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Microbial communication in the rhizosphere: Operation of quorum sensing

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Bacterial species employ complex communication mechanisms termed quorum-sensing (QS) that link cell density with gene expression. In this process diffusible signal molecules, autoinducers like acyl-homoserine lactones, accumulate in the extracellular environment, attain a critical threshold concentration and trigger the response which leads to gene expression. Besides operation of QS in the rhizosphere, it is apparent that some cross-talk between bacterial forms also occurs: plant growth-promoting bacteria such as pseudomonads, bacilli, etc. can influence the operation of QS systems in plant pathogenic forms. At threshold cell-density level, bacteria produce substances that inhibit proliferation of pathogens; beneficial bacteria responsible for nitrogen fixation on the other hand, use QS to optimize nodule formation on plant roots. Further advancement and finer understanding of QS in the rhizosphere will facilitate sustained exploitation of bioinoculants in soil health, plant productivity, bioremediation strategies in environmental applications and operation of biodegradation mechanisms that often determine the fate of a microorganism introduced in the natural ecosystems.

THE environment surrounding the root system of plants is termed the 'rhizosphere'. It has a major influence on the health and productivity of crops. However, it is a complex system wherein a series of interactions take place, which are under the influence of both biotic and abiotic factors. Microorganisms constitute a major component of the rhizosphere, the composition of which often differs greatly from that of the surrounding soil with change in plant species and as a result of diverse plant–microbe interactions. Some of these interactions involve mutually beneficial exchange of nutrient materials and are encouraged, as in the case of nitrogen fixing bacteria or plant growth-promoting bacteria (PGPR) and mycorrhizal symbioses, while others such as attack by disease-causing pathogenic microorganisms, can result in crop damage and loss.

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It has been recognized that bacteria can behave not only as individual cells but, under appropriate conditions where their numbers reach a critical level, they can modify their behaviour to act as multicellular entities. This is based on the fact that in natural ecosystems, bacteria do not exist as solitary cells, but are typically colonial organisms that live as consortia to exploit the elaborate system of intercellular communication which facilitates adaptation to changing environmental conditions. Microbial sensing and response mechanisms in the form of cell-to-cell communication via the use of small signalling molecules have recently been uncovered[1]. Numerous cell density-dependent signalling, molecule-mediated sensing and response pathways have now been defined, among which several fall within the scope of regulation that is commonly known as quorum-sensing (QS), a term first used by Fuqua and co-workers[2]. QS relies on the production of low molecular mass signalling molecules, the autoinducers. The extracellular concentration of these molecules is related to population density of the producer organism. By detecting and reacting to these chemicals, individual cells can sense the surrounding cell population and make sure whether there are enough bacteria, i.e. quorum to initiate the change towards acting in a multicellular fashion.

Several QS plant–microbe systems have been investigated and categorized. Within the PGPR, *Burkholderia cepacia*, *Pseudomonas chlororaphis*, *P. fluorescens*, *Rhizobium elti*, *R. leguminosarum*, *Sinorhizobium meliloti*, plant pathogens, *Agrobacterium rhizogenes*, *A. tumefaciens*, *Erwinia carotovora*, *E. chrysanthemi*, *E. stewartii* and *Pseudomonas syringae*, and saprophytes, *Chromobacter violaceum*, *Nitrosomonas europaea*, *Pseudomonas corrugata* and *Pseudomonas putida* exhibit QS system based on acyl homoserine lactones (AHLs) to communicate in the rhizosphere.

Broadly, microbially-derived signalling molecules are placed in two main categories: (i) amino acids and short-peptide pheromones commonly utilized by Gram-positive bacteria[3–5], and (ii) fatty-acid derivatives such as AHLs, utilized by Gram-negative bacteria[6]. Cellular processes regulated by QS in bacteria are diverse, and range from genetic competence development, i.e. the natural ability to take up exogenous DNA in *B. subtilis* and *Streptococcus pneumoniae*[7], to virulence and biofilm formation in *Pseudomonas aeruginosa*[6,8], and bioluminescence in *Vibrio fischeri* and *V. harveyi*[9,10]. Recent observations suggest that in some bacteria, QS can also regulate the transition into stationary phase which represents a period of quiescent, non-growth[11]. Among the QS systems, the following molecules have received major attention.

QS molecules

The structure of different microbial AHLs varies with the size and composition of the acyl chain, ranging from 4 to 14 carbon atoms (Figure 1); these contain double bonds, and often, an oxo- or hydroxyl group on the third carbon.

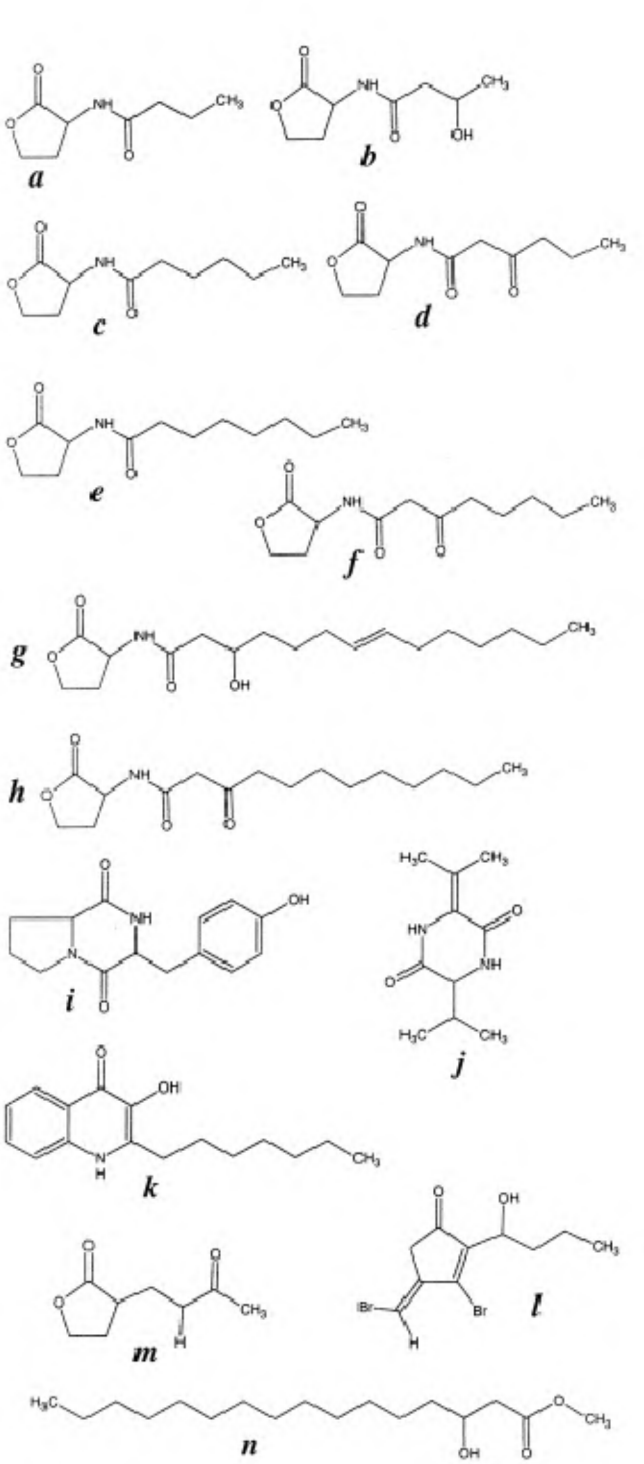

Figure 1. Some common microbial QS molecules[1]. ***a***, *N*-butanoyl-L-homoserine lactone; ***b***, *N*-(3-hydroxybutanoyl)-L-homoserine lactone; ***c***, *N*-hexanoyl-L-homoserine lactone; ***d***, *N*-(3-oxohexanoyl-L-homoserine lactone; ***e***, *N*-octanoyl-L-homoserine lactone; ***f***, *N*-(3-oxooctanoyl)-L-homoserine lactone; ***g***, *N*-(3-hydroxy-7-*cis*-tetradecenoyl)-L-homoserine lactone; ***h***, *N*-(3-oxododecanoyl)-L-homoserine lactone; ***i***, Cyclo(L-Pro-L-Tyr); ***j***, cyclo-(ΔAla-L-Val); ***k***, 2-Heptyl-3-hydroxy-4-quinolone; ***l***, 4-Bromo-5-(bromomethylene)-3-(1′-hydroxybutyl)-2(5H)-furanose; ***m***, Butyrolactone and ***n***, 3-Hydroxypalmitic acid methyl ester.

Majority of AHLs identified to date have an even number of carbons in the acyl chain, which are regulated by two-component Lux regulatory system. The type of AHL produced by a particular species is often strain-dependent. This reflects on the differing habitats in which the individual strains reside. However, based on comparative sequence analysis it has not been possible to predict which AHL(s) is likely to be synthesized by the LuxI homologue. Similarly, sequence analysis of the LuxR homologues has also not offered any clues as to which AHL is preferentially bound by each protein[12]. It was initially believed that the AHL molecules freely diffused through the cellular membranes; however, this perception has now changed. Welch *et al.*[13] have provided concrete evidence to show that the activation of CarR by a range of AHLs is dependent upon the ability of these ligands to avoid aggregation in the cellular membrane of *E. carotovora* subsp. *carotovora*. Pearson *et al.*[14] identified an active efflux pumping system, which is necessary for effective translocation of a long-chain (12-carbon) AHL in *P. aeruginosa*. Thus, it appears that while short-chain AHL molecules diffuse across the bacterial membrane, long-chain AHLs are transported actively via efflux or influx system. Molecules belonging to two new families of QS-related signalling systems have now been identified and isolated from spent culture supernatants of *P. aeruginosa*. The first group comprising diketopiperazines (DKPs) has been reported form *P. aeruginosa*, *P. fluorescens*, *P. alcaligenes*, *Proteus mirabilis*, *Citrobacter freundii* and *Enterobacter agglomerans*[15]. The second group comprising signalling molecule 2-heptyl-3-hydroxy-4-quinolone, was recovered from cell-free supernatant of *P. aeruginosa* whose synthesis is dependent on LasR[16].

Lux system

Lux system is a paradigm of AHL-based regulatory system. For example, when free-living in sea water at low cell densities, *V. fischeri* is non-luminescent. However, at high cell densities, as in specialized light organs of a small squid *Euprymna scolopes*[17,18], this bacterium luminesces with a blue-green light. The bioluminescence gene cluster of *V. fischeri* consists of eight *lux* genes (*luxA–E*, *luxG*, *luxI* and *luxR*) which are arranged in two bi-directionally transcribed operons[19] (Table 1).

On a molecular basis, bioluminescent induction involves interaction between *N*-(3-oxohexanoyl)-1L-homoserine lactone (OHHL) and the transcriptional regulator protein, LuxR. Cells of *V. fischeri* in low population densities, express *luxI* at a basal level. Therefore, the concentration of OHHL in the medium remains low. However, with increase in population density within the confines of the light organs, the concentration of OHHL increases. Once a threshold concentration of OHHL is achieved, it binds to *luxR* which then binds to a 20 bp DNA element of the diad symmetry, known as *lux* box; the latter is located around 40 bp upstream of the transcriptional start site of *luxI*[20,21]. This induces transcription of *luxICDABEG*, resulting in increased cellular levels of mRNA transcripts encoding both bioluminescence and OHHL synthesis functions. The end result is enhanced levels of both light output and AHL production.

In the rhizosphere, several systems are known to have QS-regulated interactions, among which genera *Bradyrhizobium*, *Burkholderia*, *Erwinia*, *Photobacterium*, *Pseudomonas*, *Rhizobium* and *Sinorhizobium* have expression of several important genes under QS control. Some of these are described below.

Free-living forms in the rhizosphere

QS in P. aeruginosa

P. aeruginosa is an opportunistic human pathogen that infects immunocompromised individuals and persons with cystic fibrosis. Pathogenicity of *P. aeruginosa* is dependent on its ability to secrete several virulent compounds and degradative enzymes. These include toxins, proteases and hemolysins, which are not expressed until late logarithmic phase of growth, when the cell density is high; this occurs through two known QS systems. The first is *las* system and second, *rhl* system (Figure 2). Each system has a transcriptional activator and an autoinducer synthetase. The autoinducers, PAI-1 and PAI-2, bind to specific target proteins, the transcriptional activators, and these complexes activate a large number of virulence factors. Two QS systems, *lasR/lasI* and *rhlR/rhlI* are organized into a complex hierarchy in *P. aeruginosa*, which together regulate numerous genes required for virulence[22].

The las system

The two QS systems of *P. aeruginosa* are linked to each other by the *las* system which is dominant over the

Table 1.

Gene	Probable function
luxA and *luxB*	Encode subunits of heterodimeric luciferase enzyme C and catalyse the oxidation of aldehyde and reduced flavin mononucleotide. The simultaneous liberation of excess free energy, evident as blue-green light, results in the phenotype, characteristic of bioluminescent microorganisms[84,85]
luxC–E	Encode products that or a multienzyme complex, responsible for synthesis of the aldehyde substrate used by luciferease[86,87]
luxG	Encodes flavin reductase[88]
luxI and *luxR*	Function as regulators of bioluminescence[89]

rhl system and regulates the expression of *lasB* elastase (Figure 2). It is composed of *las*, the autoinducer synthetase gene responsible for synthesis of 3-oxo-C12-HSL (*N*-[3-oxododecanoyl]-L-homoserine lactone), and *lasR* gene that codes for a transcriptional activator protein[14,23]. The *las* cell-to-cell signalling system regulates *lasB* expression and is required for optimal production of other extracellular virulence factors such as LasA protease and exotoxin A[24]. The *las* cell-to-cell signalling system is positively controlled by GacA[25] as well as by *vfr*, which is required for the transcription of *lasR*[26]. An inhibitor, RsaL, that represses the transcription of *lasI*, has also been described[27].

The rhl system

The *rhl* system controls the production of rhamnolipids and is composed of *rhlI*, 4-HSL (*N*-butyrylhomoserine lactone, previously named PAI-2 or BHL), autoinducer synthase gene, and the *rhlR* gene encoding a transcriptional activator protein[28,29]. This system regulates the expression of *rhlAB* operon that encodes a rhamnosyltransferase required for rhamnolipid production[28]. Presence of rhaminolipids reduces surface tension and thus allows *P. aeruginosa* cells to swarm over semi-solid surfaces[30]. The *rhl* system is also necessary for optimal production of LasB elastase, LasA protease, pyocyanin, cyanide and alkaline protease[25,31,32]. Significantly, transcription of *rhlI* is enhanced in the presence of RhlR–BHL; this creates a further autoregulatory loop within LasRI/RhlRI regulons. Latifi *et al.*[33] have reported that the *rhl* system also regulates the expression of *rpoS*, which encodes a stationary phase sigma factor (σ^S) involved in the regulation of various stress-response genes.

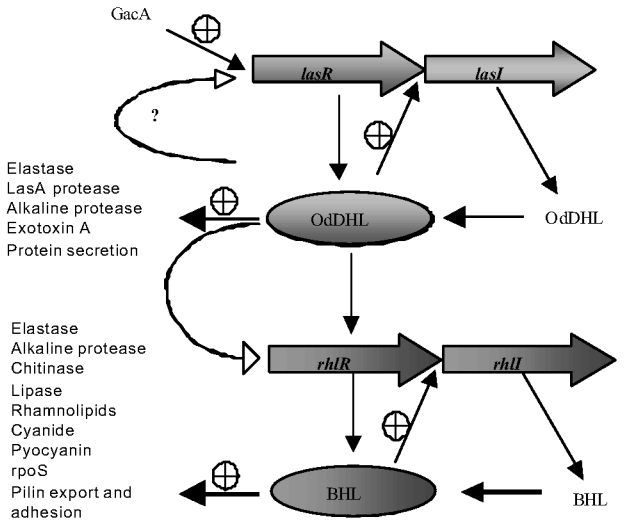

Figure 2. Quorum-sensing cascade in model system, *Pseudomonas aeruginosa* (modified from ref. 1).

However, QS regulation of σ^S in *P. aeruginosa* has been questioned by Whiteley and co-workers[34]. According to these workers, the sigma factor negatively regulates *rhlI* transcription. Like the *las* cell-to-cell signalling system, the *rhl* system, also known as *vsm* (*v*irulence *s*econdary *m*etabolites), regulates the expression of various extracellular virulence factors of *P. aeruginosa.* Studies conducted to determine the nature of interaction between LasRI and RhlRI have demonstrated that the RhlRI system is subordinate in hierarchy of regulatory commands that exist between these two QS regulons. It has also been shown that RhlRI system is functionally dependent upon the LasRI system, since transcriptional activation of *rhlR* is dependent upon LasR–OdDHL[33,35]. Thus, activation of the Las system leads to subsequent activation of the Rhl system and the two LuxR homologues together regulate the transcription of genes within their respective regulons.

Rhamnolipids are responsible for causing lysis by interacting and disrupting the plasma membrane of zoospores of *Pythium aphenidermatum*, *Phytophthora capsici* and *Plasmopara lactucae-radicis*[36]. Since rhamnolipids are regulated by the QS-dependent system, their application potential in the control of plant disease caused by zoosporic phytopathogens stands greater success with appropriate analysis of the effective molecules and mechanisms involved.

QS in other pseudomonads

Several other members of Pseudomonadaceae produce AHLs and exhibit operation of QS among which the best characterized system is that of *P. aureofaciens*. This fluorescent pseudomonad occurs naturally in the rhizosphere and produces three phenazine antibiotics, viz. phenazine 1-carboxylic acid, 2-hydroxy-phenazine 1-carboxylic acid and 2-hydroxy-phenazine. This species plays an important role in the community structure and profiling by inhibiting the phenazine-sensitive populations in the rhizosphere and the surrounding soil environment. The phenazines are potent inhibitors of fungal pathogens such as *Gaeumannomyces graminis* var. *tritici* (Ggt), the causative agent of take-all disease of wheat[37]. Regulation of phenazine production in *P. aureofaciens* is dependent on QS, involving the LuxRI homologue, PhzRI. The two genes encoding these regulators are placed close to each other on the chromosome and are transcribed convergently[38]. PhzI is responsible for the synthesis of *N*-hexanoyl-L-homoserine lactone (HHC), the cognate AHL sensed by PhzR[39]. Certain isolates of *P. fluorescens* also produce phenazines, wherein presence of *luxRI* homologues (also termed *phzRI*) has been confirmed[40]. In addition, several phytopathogenic pseudomonads such as strains of *P. corrugata*, *P. savastanoi* and five different pathovars of *P. syringae* (pvs. *syringae*, *tomato*, *angulata*, *coronofaciens* and *tabaci*) produce various AHLs.

QS in other systems

Erwinia

Many species of *Erwinia*, primarily recognized as plant pathogens, have been found to produce AHLs. At least three AHLs, OHHL, HHL, and *N*-decanoyl-L-homoserine lactone (DHL) are reported from *E. chrysanthemi*[43]. *Pantoea stewartii* subsp. *stewartii*, the causative agent of Stewart's wilt in sweet corn and leaf blight in maize, consists of LuxI homologue EsaI as a component of QS, which directs production of OHHL, and the LuxR homologue, EsaR[44].

Serratia

BHL and HHL were isolated and identified from cell-free culture supernatants of *Serratia liquefaciens* MG1. A *luxI* homologue, *swrI* was shown to encode the enzyme responsible for directing the synthesis of these two AHLs[45]; subsequently, *swrR*, a *luxR* homologue was also reported[46]. The major biosurfactant produced by *S. liquefaciens* MG1 was identified as a cyclic lipopetide called serrawettin W2, which was first isolated from *S. marcescens*[47]. This biosurfactant is able to condition surfaces prior to swarming. A functional QS system is required by *Serratia* sp. ATCC 39006 to enable production of the red pigment, 2-methyl-3-pentyl-6-methoxyprodigiosin, a secondary metabolite that possesses antimicrobial and immunosuppressive activity[48].

Yersinia

The first *Yersinia* species shown to possess a QS system was *Y. enterocolitica*, which synthesizes both HHL and OHHL via the product encoded by the *luxI* homologue, *yenI*[49,50]. A second open reading frame, termed *yenR*, lies downstream of *yenI* and encodes a LuxR homologue. Cell-free supernatants from several *Yersinia* species, including the non-pathogenic species, *Y. frederiksenii, Y. kristensenii* and *Y. intermedia,* and the pathogenic species *Y. pseudotuberculosis* and *Y. pestis* possess similar QS system.

QS in other Gram-negative bacteria

Several Gram-negative bacterial species produce AHLs or possess LuxRI homologues, viz. *Aeromonas hydrophila* and *Aeromonas salmonicida*. These common fish pathogens express LuxRI homologues, AhyRI and AsaRI, which govern the synthesis of AHLs, BHL and HHL[51]. In another fish pathogen, *Vibrio anguillarum*, expression of LuxRI homologue, VanI catalyses the synthesis of *N*-(3-oxodecanoyl)-L-homoserine lactone (ODHL).

Rhodobacter sphaeroides, a free-living microorganism also uses an AHL-based QS system and synthesizes *N*-(7-cis-tetradecenoyl)-L-homoserine lactone (tdeDHL) via expression of CerI[52]. CerI inactivation results in the formation of large aggregates of cells in liquid culture; addition of exogenous tdeDHL to CerI$^-$ strain prevents formation of cellular aggregates. In soil-dwelling *Chromobacterium violaceum*, HHL partially regulates antibiotic production, virulence factor, chitinolytic activity and purple pigment production[53,54]. A non-autoinducer-producing mutant of *C. violaceum* was commonly used as a test strain for AHL production in other bacteria on account of its ability to express pigment production in the presence of short-chained AHLs[53,55].

Non-acyl HSL-mediated QS in Gram-negative bacteria

Besides predominance of AHL-mediated QS in free-living and symbiotic bacteria dominant in the rhizosphere, non-acyl homo-serine lactone (HSL) QS system of the soil-borne plant pathogens, *Ralstonia solanacearum* and *Xanthomonas campestris* is well characterized.

QS in R. solanacearum: The phytopathogenicity of *R. solanacearum* is dependent on the production of an acidic extracellular polysaccharide (EPS) and plant cell-well degrading extracellular enzymes. Maximal expression of the virulence factors occurs at high cell-density[56]. It has been reported that LasR-type regulator, PhcA, is responsible for regulation of EPS and extracellular enzymes. PhcA activity is regulated by a two-component regulatory system, which in turn is responsive to the QS signal molecule, 3-hydroxypalmitic acid methyl ester (3OH PAME)[57]. The proteins PhcS and PhcR make up the two-component system responsive to 3OH PAME, wherein PhcS is a histidine kinase sensor and PhcR is similar to response regulators. The exact mechanism by which PhcS and PhcR relay sensory information to PhcA is not known, but genetic tests show that PhcS and PhcR negatively regulate the expression of PhcA-regulated genes in the absence of 3OH PAME[58]. This bacterium also contains an AHL-based QS system, which operates through two signals, an OHL and another yet unidentified molecule[59]. Homologues of *luxI* and *luxR*, identified in this bacterium have been designated as *solI* and *solR*. Expression of *solR* and *solI* is regulated by 3OH PAME-dependent system via PhcA, wherein they exhibit nearly similar cell-density associated expression as other PhcA-dependent virulence genes. In addition, RpoS is also required in the AHL QS system in *R. solanacearum*.

QS in X. campestris: Pathogenicity of *X. campestris* pv. *campestris* (Xcc) is dependent on various extracellular enzymes, viz. proteases, pectinases and exoglucanases,

and EPS. Production of exocellular enzymes and EPS formation in Xcc 8004 is regulated by *rpf* (*r*egulation of *p*athogenicity *f*actors) cluster, which comprises of nine genes (*rpfA–I*). Two of these genes, *rpfB* and *rpfF*, are implicated in the regulation mediated by a small diffusible molecule called DSF (diffusion signal factor). Slater and co-workers[60] have reported a functional connection between the DSF system and a two-component regulatory system encoded by *rpfGHC* operon that is located immediately adjacent to *rpfB* and *rpfF*, and is convergently transcribed. RpfC encodes a hybrid, two-component regulator that contains both sensor kinase and response regulator domains. RpfH is structurally related to the membrane-spanning sensor domain of RpfC, but does not contain a histidine-kinase domain. RpfG encodes a response regulator protein that contains a receiver domain attached to a specialized version of a HD domain and belongs to the HD–GYP subgroup of the HD superfamily[61].

Symbiotic relationship in the rhizosphere

Rhizobium

The ability of *Rhizobium* spp. to sustain symbiotic relationships with leguminous plants via the formation of nitrogen-fixing nodules on roots is well known. Operation of QS in *Rhizobium elti* and *R. leguminosarum* has been characterized, besides the role it plays in symbiotic relationship with legume hosts. Production of AHLs by rhizobial strains was identified by Cha *et al.*[42] and Shaw *et al.*[62]. Many of the gene products required for this symbiotic relationship are encoded by symbiosis (Sym) plasmids. The QS system of *R. leguminosarum* is regulated by chromosomally encoded CinRI proteins[63]. The product of *cinI* gene is responsible for synthesis of *N*-(3-hydroxy-7-*cis*-tetrade-cenoyl)-L-homoserine lactone (htdeDHL), whose expression is dependent on HtdeDHL-activated CinR. The products of *cinI* and *cinR* additionally regulate the expression of at least another chromosomally encoded AHL synthase, whereas two other HSL synthases are encoded on the Sym plasmid, pRL1J1, of *R. leguminosarum*; other rhizobial species such as *R. meliloti* produce an array of compounds with AHL-like activity, but *R. fredii* produces just one strongly non-polar compound with such activity[42,62]. CinR is a LuxR-type regulator which positively regulates *cinI* expression in reponse to htdeDHL (3OH-$C_{14:1}$-HSL). The products of *cinR* and *cinI* were previously thought to be a bacteriocin named *small*[64]. However, the purified *small* bacteriocin molecule turned out to be an AHL, identical in structure to the 3OH-$C_{14:1}$-HSL[63]. Mutation in *cinI* or *cinR* greatly reduces the expression of *rhiABC* operon[63]. These genes are expressed in the legume rhizosphere and influence the operation of symbiotic nitrogen-fixing system in Vetch[65]. The effect of *cinIR* on *rhiABC* expression is mediated via *rhiI* and *rhiR*; among these, RhiI produces *N*-hexanoyl-, *N*-heptanoyl- and *N*-octanoyl-L-HSLs, which stimulate RhiR and in turn induce *rhiABC* and *rhiI* operons[63,66]. The growth of *R. leguminosarum* is inhibited by two LuxR-type QS genes, *bisR* and *triR*, that are also known to regulate plasmid transfer in *Agrobacterium tumefaciens*[67].

Sinorhizobium

Sinorhizobium meliloti, a soil bacterium capable of establishing a symbiotic relationship in alfalfa plant (*Medicago sativa*), has been found to possess a pair of genes, *sinR* and *sinI*, which were identified as potential components of the QS system, responsible for the production of long chain ($>C_{14}$) AHLs. Their role in symbiosis has been proven by mutation in *sinI* or *sinR* genes, which resulted in decreased number of pink nodules during nodulation assay in *Medicago sativa*[68], indicating a role for QS in symbiosis.

Bradyrhizobium

Bradyrhizobium japonicum has a NolA global regulatory component which is involved in repression of the nodulation genes, i.e. *nod*, *nol* and *noe*. NolA plays a key regulatory role in the feedback repression of *nod* gene transcription in response to intracellular Nod signal production[69]. Interestingly, expression of *nolA* is regulated in a population-density-dependent manner by an extracellular factor that accumulates in the supernatant of *B. japonicum*[70]. Loh and co-workers[71] have described a two-component population-density-dependent regulator, NwsB, which is responsible for the expression of *B. japonicum* nodulation genes.

AHL-based cross-talk between microorganisms

The widespread occurrence of AHL-based cell signalling in Gram-negative bacterial species and their structural similarities have led to serious investigations of interspecies communication in the environment, where different AHL-producing bacterial species inhabit a common niche. Interaction of various LuxR homologues with non-cognate AHL molecule has been indicated in several studies[13,42]. Available information suggests that in the natural environment, one bacterial community is likely to produce AHLs that will inhibit the QS phenotypes expressed by another community. Also, bacterial species respond towards alien AHLs by utilizing them, and thus up- or down-regulate competitively advantageous phenotypes. Pierson *et al.*[72] have shown that *P. aureofaciens* populations share their wheat rhizosphere environment with many AHL-producing microorganisms. Yet, Cha *et*

al.[42] demonstrated that TraR of *A. tumefaciens* was responsive to signals (cognate and non-cognate) produced by other microorganisms that occupied the same habitat. Thus, it is imperative to consider the contribution of total number of cells within the heterogenous bacterial communities when analysing the dynamics of a single AHL-receptive species in the environment. In contrast to the use of AHLs for their own advantage, recent evidence suggests that several bacteria attempt to disturb cell-to-cell communication by destroying the message. Dong *et al.*[73] have isolated an enzyme *AiiA* from a strain of *B. subtilis*, which was capable of inactivating AHL. Expression of *aiiA* in *Ecc* (*Erwinia carotovora* pv. *carotovora*) caused a reduction in the secretion of extracellular enzymes and an attenuated pathogenicity phenotype.

Bacterial–eukaryotic communication

Many AHL-producing bacteria are associated with eukaryotes, either in pathogenic or symbiotic relationship. Higher organisms appear to have evolved mechanisms that enable them to detect and respond to AHL signalling systems in order to prevent or limit infection. The microalga, *Delisea pulchera*, for example, produces compounds known as furanones, which can specifically interfere with AHL-mediated QS systems. Teplitski *et al.*[76] have shown that higher plants such as pea, crown vetch and tomato all produce unidentified compounds that are capable of interacting with AHL-dependent QS systems.

Some AHLs have been reported to act as virulence factors. For example, a *P. aeruginosa*-produced molecule, OdDHL, acts as a potential modulatory agent of mammalian immune systems[77]; this molecule could inhibit the proliferation of lymphocytes and tumour necrosis factor production by macrophages. Saleh and co-workers[78] have shown that AHLs can hinder nucleotide-stimulated production of an antibacteral factor by CF human tracheal cells; decreased virulence was observed in pathogens with mutation in QS system, resulting in disruption of signalling pathways which could therefore be used as an alternative to antimicrobial therapy[79]. One such target for therapy could involve inhibition of AHL synthesis by LuxI homologues; another would be the prevention of AHL-mediated activation of LuxR homologues by the use of furanones or other synthetic AHL analogues[80].

Biofilm formation

QS has a definite role in formation and maintenance of biofilms[81,82]. Strains of *P. aeruginosa* defective in OdDHL production form abnormal monospecies biofilms, which, in contrast to wild-type biofilms, are sensitive to low concentration of biocides[82]. A mutant *lasI*, defective in the production of AHLs had a dramatic effect on the maturation of *P. aeruginosa* biofilm that lacked the three-dimensional architecture observed in the parent strain[82]. Li and co-workers[83] showed that a QS signalling system was essential for genetic competence in *Streptococcus mutans* during biofilm formation.

Conclusion

The number of documented QS regulatory systems has grown exponentially during the past five years, even in areas other than human–microbe interaction. One such domain pertains to molecular ecology of the rhizosphere ecosystem, wherein it has been relatively straightforward to screen rhizobacterial isolates for autoinducers, because some of these molecules are synthesized only under a particular set of environmental conditions. However, this approach under-represents the occurrence of QS systems on account of the operation of specific environmental variables. Most of the currently described QS systems were discovered in the course of dissecting the regulation of particular target genes. An impressive range of target genes are controlled by cell-density-dependent signals, and frequently the products of these genes are of considerable importance in determining microbial population structure in the rhizosphere.

Several of the known QS-dependent systems play a definite role in interactions involving bacteria and eukaryotic host organisms such as fungi. This includes the pathogens *A. tumefaciens*, *P. aeruginosa* and *Erwinia* spp. and the symbiont *Rhizobium* spp. However, it is pertinent to note that a bacterial species can also respond to the presence of foreign AHLs by utilizing the heterologous signalling molecules to, up- or down-regulate competitively advantageous phenotypes; such beneficial processes include expression of competitor-inhibitory antibiotics. For example, under *in vivo* study, it has been demonstrated that phenazine biosynthesis can be heterologously stimulated in one population of *P. aureofaciens* by AHLs[40]. The operation of PhzRI system in *P. aureofaciens* regulates phenazine production which effectively inhibits fungal pathogen Ggt in the wheat rhizosphere. In contrast, quorum-dependent system in *R. solanacearum* provokes production of acidic exopolysaccharide and plant-cell-degrading enzymes[56]. Some strains of *B. subtilis* destroy the signal molecules of other systems in the rhizosphere and thus disturb the rhizosphere equilibrium[73].

Molecules involved in QS have gained special attention in N-fixation and the associated symbiotic processes. Several gene products required in symbiosis are encoded by Sym plasmid, which also carries many important AHL synthase genes. Among these, *rhiABC* genes which are implicated in rhizosphere establishment, are also controlled by the QS system[63]. Also, QS genes, *bisR* and *triR*, are responsible for transfer of plasmid in *A. tumefaciens*[67], an organism provided with excellent properties to serve as a model in gene transfer.

The operation of QS system in the rhizosphere appears to hold great promise in control of disease caused by zoosporic fungi, i.e. damping-off of vegetable in nurseries, etc. In the control of zoospore spread in the rhizosphere, which causes rapid and severe seedling loss, rhamnolipid production controlled by *rhlRI* system plays a crucial role[28,29].

Indeed, in terms of AHL-mediated communication, the intricacies of a language that once seemed alien appear now to enter a new era, with innovative technologies presenting more opportunities to rapidly enhance our understanding of QS systems. Among these innovations, high-throughput of bacterial genes and proteins that fall under the regulatory umbrella of proteins such as LuxRI homologues, are of particular interest. Further, it is likely that many more physiological processes regulated by the bacterial QS systems, will be characterized over the next few years. While current efforts are directed towards laboratory-based assays of molecules involved in QS systems, their operation *in situ* in the rhizosphere appears imminent. Such information will permit delivery of not only more appropriate and effective bioinoculants for plant and soil health, but also cell-density-dependent control of *in situ* biological equilibrium, a feature of consequence in minimizing competition with indigenous microorganisms for the limited resources available in this unique ecosystem.

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