>2,5</sup> *V. fischeri* is bioluminescent, but produces light primarily at high bacterial cell density. Quorum sensing relies upon the interaction of a small diffusible signal molecule with a sensor or the transcriptional activator to initiate gene expression for coordinated activities.

Quorum sensing systems in bacteria have been generally divided into at least three classes<sup>6</sup>: (1) LuxI/LuxR-type quorum sensing in Gram-negative bacteria, which use acyl-homoserine lactones (AHL) as signal molecules; (2) oligopeptide-two-component-type quorum sensing in Gram-positive bacteria, which use small peptides as signal molecules; and (3) LuxS-encoded autoinducer (AI)-2 quorum sensing in both Gram-negative and Gram-positive bacteria.

A study was conducted by Frias et al<sup>5</sup> to examine the production of quorum sensing signaling molecules in bacteria isolated from dental plaque, especially in major putative periodontal pathogens such as *Porphyromonas gingivalis* or *Aggregatibacter actinomycetemcomitans*. This study revealed that at least three genera of periodontal isolates, *Fusobacterium*, *Prevotella*, and *Porphyromonas gingivalis*, were able to stimulate the production of light in *Vibrio harveyi* BB 170, which responds to autoinducer AI-2.<sup>5</sup>

## Quorum sensing in Gram-positive bacteria

A number of Gram-positive bacteria are known to use quorum sensing systems. The nature of the signaling molecules used in these systems differs from those of Gram-negative organisms, and to date, no Gram-positive bacteria have been shown to produce AHLs. Gram-positive quorum sensing systems typically make use of small post-translationally processed peptide signaling molecules. These peptide signals interact with the sensor element of a histidine kinase two-component signal transduction system.<sup>7,8</sup>

## Quorum sensing in Gram-negative bacteria

Most Gram-negative quorum sensing systems that have been studied thus far utilize AHL as a signaling molecule. When in high concentration, these molecules can bind to and activate a transcriptional activator, or R protein, which in turn induces expression of target genes.<sup>7,9</sup>

**Table 1** Various mechanisms involved in quorum sensing and quorum sensing inhibition. AHL = acyl homoserine lactone; AI = autoinducer.

Quorum sensing inducers	Quorum sensing inhibitors
Als	AHL-lactonase
• acyl homoserine lactone	AHL-acylase
• AI-2	Paraoxonase
• cyclic dipeptides	L-Canavanine
• bradyoxetin	Furanones
AI synthases	Human hormones
• AHL synthases	Other compounds
• AI-2 synthase	

## Quorum quenching

The biofilm formation can be disrupted by alarming the quorum sensing mechanism utilized by the various species of bacteria that together form the plaque biofilm. The inhibition of quorum sensing is commonly referred to as quorum quenching.

Inhibition of quorum sensing can be accomplished in several ways, including<sup>7</sup>: enzymatic degradation of signaling molecules; blocking signal generation; and blocking signal reception. The inhibitors of quorum sensing can be roughly grouped into two categories according to their structures and functions.<sup>10</sup> One group consists of molecules that structurally mimic quorum sensing signals, such as halogenated furanones and synthetic AI peptides (AIPs) that are similar to AHL and AIP signals, respectively. These inhibitors interfere with the binding of the corresponding signal to the receptor or decrease the receptor concentration. The other groups of small chemicals include enzyme inhibitors. For example, triclosan, a potent inhibitor of the enoyl-acyl carrier protein (ACP) reductase that is involved in the synthesis of acyl-ACP, one of the essential intermediates in AHL biosynthesis, reduces AHL production, and closantel is a potent inhibitor of histidine kinase sensor of the two-component system.

Various mechanisms involved in quorum sensing and quorum sensing inhibition are listed in [Table 1](#).<sup>7,9,11</sup>

## Mechanisms of small quorum-sensing inhibitors

The known small chemicals that inhibit quorum sensing can be roughly grouped into two categories according to their structures and functions. One group is the structural mimics of quorum-sensing signals, such as the halogenated furanones and the synthetic AIPs that are similar to the AHL and AIP signals, respectively. Evidence shows that these inhibitors act by interfering with the corresponding signal binding to the receptor,<sup>12</sup> or by decreasing the receptor concentration.<sup>13</sup> The other group of small chemicals is the enzyme inhibitors. For example, triclosan inhibits enoyl-ACP reductase whose product is the essential intermediate in AHL biosynthesis, and closantel is a potent inhibitor of histidine kinase sensor of the two-component system.

## Mechanisms of AHL-lactonases

The enzyme was proposed as a member of the metallo-hydrolase super-family, as it contains a His-X-His-X-Asp-His motif that resembles the zinc-binding motif of several metalloenzymes, including glyoxalase II, arylsulfatase, and  $\beta$ -lactamase. AHL lactonases hydrolyze the lactone ring in the homoserine moiety of AHLs, without affecting the rest of the signal molecule structure.<sup>4,9</sup>

## Mechanisms of AHL-acylase

Several bacterial species, including *Variovorax paradoxus*, *Ralstonia* isolates, *Pseudomonas aeruginosa* PAO1, and *Streptomyces* sp. have been reported to encode AHL-acylase for degradation of AHL signals by hydrolysing the amide bond of AHLs and producing corresponding fatty acids and homoserine lactone.<sup>14</sup> The three identified AHL-acylases are, AiiD from *Ralstonia* sp. XJ12B, PvdQ from *Pseudomonas aeruginosa* PAO1, and AhIM from *Streptomyces* sp.<sup>4</sup>

## Mechanisms of paraoxonase (PON) enzymes

Strong AHL inactivation activity was first observed in human epithelial cells.<sup>15</sup> Later, it was found to be widely conserved in the sera of all six of the tested mammalian species: humans, rabbits, mice, horses, sheep, and cattle. The characteristics of these AHL inactivation enzymes, such as dependence on  $\text{Ca}^{2+}$  ions and lactonase-like activity, are suggestive of those of paraoxonases (PONs). PONs, including PON1, PON2, and PON3, exhibit a wide range of physiologically important hydrolytic activities, including drug metabolism and organophosphate detoxification.

## L-Canavanine

L-Canavanine is an arginine analog and found exclusively in seeds of legumes. L-Canavanine is known to serve as an allelopathic substance by inhibiting the growth of certain bacteria.<sup>11,16</sup> Emmert et al<sup>17</sup> showed that canavanine exuded from alfalfa seeds has the potential to affect the population biology of *Bacillus cereus*. L-Canavanine is incorporated in place of L-arginine into nascent protein chains during synthesis, resulting in altered protein structure and function, and eventually leading to death of the targeted cell. Initial screening for quorum sensing inhibitory compounds from alfalfa (*Medicago sativa*) exudates detected several signal molecules that appeared to specifically affect quorum sensing of different bacterial strains. Some compounds had quorum-sensing-inhibitory effects, while others seemed to activate quorum sensing.<sup>4</sup>

## Furanones: Structural mimics

Halogenated furanones produced by Australian red alga *Delisea pulchra* have structural similarity to AHLs.<sup>4</sup> Biochemical studies on the effect of specific halogenated furanones on LuxR protein overexpressed in *Escherichia coli*

indicate that the furanones bind LuxR, and the complex appears to be unstable.<sup>11</sup> Binding of furanones to LuxR renders it highly unstable and accelerates its turnover rate. Interestingly, recent work by Ren et al,<sup>18</sup> 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.<sup>18</sup>

They also analyzed the effect of the furanones on AI-1 and AI-2 indicator strains of *V. harveyi* and found that luminescence was greatly inhibited in both reporter strains. This luminescence suppression was reversible when excess concentrations of either autoinducer signal were present.

## Human hormones

A recent study conducted by Sperandio et al,<sup>19</sup> on enterohemorrhagic *Escherichia coli* (EHEC) showed that human hormones cross communicated with the bacterial quorum-sensing system. Sperandio et al<sup>19</sup> also suggested the presence of a third type of autoinducer, AI-3, that is dependent on the *luxS* system. They proposed that the pathogenic genes in enterohemorrhagic *E. coli* are regulated by AI-3/epinephrine/norepinephrine signals and this might influence the infection process by coordinating the appropriate time and environment for the onset of virulence.

## Other compounds

An interesting report by Teplitski et al<sup>20</sup> described the discovery of several unidentified compounds from higher plants, which were termed AHL-mimic compounds, as they appeared to activate the expression of several quorum-sensing-regulated genes, and analyzed the presence of different AHL-mimic activities in a variety of higher plants, and found that the nature of these activities varied depending on the plant species. Another study on *Medicago truncatula* indicated that it produces at least 15–20 compounds that are capable of specifically activating or inhibiting quorum-sensing-regulated behavior in different bacterial strains.

Screening for quorum sensing inhibitory compounds is greatly facilitated by the recent creation of bacterial strains designed to detect such compounds. Rasmussen et al<sup>21</sup> constructed bacterial indicator strains to detect the presence of quorum-sensing-inhibitory compounds in a given sample. They designed two types of quorum-sensing inhibitor selector systems. The first type had a lethal gene fused to a LuxR-regulated promoter. Therefore, in the presence of AHL signaling molecules, the lethal gene is expressed, resulting in death of the indicator strains. The presence of quorum-sensing-inhibitory compounds results in neutralizing the activity of the AHL, and therefore the lethal gene is not expressed, allowing the growth of the bacteria on the test plates.

The second type of quorum-sensing inhibitor selector system uses an antibiotic resistance gene that is controlled by a repressor. The repressor in turn is controlled by LuxR. In the presence of AHL, the repressor prevents the

expression of the antibiotic resistance. Therefore, the indicator strain is unable to grow in the presence of the antibiotic and AHLs, unless it encounters a quorum-sensing-inhibitory compound that will allow the expression of the antibiotic resistance gene.

## Quorum quenching in periodontal bacteria

Many oral bacteria like *Porphyromonas gingivalis*, *A. actinomycetemcomitans*, and *Streptococcus* sp. are known to communicate and coordinate their pathogenic behavior through quorum sensing. Various means of inhibiting quorum sensing that have been discussed above may have a role to play in controlling periodontal infections. These methods along with mechanical plaque removal and daily oral hygiene may help to reduce periodontal disease severity. Many plant products have quorum quenching potential, and the use of such plant-based molecules may have some benefit in the oral cavity.<sup>6</sup>

Various plants, algae, and fungi produce molecules that might play a role in inhibiting quorum sensing in bacteria. These include:

- horseradish-iberin<sup>22</sup>
- garlic-ajoene<sup>22</sup>
- turmeric-curcumin<sup>22</sup>
- citrus flavinoids-flavonine naringenin<sup>22</sup>
- sponge *Agelas oroides*-alkaloid oroidin<sup>22</sup>
- red marine alga known as *D. pulchra*-halogenated furanones<sup>23</sup>
- grapefruit extract-furocoumarins, carotenoids, limonoids, pectin, and coumarin<sup>23</sup>
- nutmeg (*Myristica cinnamomea*)-alabaricone C5<sup>23</sup>
- sweet basil-osmarinic acid<sup>23</sup>
- garlic-disulfides and trisulphides<sup>23</sup>
- clove extract-eugenol<sup>24</sup>
- clove extract-hexane and methanol<sup>24</sup>
- *Piper nigrum*, *Piper betle* and *Gnetum gnemon*-hexane, chloroform, and methanol<sup>25</sup>

## Conclusion and future aspects

Quorum sensing inhibition in periodontal treatment is still in the research stage, and more research needs to be done on natural products that can inhibit quorum sensing in periodontal pathogens.

## Conflicts of interest

The authors have no conflicts of interest relevant to this article.

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