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Bacterial Quorum Sensing: Functional Features and Potential Applications in Biotechnology

Neelam Mangwani[a] Hirak Ranjan Dash[a] Ashvini Chauhan[b] Surajit Das[a]

[a]Laboratory of Environmental Microbiology and Ecology, Department of Life Science, National Institute of Technology, Rourkela, India; [b]Environmental Biotechnology Laboratory, School of the Environment, Florida A&M University, Tallahassee, Fla., USA

Key Words
Quorum sensing · Bacteria · Autoinducers · Biotechnology

Abstract
Quorum sensing (QS) represents an exceptional pattern of cell-to-cell communication in bacteria using self-synthesized signalling molecules known as autoinducers. Various features regulated by QS in bacteria include virulence, biofilm formation, sporulation, genetic competence and bioluminescence, among others. Other than the diverse signalling properties of autoinducers, there are non-signalling properties also associated with these signalling molecules which make them potential antimicrobial agents and metal chelators. Additionally, QS signal antagonism has also been shown to be a promising alternative for blocking pathogenic diseases. Besides, QS has impressive design features useful in tissue engineering and biosensor technology. Although many aspects of QS are well understood, several other features remain largely unknown, especially in biotechnology applications. This review focuses on the functional features and potential applications of QS signalling molecules in biotechnology.

Introduction

Communication, a means of sharing or interaction among individuals, is recognized as an integral element of organised social behaviour. Different organisms have diverse modes of communication depending upon their lifestyles. One such communication phenomenon, specific to bacteria, is termed quorum sensing (QS), which is an exceptional type of organised signalling regulated by the local cell population density [Waters and Bassler, 2006]. Signalling molecules, known as autoinducers, are exported to the surrounding environment by the cells themselves, which upon attainment of a threshold level are sensed by a population of cells, thus initiating a signalling cascade for the expression of QS genes. Habitual processes controlled by QS are not productive when assumed as an individual practice but become an effective response when carried out in an assemblage [Bassler and Losick, 2006]. The QS phenomenon was originally discovered in the luminescent marine bacteria *Vibrio fischeri* and *V. harveyi*, which were found to be luminescent at high cell density [Nealson et al., 1970]. The distinct practice of QS and the role of autoinducers in gene regulation has consequently been extensively studied in Gram-negative bacteria, which mediate a QS response via acyl ho-

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Surajit Das
Laboratory of Environmental Microbiology and Ecology (LEnME)
Department of Life Science, National Institute of Technology
Rourkela, Odisha 769008 (India)
E-Mail surajit@nitrkl.ac.in and surajit@myself.com

Table 1. QS regulatory genes found in bacterial species with various biological responses

Bacterial species	QS regulatory gene	Autoinducer class	Biological response
Vibrio harveyi	*luxM/N*	AHL and AI-2	virulence [Henke and Bassler, 2004]
V. fischeri	*luxI/R*	AHL	bioluminescence [Eberhard et al., 1981]
Agrobacterium tumefaciens	*traR*	AHL	conjugation and transfer [Winans et al., 1999]
Pseudomonas aeruginosa	*lasI/R, rhlI/R*	AHL	virulence [Pearson et al., 1995]
Staphylococcus aureus	*agr* system	AIPs	biofilms and virulence [Lyon and Muir, 2003]
Erwinia carotovora	*expI/expR-carI/carR*	AHL	virulence and antibiotic production [Dong et al., 2004]
Yersinia pseudotuberculosis	*ypsRI* and *ytb*RI	AHL	virulence, motility and clumping [Atkinson et al., 1999, 2006]
Bacillus subtilis	*comX/comA*	CSF	competence and sporulation [Dunny and Leonard, 1997; Lazazzera, 2000]

moserine lactone (AHL) autoinducers [Kovacikova and Skorupski, 2002], whereas Gram-positive bacteria synthesize and process peptides for cell-to-cell signalling [Barbarossa et al., 2010]. However, there are some signalling molecules, like autoinducer-2 (AI-2), produced by both groups of bacteria that also facilitate interspecies communication [Federle and Bassler, 2003].

QS is a complex process and serves as a fundamental event in bacterial life cycles to regulate important behaviours like competence, virulence, biofilm development, sporulation, bioluminescence, exopolysaccharide secretion and stress responses [De Kievit, 2009; Suga and Smith, 2003; Weber et al., 2009]. Apart from regulatory functions, there are also many non-signalling properties of autoinducers, such as iron chelation, membrane modification and antibiotic activity [Schertzer et al., 2009]. Engineered QS systems can also be used for the production of valuable biochemicals, pathogen diagnostics and therapeutics, cancer detection and biocontrol (via quorum quenching). Hence, it is necessary to understand the functioning of autoinducers and their diverse signalling and non-signalling aspects in view of potential applications in biotechnology.

Spectrum of Autoinducers and QS Regulatory Mechanisms in Bacteria

Synthesised autoinducing molecules produced by bacterial cells diffuse into proximate environments but have limited effect at low concentrations. With attainment of optimal cell density and threshold concentrations of autoinducers, signalling cascades enable cells to monitor their population density. The expression of QS relevant genes is mediated by binding of autoinducers to transcriptional factors or cell surface receptors, followed by establishment of signalling cascades. Therefore, QS signalling molecules (autoinducers) produced by cells are unambiguous in various bacterial groups (table 1).

AHL Signalling in Gram-Negative Bacteria

AHLs include signalling molecules which are specifically involved in cell-to-cell communication in Gram-negative bacteria. Structurally, they consist of homoserine lactone moieties attached to an acyl side chain by amide linkage [Watson et al., 2002]. AHLs from various Gram-negative bacteria are related in structure, but they can vary in the acyl side chain component. In AHL-mediated QS, a cell responds to an AHL using the specificity of interaction between the AHL and a transcription factor [Lazdunski et al., 2004]. A number of studies have been performed on different groups of organisms to obtain a comprehensive understanding of the regulatory mechanism of AHL-mediated QS.

Bioluminescence in the marine bacterium *V. fischeri* is a quorum-dependent phenomenon regulated by the *luxI/luxR* gene family. LuxI catalyses the formation of the AHL signal, and LuxR is a transcriptional activator which binds to the AHL. LuxI family synthases are engaged in biosynthesis of AHLs, which on association with the LuxR family of transcriptional regulators trigger gene expression in response to autoinducers [Eberhard et al., 1981; Engerbrecht and Silverman, 1987; Fuqua et al., 1996] (fig. 1). To date, two other classes of AHL synthases, LuxM and HdtS, have been characterized. LuxM has been exclusively studied in *Vibrio* spp. [Milton, 2006], and HdtS was originally reported from *Pseudomonas fluorescens* [Laue et al., 2006]. Its homologue (Act) has been

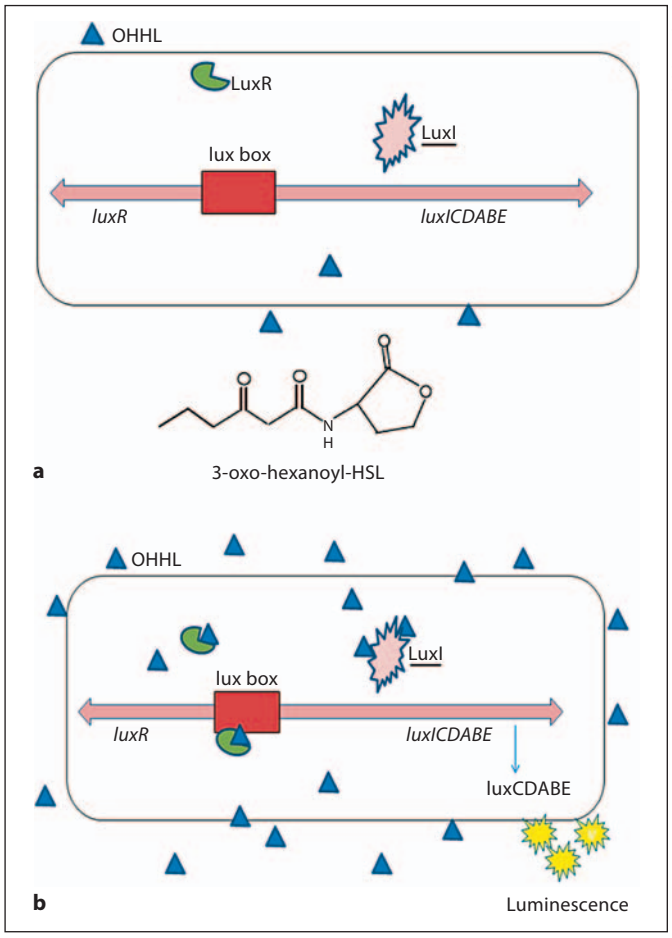

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Fig. 1. AHL signalling: regulation of bioluminescence by *luxI/R* regulators in *V. fischeri*. **a** At low cell density, *luxR* and *luxI* are transcribed at low levels and there is insufficient accumulation of OHHL. **b** At high cell density, when the intracellular concentration of OHHL reaches a certain threshold, a LuxR-OHHL complex activates transcription of the *luxICDABE* operon, resulting in increased levels of OHHL and bioluminescence. HSL = Homoserine lactone.

identified in *Acidithiobacillus ferrooxidans* [Rivas et al., 2007]. Recently, AHL-mediated QS has been reported in *Pseudomans aureofaciens* and *Xenorhabdus nematophila* [Kabir et al., 2010]. AHL-mediated QS systems have also been extensively characterized in bacteria such as *Rhizobium* [Sanchez-Contreras et al., 2007], *Erwinia* [Barnard et al., 2007], *Agrobacterium* [White and Winans, 2007] and *Yersinia* [Atkinson et al., 2006].

Peptide Signalling in Gram-Positive Bacteria

Peptide-based QS is prevalent in Gram-positive bacteria, where post-transcriptionally processed small peptides serve as signalling molecules [Dunny and Leonard, 1997]. Autoinducing peptides (AIPs) are ribosomally synthesised and exhibit a variety of linear and cyclic structures [Horinouchi, 1999]. The key event involved in peptide-based signalling is the processing of pro-peptide signals in the cytoplasm. They may incorporate unusual and modified amino acids that add to their stability and functionality. Modified and fully processed peptide signals are then exported from the cell via membrane transporters which subsequently pass on the signal to a DNA-binding regulator. Usually, peptides are secreted by membrane-associated transporters, for example ATP-binding cassette transporters. A two-component regulatory signal transduction system consisting of a membrane-located sensory kinase and intracellular response regulator assists in signal transduction and autoinduction. Peptides interact with membrane-associated sensory kinases that transduce a signal across the cell membrane. In a few cases, transport of AIPs into the cell is assisted by oligopeptide permeases, followed by interaction with intracellular receptors [Gera and Srivastava, 2006]. Binding of the peptide to a receptor can trigger a series of phosphorylation events which are concluded by phosphorylation of the cognate response regulatory protein. Phosphorylation activates the response regulators which then bind to the target DNA and regulate transcription of the QS-controlled gene(s). Peptide-based quorum signalling has been studied in *Staphylococcus aureus,* in which the *agr* genes are involved in QS regulation (fig. 2). The *agr* system is evolutionarily conserved in Gram-positive bacteria, including *Lactobacillus plantarum, Clostridium botulinum, C. perfringens, C. difficile, Listeria monocytogenes* and *Enterococcus faecalis* [Wuster and Babu, 2008].

AI-2 Signalling for Interspecies Communication

Although AHLs and processed peptides are quite specific to Gram-negative and Gram-positive bacteria, there are some signalling molecules which are sensed and synthesised by both groups. Earlier it was believed that the QS regulatory mechanism is specific to these groups with specified classes of autoinducers. However, there are some organisms that can have more than one QS regulator and function based on the system requirement [Barbarossa et al., 2010; Federle and Bassler, 2003]. AI-2, produced and sensed by both Gram-negative and Gram-positive bacteria, has been identified as a modified product of 4,5-dihydroxy-2,3-pentanedione (DPD), a product of the activated methyl cycle. S-Ribosylhomocysteinase (LuxS) converts S-ribosylhomocysteine to homocysteine and DPD. DPD undergoes rapid cyclization to form AI-2 and is exported outside the cell [Pei and Zhu, 2004]. At

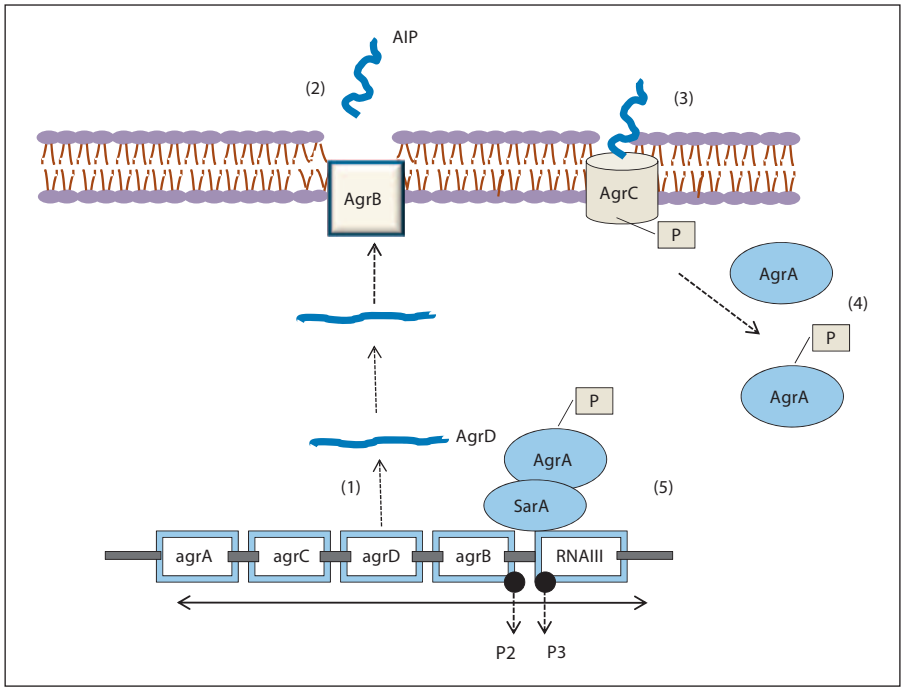

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Fig. 2. Peptide signalling: *S. aureus* Agr QS (modified from Fuqua and Greenberg [2002]). (1) The *agr* genes are linked to the RNAIII locus, and the *agrD* gene codes for a prepropeptide. (2) The prepropeptide is processed by and secreted through the product of the *agrB* gene. (3) The processed AIP interacts with a histidine sensor kinase receptor AgrC and sets off a series of phosphorylation events. (4) The phosphorylation of response regulator AgrA gene activates transcription of the regulatory RNA (RNAIII). (5) SarA is an additional transcription factor that activates both promoter 2 (P2) and promoter 3 (P3) in conjunction with AgrA. P = Phosphorus.

the threshold concentration of AI-2, a signal transduction cascade for the uptake and processing of AI-2 is triggered. AI-2 is imported inside the cell by membrane transporters. Inside the cell, AI-2 is phosphorylated by kinases and activates the QS circuit by binding to the repressor and activation of the relevant gene (fig. 3). This system was originally discovered in *V. harveyi,* in which it is regulated by an *luxS*-regulated operon *(lsr).* Different homologues of LuxS identified so far are involved in the synthesis of AI-2, suggesting the association of AI-2 and QS in both Gram-positive and Gram-negative bacteria for regulation of a range of features. The AI-2 structure is found to be associated with boron in *V. harveyi,* whereas in AI-2 from *Salmonella typhi,* boron is not present [Miller et al., 2004]. Boron is not essential in all QS, and its structure is slightly significant for *V. harveyi* AI-2. It is not clear how boron participates in signal transduction, but the presence of boron in *V. harveyi* AI-2 may be helpful for specific interaction with *V. harveyi* signal transduction [Aharoni et al., 2008]. A large number of bacteria produce high levels of AI-2 molecule for interspecies communication [Rader et al., 2007]. Recently, a group of scientists found that a transcription factor, AphA, is a master regulator of QS which operates at low cell density in *V. harveyi* and *Vibrio cholerae,* in contrast to LuxR and HapR, respectively, which operate at high cell density in these two organisms [Rutherford et al., 2011].

Autoinducing Molecules at the Signalling Phase

It is conceivable that the aim of bacteria is to multiply their population, and each cell in the population has an independent existence which is not related to that of other cells in the population. However, several genetic and biochemical studies have revealed the existence of well-organised communication between cells within a population that is mediated by means of signalling molecules [Horinouchi, 1999; Kleerebezem, 2004; Pesci et al., 1997; Pontes et al., 2008]. Extensive work has been carried out to understand the involvement of QS in virulence, symbiosis, competence, secondary metabolite production, bioluminescence, extracellular enzyme production, motility and biofilm formation, among others (table 1).

Virulence Factors

Bacteria make use of QS to establish disease and escape from the immune response of host organisms. In recent years, a number of studies have been conducted on *Pseudomonas aeruginosa,* an opportunistic human pathogen which produces multiple extracellular virulence factors to cause extensive host tissue damage. Two pairs of LuxI/LuxR homologues, LasI/LasR21 and RhlI/RhlR22, are known to participate in the production of multiple virulence factors including elastase *(lasB),* protease *(lasA),* endotoxin A *(toxA)* and alkaline phospha-

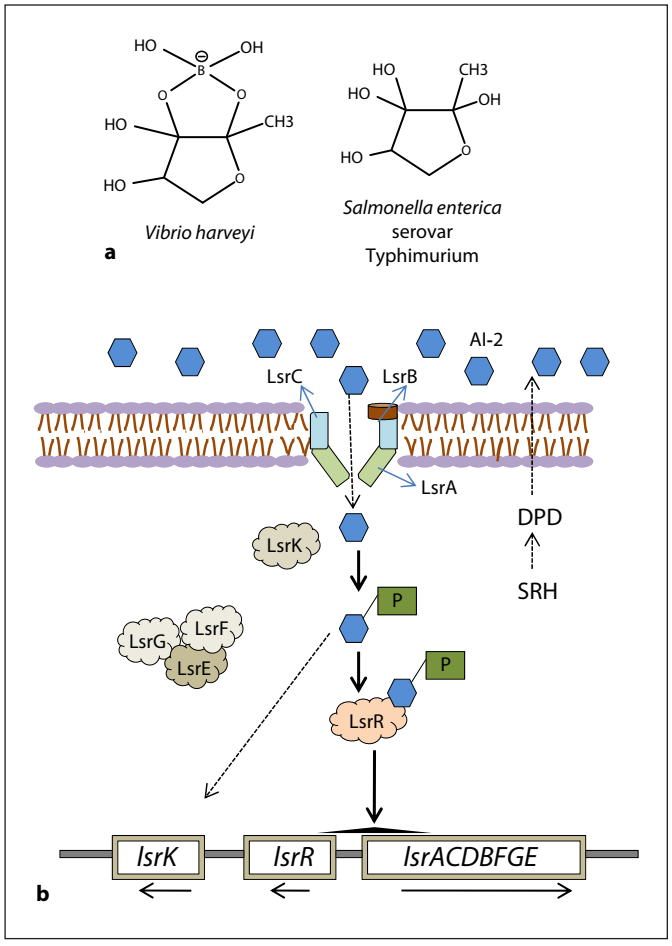

Fig. 3. a Structure of AI-2. **b** AI-2 signalling regulation in *Salmonella enterica* serovar Typhimurium (modified from Roy et al. [2011]). At the threshold concentration of AI-2, a signalling cascade is triggered. AI-2 is imported into the cell via an Lsr transporter, synthesized in an operon *(lsrACDBFG),* and LsrB is exposed at the cell surface. Inside the cell, AI-2 is phosphorylated by kinase, LsrK, to form phospho-AI-2. Phospho-AI-2 activates the QS circuit by binding to the repressor, LsrR, causing its release. In the absence of phospho-AI-2, the repressor is bound to the lsr promoter region and prevents operon expression. Once phopsho-AI-2 induces the *lsr* operon, the circuit uses a positive feedback mechanism causing increased expression of the transporter on the cell surface, which results in increased internalization of AI-2. Also upregulated is LsrG, which is involved in the degradation of phospho-AI-2. LsrE, which is a putative sugar epimerase, is present in *S. enterica* serovar Typhimurium. SRH = S-Ribosylhomocysteine; P = phosphorus.

tase *(aprA).* Both LasI and RhlI are autoinducer synthases, catalysing the formation of N-(3-oxo-dodecanoyl)-homoserine lactone and N-(butanoyl)-homoserine lactone, respectively [Brint and Ohman, 1995; Pearson et al., 1995; Pesci et al., 1997]. In Gram-positive bacteria, the involvement of QS in virulence regulation is not well understood; however, in *S. aureus,* expression of some virulence genes were reported to be quorum dependent [Kleerebezem et al., 1997]. Diseases caused by *Vibrio, Xanthomonas, Yersinia* and *Aeromonas* in humans and aquatic organisms are also regulated by quorum-based signalling cascades [Milton, 2006].

V. cholerae is reported to possess the QS signalling molecule Ea-CAI-1 to control biofilm formation and virulence factor production. This newly discovered 3-aminotridec-2-en-4-one (Ea-CAI-1) QS signalling molecule is formed by the coupling of the CqsA protein S-adenosylmethionine and decanoyl coenzyme A. However, fidelity is maintained in signal transduction systems in *Vibrio* species [Ng et al., 2011].

Starvation and Genetic Competence

Quorum responses are likely to be involved in gene products to work more efficiently at high cell density during transition into the stationary phase [Lazazzera, 2000]. Genetic competence, sporulation and multicellular behaviour are a few of the QS-induced behaviours regulated at starvation. Genetic competence development in *Bacillus subtilis* is stimulated by accumulation of two extracellular signalling peptides, ComX pheromone and competence-stimulating factor (CSF), which further regulates the activity of transcription factor ComA [Lazazzera et al., 1999; Solomon et al., 1996]. CSF serves not only as an indicator of cell density but also as an indicator of starvation. *Streptococcus pneumoniae* is known for its ability to enter into a state of genetic competence under conditions of high cell population density in response to a secreted signalling peptide. In contrast to *B. subtilis,* only a fraction of the *S. pneumoniae* cells in the population become competent in response to the peptide autoinducer [Guiral et al., 2002].

Autoinducers also control synthesis of stationary-phase regulatory proteins. For example, in *B. subtilis,* the Spo0A transcriptional factor is involved in expression of early sporulation genes regulated by the peptides CSF and PhrA [Ireton et al., 1993]. Studies on *Vibrio* sp. strain S14 [Srinivasan et al., 1998] and *Rhizobium leguminosarum* bv. *phaseoli* [Ireton et al., 1993] also support the involvement of QS in cell survival during the stationary phase at high cell density.

Starvation regulates the production of cell-to-cell signalling in bacteria with a multicellular lifestyle (e.g. *Myxococcus xanthus* and *Dictyostelium discoideum*) to form multicellular structures to disperse spores to new environments at high cell density [Kaplan and Plamann,

1996; Soderbon and Loomis, 1998]. In *M. xanthus,* production of the cell density signal (a mixture of amino acids termed A factor) is stimulated by starvation [Harris et al., 1998], similar to *D. discoideum* [Kolbinger et al., 2005].

Stress Responses

Flexibility in communication allows cells to respond to any stress condition and solve the stress in a cooperative way. In studies on the coordinated behaviour of cells at a high population density, QS-dependent responses have shown to result from various stresses such as toxic substances and antibiotic exposure during the stationary phase or oxidative stress [Pontes et al., 2008; Rothfork et al., 2004]. Studies on *V. cholerae* established that maintenance of viability under stress conditions is regulated by the expression of RpoS. This regulation acts through HapR, suggesting a role of the QS-enhanced stress response in *V. cholerae* [Joelsson et al., 2007]. *Burkholderia pseudomallei,* a causative agent of melioidosis in humans, responds to oxidative stress via BpsR/octanoyl homoserine lactone-dependent regulation of DpsA, a non-specific DNA-binding protein. It protects *B. pseudomallei* from oxidative stress [Guiral et al., 2002]. Universal stress protein genes *(usp)* in *Burkholderia glumae* are positively regulated by QS under heat shock conditions [Kim et al., 2012]. Similarly, *Streptococcus mutans* bacterial persistence via QS has been reported during the formation of stress-induced multidrug tolerance [Leung and Lévesque, 2012].

Sodalis glossinidius, a maternally transmitted bacterial endosymbiont of tsetse flies (*Glossina* spp.), uses an AHL-based QS system to modulate gene expression in accordance with bacterial cell density [Gonzalez and Keshavan, 2006]. Pontes et al. [2008] studied the *S. glossinidius* QS system, which relies on the function of two regulatory proteins, namely SogI (a LuxI homolog), which synthesizes a signalling molecule characterized as N-(3-oxo-hexanoyl) homoserine lactone (OHHL), and SogR1 (a LuxR homolog). These interact with OHHL to modulate transcription of specific target genes during oxidative stress.

Bioluminescence and Pigment Production

Bioluminescence is the production and emission of light by a living organism as a result of a chemical reaction in which chemical energy is converted to light energy. Bacteria like *V. harveyi* and *V. fischeri* produce blue-green luminescence at high cell density but are found to be non-luminescent when grown at low cell density [Nealson et al., 1970]. The *V. fischeri* bioluminescence gene cluster consists of eight *lux* genes *(luxA–E*, *luxG*, *luxI* and *luxR)* that are present in two bi-directionally transcribed operons (fig. 3).

The *luxR* operon is involved in the synthesis of the LuxR protein, which, in combination with autoinducers, induces expression of the second operon, i.e. the *luxICDABEG* operon. The products of both the *luxI* and *luxR* genes function as regulators of bioluminescence [Fuqua et al., 1996]. The *luxI* gene of *V. fischeri* participates in the synthesis of 3-oxo-hexanoyl homoserine lactone. The *luxA* and *luxB* genes encode luciferase enzyme subunits which catalyse the oxidation of an aldehyde and reduce flavin mononucleotide. The products of this reaction are a long-chain fatty acid, water and light [Gera and Srivastava, 2006]. *Chromobacterium violaceum,* a Gram-negative bacterium commonly found in soil and water, produces a characteristic purple pigment, violacein, via AHL N-hexanoyl-L-homoserine lactone-mediated QS [McClean et al., 1997].

Biofilm Development

Bacteria form multicellular sessile communities known as biofilms, in which cells of a community are embedded in a matrix of secreted extracellular polymeric substances. Biofilm formation and QS are fundamental and often interrelated features of bacterial social life [Li and Tian, 2012]. Biofilm formation is a kind of developmental cycle of microbial communities, and QS may serve as a checkpoint of various phases in the biofilm progress [Irie and Parsek, 2008]. Distinguishing the primary QS system responsible in the biofilm-related phenotype may be a difficult task, but there are many steps in biofilm development which are regulated by QS-related mechanisms. Studies on different QS mutant strains have revealed the involvement of quorum regulatory units in biofilm formation and maturation [Kjelleberg and Molin, 2002]. However, both the species and experimental conditions also have variable effects on biofilm development, and QS serves as one of the major factors influencing biofilm architecture [Nadell et al., 2008]. Table 2 lists disabled QS systems and the subsequent effect on biofilm formation in different bacteria.

Plant-Microbe Interaction

QS signals are used by an array of microorganisms that are important for association with higher organisms. Many plant-associated bacteria utilize QS to regulate expression of a number of genes that are required for the establishment of these interactions (table 3). However, deviation from the classical LuxR/LuxI trend is observed in

Table 2. Disabled QS systems and effects on biofilm formation in different bacteria

Bacterial species	Genes involved	Effect on biofilm
Aeromonas hydrophila	*ahy* (AHL)	defective maturation of biofilm [Lynch et al., 2002]
Serratia liquefaciens	*swr* (AHL)	thinner biofilm, lacking cell aggregates and cell chains [Labbate et al., 2004]
Staphylococcus aureus	*agr* (peptide)	varies with growth conditions [Yarwood et al., 2004]
Vibrio cholerae	*luxO (AI-2* and *CAI-1), hapR*	failure to produce thicker biofilm [Zhu and Mekalanos, 2003]
Klebsiella pneumoniae	AI-2	delayed biofilm development [Balestrino et al., 2005]
Pseudomonas aeruginosa	*las* (AHL)	flat, unstructured biofilm [Davies et al., 1998]
Eikenella corrodens	*luxS* (AI-2)	inhibition of biofilm formation [Azakami et al., 2006]

Table 3. Bacteria found in association with plants and their QS properties

Bacterial species	Autoinducers	Functions
Agrobacterium tumefaciens	N-(3-oxo-octanoyl)-L-homoserine lactone	conjugal transfer of Ti plasmids [Fuqua and Winans, 1994]
Erwinia carotovora	N-(3-oxo-hexanoyl)-L-homoserine lactone	carbapenem biosynthesis [Bainton et al., 1992]
Rhizobium leguminosarum	N-(3-hydroxy-7-*cis*-tetradecanoyl)-L-homoserine lactone	rhizosphere genes, growth inhibition, plasmid transfer [Lithgow et al., 2000; Schripsema et al., 1996]
Serratia liquefaciens	N-butyryl-L-homoserine lactone and N-hexanoyl-L-homoserine lactone	swarmer cell differentiation [Eberl et al., 1996]
Rhizobium etli	complex mixture	nodulation and growth [Daniels et al., 2002]

groups that are associated with plant or other higher organisms. Microbes inhabiting plants respond to a diverse mixture of AHLs, and the production of AHL analogs by plants and AHL-degrading enzymes by bacteria suggests that bacteria and plants have evolved in parallel to utilize AHL signalling as a key control point for influencing the outcome of interactions between microbes and plants. Bacteria often exist in association with plants as members of polymicrobial biofilm communities. It has been reported that more AHL-producing bacteria live within the rhizosphere in association with plants than within the bulk soil population [Cha et al., 1998; Danhorn and Fuqua, 2007].

Non-Signalling Aspects of QS Signalling Molecules

A QS molecule involved in cell-to-cell signalling allows bacteria to communicate via autoinducers. However, complex signalling molecules (autoinducers) are not just a means of communication; they are also involved in other cell phenomena that are not part of the usual signalling properties [Schertzer et al., 2009]. These signalling molecules possess antimicrobial and iron chelating properties, making them an alternative mediator for iron uptake used by many bacteria. The best-known examples of non-signalling properties of autoinducers are lantibiotics like nisin and subtilin from *Lactobacillus lactis* and *B. subtilis,* respectively. Lantibiotics are processed polypeptides with a unique antimicrobial property; they are used as antimicrobial agents to counteract bacterial infection [Kleerebezem, 2004].

Signalling Molecules with Antimicrobial Properties

Antimicrobial compounds secreted by different groups of microorganisms are largely well-known natural products; some of them act as signalling molecules at minimal concentration. Autoinducers from *P. aeruginosa*, *Lactococcus lactis* and *B. subtilis* are known to possess antimicrobial activities. *Pseudomonas* quinolone signals (PQS) have been well studied for their concentration-dependent antimicrobial activities [Haussler and Becker, 2008]. PQS are reported to be involved in stimulatory production of phycocyanin, a secretory product of *P. ae-*

ruginosa that stimulates expression of a precise set of genes and controls colony morphology. There are reports which suggest that by having potent antibiotic properties, pyocyanin also works as a terminal signalling factor in the QS network of *P. aeruginosa* [Dietrich et al., 2006, 2008]. Likewise, Gram-positive bacteria which utilize short AIPs for cell-to-cell communication are also augmented with antibiotic properties. Well-studied AIPs with antibiotic properties are lantibiotics that are considered to be related to AIPs because of their structural similarities with AIPs (i.e. presence of lanthionine or β-methyllanthionine thioether linkages that contribute to their polycyclic structures). Gram-positive bacteria like *L. lactis* and *B. subtilis* have been studied for the lantibiotics nisin and subtilin, respectively [Kleerebezem, 2004]. Like AIPs, lantibiotics autoregulate their own production in a density-dependent manner via two-component cell surface receptor-mediated cascades, indicating their signalling roles relevant to AIPs. Lantibiotics are antimicrobial signalling peptides which greatly alter bacterial expression patterns without any effect on growth at subinhibitory concentrations [Atkinson et al., 1999]. Despite the paucity of information on antimicrobial properties of signalling molecules, they may serve as global stock to fulfill the requirements of effective antibiotic compounds in the future.

Autoinducing Molecules as Iron Chelators

QS molecules often have metal chelating properties and are employed for scavenging metals by many microbes. Like QS, iron scavenging is also an integral part of virulence for many pathogens. Iron chelating and signalling functions of autoinducers have been shown by many bacteria. *P. aeruginosa,* an opportunistic pathogen, makes use of numerous endogenous and exogenous siderophores to satisfy its iron requirement; in addition, this bacterium uses PQS to seize iron stores of competing bacteria [Diggle et al., 2007]. This pathogen is well studied with regard to QS, and it was found that it makes use of its PQS to obtain iron in a host environment as iron is required for its growth. An additional iron limitation is faced by many pathogens in host environments due to the presence of iron-associated molecules like heme, lactoferrin and transferrin, which make iron capture more challenging [Weinberg, 2009]. The quinolobactin siderophore from *P. fluorescens* is structurally related to PQS. Soluble anaerobically, iron can be acquired by microorganisms via ferrous transporters, yet because of its insolubility under aerobic conditions, ferric iron acquisition typically requires high-affinity chelators or siderophores and their dedicated transport systems. However, metal chelation is not restricted to iron. There are reports which suggest that QS molecules can also chelate metals like Mg^{2+} and Ca^{2+} [Flemming et al., 2000].

Novel Applications of QS Signalling Systems

Signalling molecules involved in QS regulate several sets of genes by many signalling cascades. Besides, the use of modified QS molecules in the field of application-oriented biotechnology is of the highest importance, including production of biochemicals, pathogenic diagnostics and therapeutics, tissue engineering, mixed species fermentation, building QS-based biosensors, QS-based biocontrol strategies, reduction of biofouling, biofuel production, development of biomaterials and construction of artificial genetic circuits (table 4).

Biosensors

The bioluminescence properties of *V. fischeri* and pigment production in *C. violaceum* make them useful as biosensors for various studies [McClean et al., 1997; Zhu and Mekalanos, 2003]. Microbial biosensors can be designed to recognise cancer cell aggregation in vivo and can be exploited to create biosensors that can target cancer cells [Lindum et al., 1998; Oliver et al., 2009].

Production of Biosurfactant

QS mechanisms control the production of biosurfactants showing surface tension-reducing activities [Lindum et al., 1998]. AHLs, peptides and AI-2 are useful for large-scale production of these biochemicals. In addition, genetic modification of QS-producing microorganisms can be useful for enhanced production of QS molecules. Many bacteria are capable of synthesizing surfactants used for metal removal from contaminated sites. Moreover, biosurfactant application facilitates the bioavailability of hydrophobic pollutants (e.g. polycyclic aromatic hydrocarbon, n-alkane) that can be used for enhanced remediation of toxic compounds [Paul et al., 2005].

Pathogenic Diagnostics and Therapeutics

It has been suggested that QS signals alone can be used as markers for the presence of pathogenic bacteria in clinical and environmental samples [Kohler et al., 2009]. Autoinducers can also be used to detect the presence of pathogenic microorganisms in contaminated ground water, dairy and meat products [Choudhary and Schmidt-Dannert, 2010]. 3-Oxo-dodecanoyl homoserine lactone,

Table 4. Various applications of QS in biotechnology

Application	Bacterial species	Targeted QS system	Approaches
Biosensors			
Pathogen detection	*V. fischeri*	AHL, AI-2	transcriptional regulation and gene expression
	C. violaceum	AHL	[March and Bentley, 2004; McClean et al., 1997]
	Agrobacterium tumefaciens	AHL	
Cancer detection	*P. aeruginosa*	AHL	targeted microbial localization [Choudhary and Schmidt-Dannert, 2010]
Therapeutics			
Cancer therapeutics	*P. aeruginosa*	3-oxo-C12-HSL	autoinducer induces apoptosis in human breast cancer cell lines [Anderson et al., 2006]
Antimicrobial compounds	*L. lactis* and *B. subtilis*	peptides	antimicrobial activity of autoinducer molecules [Kleerebezem, 2004]
	P. aeruginosa	PQS	
Biocontrol			
Pest management	*Erwinia carotovora*	AHL	quorum quenching [March and Bentley, 2004]
	Pectobacterium carotovorum	AHL	
	Burkholderia cepacia	C6-HSL, C8-HSL	
	Agrobacterium tumefaciens	AHL	
	Pseudomonas spp.	PQS, AI-2	

C12-HSL = Dodecanoyl homoserine lactone; C6-HSL = hexanoyl homoserine lactone; C8-HSL = octanoyl homoserine lactone.

the QS signal of *P. aeruginosa* which induces apoptosis by inhibiting proliferation in human breast cancer cell lines, can also be used as a good starting point for the development of a synthetic AHL homologue for the detection of cancer [Tateda et al., 2003]. QS regulatory networks in *Streptomyces coelicolor* were used to design a mammalian regulation system to repress transcription from mammalian transsilencer-specific operator-containing promoters [Weber et al., 2005], suggesting bacterial QS regulons as a near-infinite source for the design of mammalian gene control systems relevant to future gene therapy and tissue engineering scenarios.

Biocontrol Strategies

Disruption of QS-based regulatory circuits can prove to be an effective strategy for prevention of disease outbreaks caused by many bacteria. There are many experimental efforts available in the literature that focus particularly on disruption of QS. Disruption of QS by inhibiting QS receptors has been studied in *C. violaceum* of the LuxR family [Chen et al., 2011]. These strategies, which make use of autoinducers for disruption of QS, include inhibition of signal molecule biosynthesis using S-adenosylmethionine as a substrate to block LuxI expression and use of QS antagonists. Studies indicate that halogenated furanones that have structural similarity with AHLs bind to LuxR and suppress further gene expression, and AHLs with long carbon chains have inhibitory roles [Choudhary and Schmidt-Dannert, 2010]. Moreover, degradation of AHLs may not only be a preventive tool, but also a curative biocontrol activity. It is a promising alternative to antibiotics in fighting bacterial infections, and this approach also has value in aquaculture, since a link between QS and virulence factor production in several aquatic pathogens has been demonstrated [Defoirdt et al., 2004].

The ability to generate bacterial QS signalling molecules in transgenic plants offers the opportunity for disease control and for manipulating plant-microbe interactions in order to obtain improved crop production and pest management (table 4). In addition, involvement of QS in antibiotic synthesis and antibiotic properties of autoinducers provides an alternative source for the production of diverse classes of medically important compounds.

Reduction of Biofouling

Formation of a biofilm is considered to be an initial step in the development of biofouling; hence, understanding the direct and indirect effects of QS signals and inhibitors or controllers in the process of marine biofouling is important [reviewed by Dobretsov et al., 2009]. There are three basic ways of controlling AHL QS, namely by blockage of AHL production, interference with the signal receptor or

inactivation of AHL signal molecules. In the process of continuous bioreactors, there is evidence for the production of AHLs, of which N-octanoyl-homoserine lactone is most abundant, confirming the close association between QS and membrane fouling. Porcine kidney acylase I, which can inactivate the AHL molecule by amide bond cleavage, prevents membrane biofouling by quenching AHL autoinducers [Yeon et al., 2009]. Thus, biochemical control of interfering QS could be an effective alternative to the control of biofilm formation [Kim et al., 2009].

Biofuel Production

Current technologies for converting lignocellulose materials to biofuels are hampered by costly processing steps like pretreatment, saccharification and product recovery. QS-mediated biofilm-based processes may improve the potential of biofuel production. Advantages of this process include concentration of cell-associated hydrolytic enzymes at the biofilm-substrate interface to increase reaction rates and the possibility of fungal-bacterial symbioses that allow simultaneous delignification and saccharification [Wang and Chen, 2009]. Hence, the transfer of QS-based biofilm technology has potential in the optimization of biofuel production.

Biodegradation

Growing awareness about the harmful effects of environmental pollution has led to a marked increase in various strategies that might be used to clean up pollutants from contaminated sites. QS-based bioremediation of organic pollutants can be employed in environmental restoration. Yong and Zhong [2010] reported improved biodegradation of phenol by *P. aeruginosa* by exogenous addition of AHLs. Reports on the contribution of QS to hexadecane degradation by *Acinetobacter* sp. are also available [Kang and Park, 2010]. However, the involvement of QS in biodegradation needs further research.

Conclusions and Future Perspectives

QS in bacteria is associated with signalling molecules that monitor their population density. These signalling molecules are very specific in their responses and regulate relevant gene expression. However, some bacteria also make use of these molecules to establish associations with other organisms. These signalling molecules regulate an array of features in bacteria such as competence, virulence, biofilm development, sporulation, bioluminescence, exopolysaccharide secretion, secondary metabolism, symbiosis and stress responses. However, these signalling molecules are not just a means of communication, as they have many supplementary non-signalling features, including antimicrobial activities and iron chelation. These features could potentially be used to develop novel methods for treating emergent diseases. Different classes of signalling molecules have been reported to be associated with antimicrobial effects which make them useful as alternative sources of effective antibiotics. Signalling molecules like PQS and AI-2 have also been reported to be associated with some metals. PQS are frequently found to be involved in *Pseudomonas* for iron uptake from host cells. Although bacterial QS are well understood, the mechanisms and genes involved in QS remain complicated to understand. Extensive research will be required to further explore new facets of cell-to-cell signalling as well as non-signalling aspects of autoinducing molecules for potential applications in modern biotechnology and to solve key societal problems.

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