Microbial Chit-Chat: Quorum Sensing

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It has been widely recognized that bacteria do not live in isolation but exist in the form of communities and carry out intercellular signaling. This type of communication termed as quorum sensing, is a function of cell density. Despite few similarities, gram positive and gram negative bacteria significantly differ in their mechanisms for quorum sensing. The phenomenon of quorum sensing is affected by a variety of environmental factors. Fundamental studies on quorum sensing have enormous potential for practical applications in medicine and agriculture.

Keywords: Quorum Sensing (QS), Autoinducers, N-acyl Homoserine Lactone (AHL), Intraspecies communication

Introduction

Imagine a city with half a million people; if they don't communicate, chaos would result.

– Alan Decho

It is very interesting to know how microbes communicate with each other and regulate their gene expression. Microorganisms, may it be prokaryotes or eukaryotes, employ highly complex communication mechanism termed 'quorum sensing', that link cell density with processes like the production of extracellular polysaccharides, degradative enzymes, siderophores, pigments, and antibiotics, Hrp protein secretion, motility, biofilm formation, Ti plasmid transfer, epiphytic fitness, etc. (Miller and Bassler, 2001). The word quorum sensing was coined by E P Greenberg in 1994 (March and Bentley, 2004). Signal transduction processes in quorum sensing involve the production and release, and response to hormone like molecules known as autoinducers that accumulate in the environment of the cell as cell population increases.

Quorum sensing helps the bacteria to act as a multicellular organism, get involved in the decision-making process and capable of bringing about alterations in its surrounding environment, which is not possible in the case of a single bacterium. This review describes

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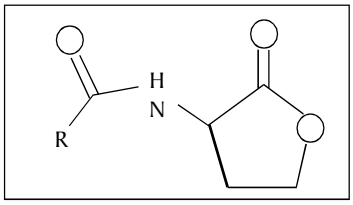
some well-characterized quorum sensing systems in gram positive and gram negative bacteria along with their similarities and differences. Similarities may exist because of common aim of communication. Differences may arise because of the differences in the surrounding environment of each type of bacteria (Waters and Bassler, 2005). This review also highlights quorum sensing in eukaryotes, i.e., yeast which explains the diversity of the quorum sensing operations along with its complexity.

Quorum Sensing in Gram Negative Bacteria

Quorum sensing is practiced by gram negative bacteria through production of the autoinducers—N-acyl homoserine lactones (Taga and Bassler, 2003). These molecules in gram negative bacteria mediate the species specific quorum sensing. The structure of all AHL molecules found in different gram negative bacteria consists of a homoserine lactone ring with N-acyl side chain, varying in length from 4 to 14 carbon atoms. At C_3 position of the side chain, there are various substitutions (AHLs, Figure 1).

Figure 1: N-acyl Homoserine Lactone (Core Molecule)



The major AHLs discovered are 3-oxo-C_{10} HSL (*Vibrio anguillarum)*, 3-hydroxy-7-cis-C_{14}-HSL (*Rhizobium leguminosarum*), C_4-HSL (*Aeromonas* spp.) and C_6-HSL (*Pseudomonas aureofaciens*). There are a number of other signaling molecules, produced by gram negative bacteria. e.g., Pseudomonas Quinolone Signal (PQS), HHQ (2-heptyl-3-hydroxy-4(1H)-quinolone), Diffusible Factor (DSF), 3-OH-PAME (hydroxyl-palmitic acid methyl ester), AIP-1 (staphylococcal autoinducing peptide 1) and DPD (4, 5 dihydroxy-2, 3-pentanedione) (Williams, 2007).

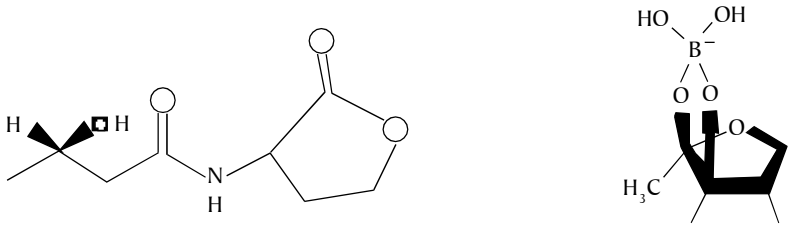
Two regulatory proteins are involved in the AHL-mediated quorum sensing: LuxI and LuxR. The LuxI protein has a role in the synthesis of autoinducer. The second regulatory protein, LuxR binds to this cognate autoinducer along with DNA at one end. The LuxR-AHL complex finally leads to the expression of target structural gene(s). The type of AHL produced depends upon the bacterial strain and its surrounding environment (Gera and Srivastava, 2006). A number of homologues of LuxI/LuxR proteins exist like VanI/VanR (*V. anguillarum*), RhiI/RhiR (*R. leguminosarum*), AhyI/AhyR (*Aeromonas hydrophila*) PhzI/PhzR (*P. aureofaciens*), etc. (Bassler and Miller, 2006).

The mechanism of quorum sensing in gram negative bacteria can be explained well by the example of *Vibrio cholerae. V. cholerae*, the agent of cholera in humans, are curved-rods, whose colonies are found in gastrointestinal tract adhered to villi. It produces proteases, which help it to enter the human gastrointestinal tract. *In vitro* studies of quorum sensing in *V. cholerae* indicates that there are three autoinducer responsive systems involved. These repress the expression of genes responsible for virulence, only when the bacterial population

is high (Tanouchi *et al.*, 2008). System 1 includes a putative synthase CqsA and a hybrid sensor/kinase CqsS. System 2 includes LuxSPQ. System 3 is uncharacterized (Cámara *et al.*, 2002; and Miller *et al.*, 2002).

Another important feature of this mechanism is the production of two autoinducer molecules, AH1 and AH2 by system 1 and system 2, respectively (Miller and Bassler, 2001). The AH1 is a homoserine lactone autoinducer ((S)-3-hydroxytridecan-4-one) (Figure 2). A gene Cholera quorum sensing autoinducer (CqsA) encodes a signal synthase CqsA that produces AH1 called as CAI-1 (CqsA dependent autoinducer/Cholera autoinducer1). The sensor protein for this AI-1 is LuxN and it binds to a distinct ligand. It is therefore termed as Cholera quorum sensing sensor (CqsS). AI-2 is a furanosyl borate diester ((2S, 4S)-2-methyl-2, 3, 3, 4-tetrahydroxy tetrahydrofuran borate) (Waters *et al.*, 2008). It is produced by LuxS and the sensors are LuxP and LuxQ. LuxP is a periplasmic protein (McNab and Lamont, 2003) and LuxQ is a hybrid sensor/kinase protein like CqsS. Both the proteins bind and interact with the signal molecule AI-2. The CAI-1 system 1 is responsible for intraspecies communication and the AI-2 system 2 for interspecies communication. It is believed that system 3 responds to an intracellular signal molecule which may be cyclic AMP because cyclic AMP receptor protein is required for expression of *hapA* and therefore should be regulated by QS (Teresa and Iglewski, 2000).

Figure 2: Autoinducer Molecules in *V. cholerae*



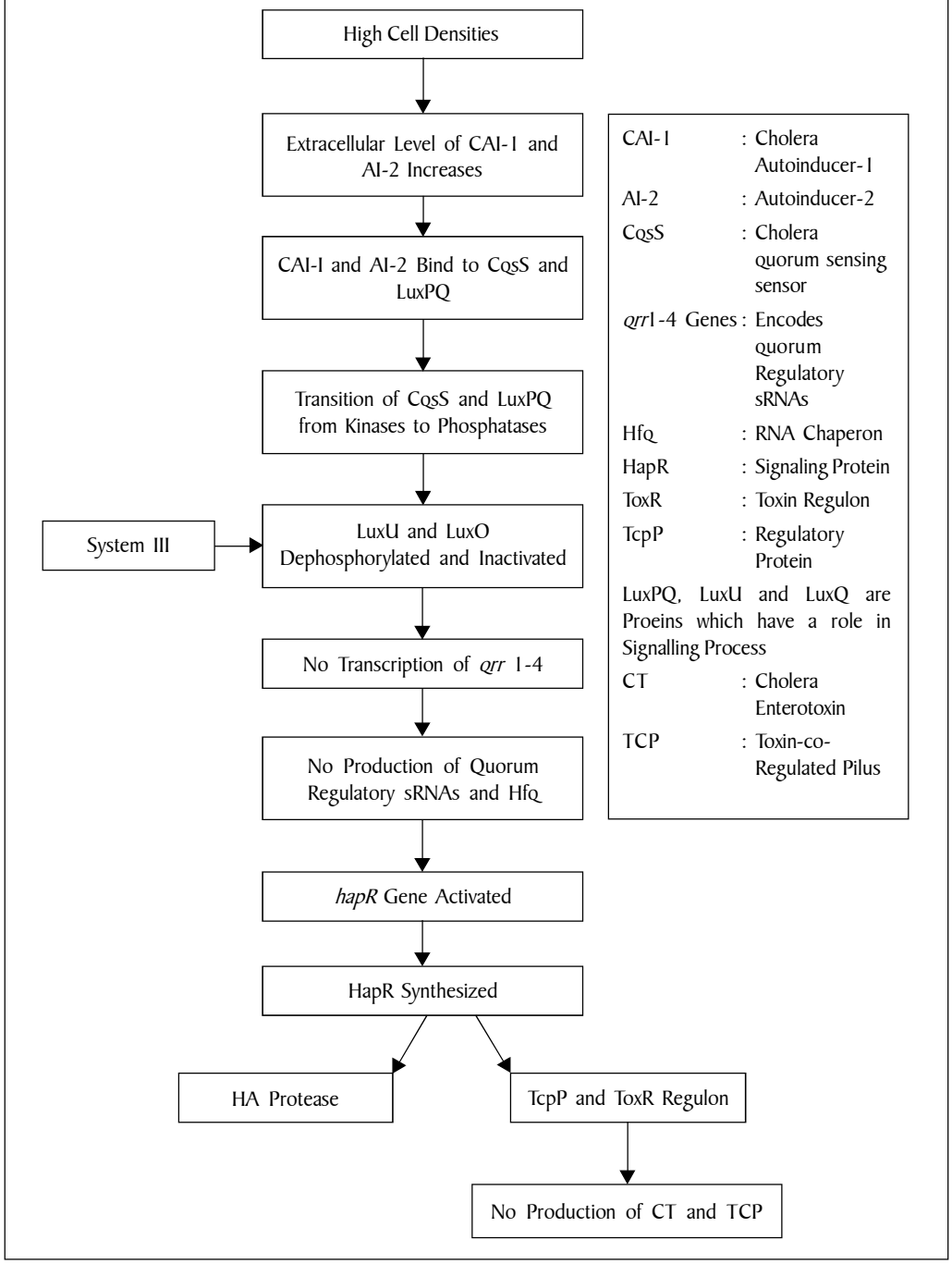
AH1

AH2

The three systems function through LuxU, LuxO and HapR signaling protein (Cámara *et al.*, 2002). LuxU is a histidine phosphotransferase protein. The LuxO is a response regulator protein. It requires a factor σ^{54} for its activity. HapR is the master regulator of QS and represses the expression of tcpP and ToxR regulon virulence genes which produce the primary colonization factor–TCP (toxin-co-regulated pilus) and cholera enterotoxin CT (Tanouchi *et al.*, 2008). The HapR regulator is encoded by *hapR* gene, which is regulated by *qrr* encoding four quorum regulatory small RNAs and a RNA chaperon Hfq. The transcription of *qrr* genes is enhanced by a small nucleoid Fis at low cell density (Lenz and Bassler, 2006). Thus at low cell density, the HapR is repressed by the QrrsRNAs and Hfq enabling the expression of genes for virulence and inhibiting HA protease (Svenningsen *et al.*, 2008). HA proteases at higher cell densities induce new infection centers in the

body of host, caused by detachment of bacteria from intestinal wall (Zhu *et al.*, 2002). The entire signaling process in *V. cholerae* is presented schematically in Figure 3.

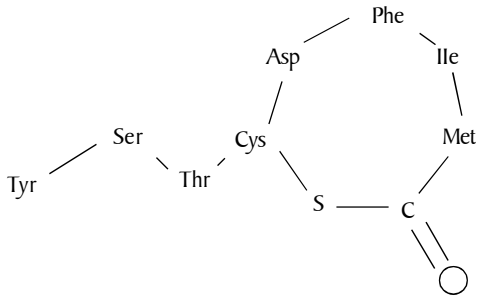
Figure 3: QS Signaling Process in *V. cholerae*



Quorum Sensing in Gram Positive Bacteria

Unlike gram negative bacteria, gram positive bacteria do not use acylated lactones as signaling molecules except *Streptomyces* that uses γ-butyrolactone. Instead they carry out quorum sensing through small peptides called Autoinducing Polypeptides (AIPs) or pheromones (Figure 4). Initially AIPs are produced as precursor peptides encoded by specific DNA sequences in an autoregulated manner. These precursor peptides are subsequently cleaved into peptide signals and are exported outside the cell by an ATP Binding Cassette (ABC) transporter.

Figure 4: Peptide Pheromone of *Staphylococcus aureus*



The peptide pheromones are responsible for bacterial communication within the species. An AIP contains a thiolactone ring having conserved cysteine residues at five amino acids from C-terminus. It may be cyclic (*Staphylococcus, Enterococcus faecalis*) or linear (*Streptococcus*). It may be post-translationally unmodified (*Streptococcus*) or modified. Some peptide pheromones are diffusible across the membrane, transducing signal for quorum sensing and some are transported to bind to intracellular receptors with the help of oligopeptide permease (Kleerebezem, 1997). The different AIPs and their precursor peptides in some spp. of bacteria are listed in Table 1.

Table 1: Different AIPs and Their Precursor Peptides

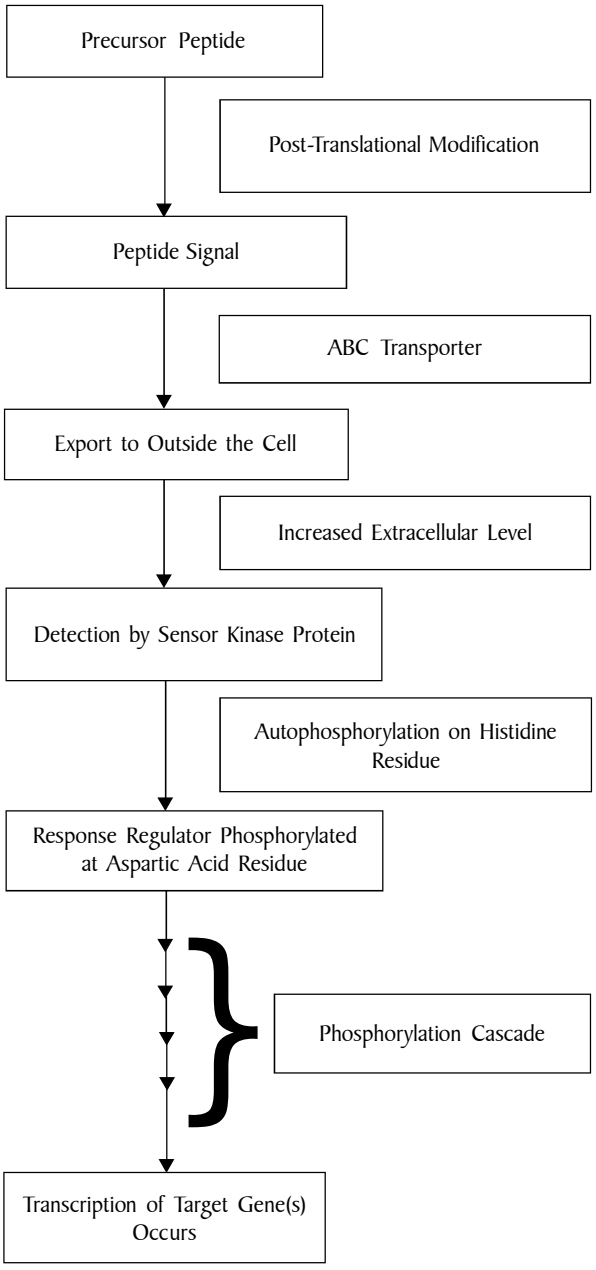
Bacteria	Peptide Precursor	Peptide Signal
Streptococcus pneumoniae	ComC	Competent Stimulating Peptide (CSP)
Bacillus subtilis	ComX Precursor Phr	ComX Competence and Sporulation Factor (CSF)
S. aureus	AgrD Precursor Protein	An Octapeptide

Source: Bassler and Miller (2006)

Here, the task of LuxR protein of gram negative bacteria is accomplished by a bicomponent membrane bound system, consisting of a sensory kinase protein and a response regulatory protein. The sensor kinase protein detects the increased concentration of the signal peptide in the extracellular environment. This is then followed by a cascade of phosphorylation and

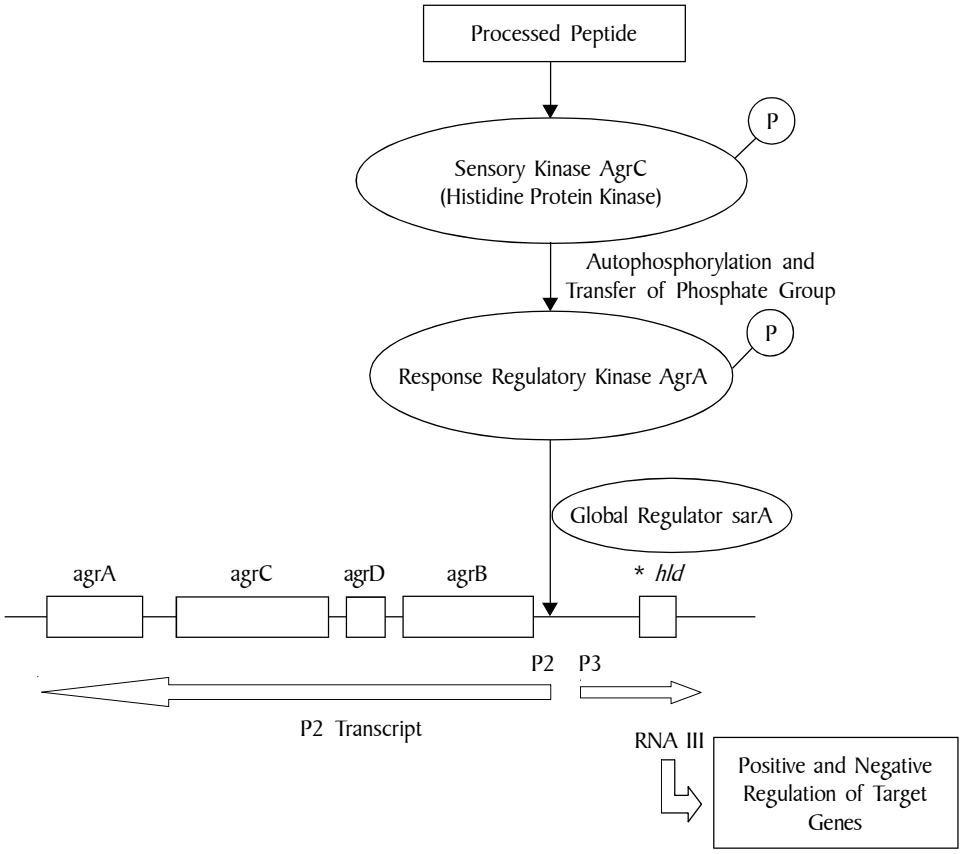
dephosphorylation reactions on conserved amino acid residues. The whole process terminates when activated response regulator protein binds to the target DNA and controls the transcription of downstream target genes. The mechanism of quorum sensing in gram positive bacteria is summarized in Figure 5.

Figure 5: Quorum Sensing Signaling in Gram Positive Bacteria



The mechanism of quorum sensing in gram positive bacteria is well understood in *S. aureus*, which is generally found on the skin and in the nose of human beings. It can cause skin infections like pimples, boils, carbuncles and abscesses by producing toxins. It can also cause meningitis, osteomyelitis, endocarditis, Toxic Shock Syndrome (TSS), septicemia and nosocomial infections. Quorum sensing in *S. aureus* includes accessory gene regulator (agr) system which has a role in its pathogenesis. The agr system includes peptide precursors (AgrA, AgrB, AgrC, and AgrD) (Teresa and Iglewski, 2000; and Bassler and Miller, 2006) whose signaling process involving P2 operon is explained in Figure 6 (Novick, 1999).

Figure 6: Probable Agr System Model in *S. aureus*



Note: * *hld* gene: Delta lysine gene. The binding of the processed peptide to AgrC present on the extracellular domain of the membranea results in its autophosphorylation. Subsequently the phosphate group is transferred to AgrA, which along with SarA activates P2 and P3 promoters of the P2 operon. This activation results in the gene regulation by RNA III.

Interspecies Communication

Interspecies communication occurs in polymicrobial communities in nature. A single autoinducer AI-1 can cause various signaling changes in a number of other unrelated

bacteria. *Streptococcus gordonii* and *Veillonella atypica* are two plaque forming bacteria found in teeth. *S. gordonii* ferments the sugars to form lactic acid, which is a substrate for *V. atypica*. It was observed during their co-culture that there was an increase in the expression of α-amylase encoding gene *amyB* of *S. gordinii* due to some sort of signaling events taking place. Moreover, *V. atypica* expressed Green Fluorescent Protein (GFP) in the presence of *S. gordinii* and those colonies which were not surrounded by *S. gordinii* did not express GFP (Egland *et al.*, 2004).

Bassler and her colleagues through their experiments on mutant *V. harveyi* strains showed that in this bacteria one system tells how many of its own species are in the area; the other tells how many other types of bacteria are around. They concluded that bacteria releases certain signals which provide it with the necessary information regarding its neighboring bacteria. The signaling molecules that regulate interspecies quorum sensing includes LuxS-encoded AI-2 molecule, epinephrine/norepinephrine responsing AI-3 molecule, Diffusible Signal Factor (DSF), cis-11-methyl-2-dodecenoicacid and related molecules like BDSF cis-2-decenoic acid, indole and antibiotics like tobramycin and azithromycin at subinhibitory concentrations (Ryan and Dow, 2008). Out of all the autoinducers mentioned above, AI-2 is the one which is produced and recognized by a broad range of bacteria (*V. harveyi*, *Salmonella*, *E. coli*, *V. cholerae*, etc.), suggesting that it provides the first known molecule that facilitates interspecies quorum sensing (Federle and Bassler, 2003; and McNab and Lamont, 2003).

Quorum Sensing in Eukaryotes

Candida albicans, a yeast responsible for candidiasis, grows in the tissues of candidiasis patients and converts its budding yeast form cells to the filamentous forms. It is due to this transformation into filamentous growth, that this fungal pathogen has the opportunity to establish an infection. At low cell densities ($<10^6$ cells/mL) of the yeast form, development of germ tube takes place. At high cell densities, farnesol is the extracellular signal molecule which blocks the transition of yeast to the filamentous form, thus mediating the eukaryotic quorum sensing (Hornby *et al.*, 2001). This yeast-mycelium dimorphism observed in fungi is given the term 'inoculum size effect' (Chen *et al.*, 2004). During the course of infection, *C. albicans* forms large thick-walled chlamydospores. The function of these chlamydospores is yet not clear but they are not found to be active in any other species. Here, the resulting elongated filamentous cells that are formed are wider than the true hyphae and are not inhibited by farnesol (Sprague and Winans, 2006).

Quorum Sensing Inhibition

The quorum sensing regulation system can be considered target for combating bacterial infections (Khmel and Metlitskaya, 2006). Several quorum sensing inhibitors are known which can be used potentially to construct antipathogenic drug. The mechanism of quorum sensing inhibition in a bacterial species and the inhibitors involved are cited in Table 2.

Table 2: Mechanism of Quorum Sensing Inhibition: Inhibitors Involved and Their Mode of Action		
Inhibition Mechanism	**Inhibitors**	**Mode of Action and Its Example**
Inhibition of binding of autoinducer to receptor proteins	Autoinducer antagonists like furanone derivatives. 1. Halogenated furanones 2. A furanone derivative (5Z)-4-bromo-5-(bromomethylene)-3-butyl-2(5H)-furanone	Compete with AHL for receptor binding Quorum sensing inhibition in *Pseudomonas aeruginosa* Inhibition of AI-2 in *E. coli* (Ren *et al.,* 2004; and Gonzalez and Keshavan, 2006)
Suppression of quorum sensing system in gram positive organisms	RNA III inhibiting peptides (RIP) or chemically synthesized analogues	Inhibit TRAP (target of RNA III activating protein) phosphorylation and suppress RNA III production in *S. aureus* (Balaban *et al.*, 2001)
Suppression of autoinducer synthesis	S-adenosylmethionine (SAM) analogues like S-adenosylhomocysteine and S-adenosylcysteine Antibiotics like Macrolides and Erythromycin at subinhibitory concentrations	Inhibit AHL synthesis in *P. aeruginosa* (Parsek *et al.*, 1999) Suppress AI production and virulence factors in *P. aeruginosa* (Sofer *et al.*, 1999; and Tateda *et al.*, 2004)
Degradation of autoinducers	AHL degrading enzymes • Lactonases • AHL acylases • Paraoxonase (PON) enzymes	 Cleave homoserine lactone ring in AHL molecule in *Erwinia carotovora* (Dong *et al.*, 2000) Detach AHL acyl chains in *Variovorax paradoxus* and *Ralstonia* strain XJ12B (Leadbetter and Greenberg, 2000; Lin *et al.*, 2003; and Liang *et al.,* 2007) Degrade 3-oxo-C_{12}-HSL in airway epithelium in *P. aeruginosa* (Williams, 2007)

Environmental Factors Affecting Quorum Sensing

Many physical, chemical and biological factors have the potential to affect signaling pathways of quorum sensing. In natural systems, the concentration of signal molecules is dependent upon the signal-production rate, the degradation rate or half-life of the signal, the diffusion properties of the signal and the external hydrodynamic or mass-transfer conditions.

The environment in which the host survives affects the stability of the transduced signal. In case of soil bacteria, where various plant metabolites are produced in response to the infection, these metabolites tend to alter the accumulation, signaling and release of the autoinducers (Newton and Fray, 2004). Due to the presence of physiological heterogeneity within the biofilms, there is formation of oxygen gradients in it as a result of which all the

cells present in the biofilm are not in the same stage of their metabolism. This phenomenon is particularly observed in *P. aeruginosa* (Parsek and Greenberg, 2000).

If the bacterial population surrounding is a liquid flow, then it will wash away the signal, diluting its concentration. This will lead to an uneven mass transfer of the bulk fluid and also disturb the geometry of the structured community. There is reduced signaling in the core of the community and so a further increase in the biomass is then required.

AHL inducer is pH sensitive and the change in pH affects its half-life, as shown in Table 3. Lantibiotics are complex peptide signals which also possess antimicrobial activity. The linear peptides are rapidly metabolized by the secreted proteases and so have a very short half-life. But due to the presence of lanthionine bridges, they can also block the activity of many proteases. With an increase in pH, there is a sudden drop in the solubility and stability of lantibiotics.

Table 3: Effect of pH on the Half-Life of the AHL Autoinducer

Alkaline Environment (pH)	AHL Half-Life (min)
Ocean Water (8)	100
Octopus Springs (8-9)	10-100
Alkaline Soils (9-10)	10
Mono Lakes, Ca (10)	1
East African Soda Lakes (11)	0.1

Signaling in the gaseous environment has been studied in *Ralstonia solanacearum* proving that quorum sensing can also occur in the absence of an aqueous surrounding. Volatile signaling takes place with the help of 3-OH palmitic acid methyl ester (3-OH PAME), an autoinducer found in this soil inhabiting plant pathogen.

The AHL stability is affected by the signal degradation property of certain bacteria. There are some AHL degrading bacteria which do not allow the signal, produced by organisms located at one place to be signaled to the bacteria at another location. This is termed as insulation (Horswill *et al.*, 2007).

Applications

Quorum Sensing for Genetic Engineering in Agriculture

Plants can be genetically modified, so as to produce quorum sensing components and communicate with bacteria. This can help in enhancing beneficial plant-microbe interactions, and in eliminating pathogenic bacteria. Such components play a very important role in plant-microbe interactions. Studies have shown that after the expression of *yenI* from *Yersinia enterocolitica* in the chloroplasts of tobacco plants, C_6-HSL and 3-oxo-C_6-HSL autoinducers are synthesized in 1:1 ratio and these are similar to the AHLs of the plant symbiont *P. aureofaciens* and the plant pathogen *E. carotovora*, respectively. Hence, the AHLs produced

by plants in this way behave in a similar manner to their symbionts and pathogens (Fray *et al.*, 1999). Similarly, the quorum-sensing components from *A. tumefaciens* were found to be useful as a gene-switch system which can be used in a variety of plants, including bryophytes (moss), dicots (carrot and arabidopsis) and monocots (barley) for the regulation and expression of desired genes (You *et al.*, 2006).

Treatment of Infections Using Quorum Sensing

An invention by Jones and Blaser (2003) provided solution for treatment of *B. anthracis* infection. These scientists proved experimentally that furanone—a compound inhibiting AI-2-LUX-S-mediated quorum sensing (interspecies communication) prevents *B. anthracis* infection by inhibiting its growth thereby inhibiting toxin production. Another approach involves the use of a live attenuated vaccine comprising LUX-S mutated *B. anthracis* cell to enhance host immune response (Wipo patent WO2005005598).

The acute *P. aeruginosa* lung infection can be treated by active immunization with 3-oxo-C_{12}-HSL-carrier protein conjugate (Suga and Smith, 2003). This vaccination does not have any direct effect on bacterial population in lungs but surely leads to an increased amount of specific antibody in serum and reduced levels of pulmonary tumour necrosis factor (TNF-α), thus preventing host inflammatory responses (Miyairi *et al.*, 2006).

In addition, quorum sensing can help in efficient waste treatment. The AHLs were added to a phenol degrading activated sludge community, which resulted in increased phenol degradation rate (Valle *et al.*, 2004).

Future Challenges

There have been many developments in the applications of the quorum sensing phenomena but the factors limiting the quorum circuit in the establishment of an infection as well the complete understanding about the prokaryote-eukaryote interaction has still been a major challenge. The full utilization of the signaling pathways for combating the spread of infections is not achieved. The levels of amplitude of the bacterial behavior can be characterized and predicted from the environmental conditions. The next few years offer the prospect of a substantial expansion of knowledge of bacterial interspecies communication, which will be provided both through an enhanced understanding of intraspecies signaling and through the further development of model systems of dual and multiple cultures to study bacterial behavior within biofilms (Ryan and Dow, 2008).

The phenomenon of quorum sensing and how bacteria talk to each other is a fascinating one, and its study could reveal fundamental principles about cell-cell communication and information flow. Additionally, if antibiotics can be designed that specifically counteract quorum sensing, these fundamental quorum sensing studies could prove to have enormous practical application (Bassler and Miller, 2006). As this is a burgeoning field of research, novel signals, unique detection and response apparatuses and additional, as yet undescribed, quorum sensing behaviors await discovery. ❋

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