# Quorum-Sensing Systems in *Pseudomonas*

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## Introduction

Quorum sensing (QS) or cell-to-cell communication is a mechanism used by bacteria to control a broad range of activities in bacteria. The modulation of gene expression by quorum sensing causes phenotypic changes in bacteria leading to their better adjustment to environmental conditions and stress during growth (Turovskiy et al. 2007). Quorum sensing involves the production, secretion, and response to small diffusible signaling molecules also known as autoinducers. Bacteria produce signaling molecules at a basal level during the stationary phase of their growth, and with the increase in cell density, the concentration of the signaling molecule in the environmental medium increases; and on reaching a threshold level, it induces phenotypic effects by regulating quorum-sensing-dependent target gene expression (Czajkowski and Jafra 2009). Quorum sensing is involved mainly in the regulation of virulence, development of genetic competence, transfer of conjugative plasmids, sporulation, biofilm formation, antimicrobial peptide synthesis, and symbiosis (Bai and Rai 2011).

There are two groups of signal molecules involved in bacterial quorum sensing. One is the peptide derivatives typically used by Gram-positive bacteria, while the fatty acid derivatives are utilized by the Gram-negative bacteria. Most bacteria utilize two general mechanisms for detecting and responding to quorum-sensing signals and in modulating the target gene expression. In the acyl-homoserine lactone (AHL)-dependent quorum-sensing systems, the quorum-sensing signal is detected by a cytosolic transcription factor, whereas the quorum-sensing signal autoinducing peptide (AIP) is detected by a membrane-associated two-component response regulatory system (Dong et al. 2005).

In AHL-mediated quorum sensing, AHL synthase (I-protein) encoded by LuxI homologue synthesizes AHL molecules using S-adenosylmethionine (SAM) and acyl chains derived from the common fatty acid biosynthesis pathway. The short-chain AHL signal passively diffuses across bacterial membranes and accumulates in the environment, and the long-chain AHL signals require active transportation mechanisms for their efflux. The bacteria produce signaling molecules at a basal level during the stationary phase of their growth. With an increase in bacterial population, the concentration of AHL signal reaches a threshold level, resulting in signal accumulation and recognition by the cognate receptors. The signal reception involves R protein which belongs to the LuxR family of transcriptional regulators and acts as a receptor for the AHLs synthesized by the LuxI proteins. The R-AHL complex is a dimer and

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binds to conserved palindromic sequences of the quorum-controlled promoters, including the promoter of the luxI-type gene, and boosts AHL production (autoinduction) and expression of other genes in the quorum-sensing regulon. Thus, the R-AHL complex is involved in autoinduction and control of quorum-sensing regulons. The AHL degradation enzyme and the cognate regulatory transcription factor(s) are involved in signal decay (Schuster et al. 2004).

The LuxI-LuxR system was first discovered in *Vibrio fischeri* during the investigation of the phenomenon of bioluminescence. Now, the LuxI/LuxR system has become the model system upon that the other quorum-sensing systems have been based. Homologous LuxI/LuxR systems have been identified in many Gram-negative bacteria, each capable of producing specific AHLs. In the opportunistic pathogens, such as *P. aeruginosa* and *Serratia marcescens*, these signaling mechanisms control the expression of the virulence factors. *Pseudomonas aeruginosa* contains two systems homologous to LuxI/LuxR. LasI/LasR has been shown to control biofilm formation and the production of extracellular enzymes, as well as transcription of another quorum-sensing system, RhlI/RhlR, adding an additional level of control through AHL signaling (de Kievit and Iglewski 2000).

Pseudomonads are ubiquitous Gram-negative bacteria capable of surviving in several environmental niches. The genus comprises of important plant pathogens (e.g., *P. syringae*) and human opportunistic pathogens (*P. aeruginosa*). Some of them are able to colonize plant-related niches, such as the rhizosphere (e.g., *P. aeruginosa*, *P. fluorescens*, *P. putida*, *P. aureofaciens*, and *P. chlororaphis*), where they can act as biocontrol agents through the production of traits that directly influence plant disease resistance and growth (Venturi 2006). Therefore, it is important to study the quorum-sensing systems in the genus *Pseudomonas* as it comprises of opportunistic pathogens, plant pathogens, biocontrol agents, and industrially relevant organisms. The quorum-sensing systems, the signaling molecules, and the QS regulated phenotypes in the genus *Pseudomonas* have been summarized in Table 1.

## Quorum Sensing in *Pseudomonas aeruginosa*

*P. aeruginosa* is an opportunistic human pathogen responsible for microbial keratitis, burn wound, and pulmonary infections in cystic fibrosis and immunocompromised patients. It is a highly environmentally adaptable pathogen having a large dynamic genome of which 10 % codes for regulatory elements including a complex multi-signal QS system. Quorum sensing plays a key role in regulating a majority of genes related to various physiological processes, virulence factor production, motility, biofilm formation, and the expression of antibiotic efflux pumps, while the QS signal molecules are involved in the host–pathogen interactions (Williams and Camara 2009).

In *P. aeruginosa*, the QS circuit is integrated by two complete sets of LuxI-LuxR systems, LasI-LasR and RhlI-RhlR, and one incomplete system, QscR which has no cognate "I" protein (Lee et al. 2006). LasR responds to *N*-3-oxododecanoyl homoserine lactone (3-oxo-C12-HSL) synthesized by LasI, while RhlR responds to *N*-butyryl homoserine lactone (C4-HSL) produced by RhlI, and QscR responds to 3-oxo-C12-HSL, sharing it with LasR (Liu et al. 2009). Through this QS circuit, the LasI-LasR system controls expression of many extracellular virulence factors, the RhlI-RhlR system regulates production of rhamnolipid and secondary metabolites, and QscR induces PA1897 and its own gene, *qscR* (Choi et al. 2011). These QS systems are well regulated in a hierarchical cascade in which LasR activates expression of the RhlI-RhlR system and LasI-producing 3-oxo-C12-HSL activates QscR, which autoactivates its own expression (Ha et al. 2012). In certain cases, the LasR and RhlR regulate some genes in a mutually antagonistic manner, and QscR represses many genes induced by LasI-LasR and RhlI-RhlR systems (Choi et al. 2011; Park et al. 2013). Thus, *P. aeruginosa* has two acyl-HSL synthases and three receptors. The LasI synthase produces 3-oxo-C12-HSL, for which there are two receptors, LasR and QscR. The

**Table 1** An overview of quorum-sensing systems in *Pseudomonas* species

| *Pseudomonas* species | Quorum-sensing systems | Quorum-sensing signals | Quorum-sensing-regulated phenotypes | References |
|---|---|---|---|---|
| *Pseudomonas aeruginosa* | LasI/R<br>RhlI/R<br>QscR<br>PQS | 3-oxo-C12-HSL<br>C4-HSL<br>PQS, HHQ | Biofilm formation, virulence factors, elastase, lipase, exotoxin A, lectins, alkaline protease, hydrogen cyanide, swarming, twitching, pyocyanin, pyoverdine, rhamnolipids, and others | Winzer et al. (2000)<br>Chugani et al. (2001)<br>Wade et al. (2005)<br>Lee et al. (2006)<br>Williams and Camara (2009)<br>Dubern and Diggle (2008)<br>Liu et al. (2009) |
| *Pseudomonas fluorescens* | MpuI/R<br>HdtS | 3-OH-C14:1-HSL, C-10-HSL<br>C6-HSL, C8-HSL, C4-HSL<br>3-OH-C6-HSL<br>3-OH-C8-HSL<br>Diketopiperazines | Mupirocin and metalloprotease biosynthesis | Laue et al. (2000)<br>Shaw et al. (1997)<br>Cha et al. (1998)<br>Cui (2004)<br>Liu et al. (2007) |
| *Pseudomonas putida* | PpuI/R | 3-oxo-C12-HSL | Biofilm development | Bertani and Venturi (2004) |
| *Pseudomonas syringae* | AhlI/R | 3-oxo-C6-HSL | Cell aggregation and epiphytic fitness | Chatterjee et al. (2003), Quinones et al. (2004) |
| *Pseudomonas aureofaciens* | PhzI/R<br>CsaI/R | C6-HSL | Phenazine antibiotics synthesis, cell surface components, and rhizosphere colonization | Zhang and Pierson (2001) |
| *Pseudomonas chlororaphis* | PhZI/R | C6-HSL | Phenazine-1-carboxamide biosynthesis | Chin et al. (2005) |
| *Pseudomonas mediterranea* | PmeI/R | C6-HSL | Virulence factor and lipodepsipeptides production | Licciardello et al. (2012) |
| *Pseudomonas corrugata* | PcoI/R | C6-HSL, 3-oxo-C6-HSL, and C8-HSL | Virulence factor and lipodepsipeptides production | Licciardello et al. (2009) |

RhlI synthase produces C4-HSL, for which the receptor is RhlR. Integrated into the acyl-HSL quorum-sensing circuits is a third signal, 2-heptyl-3-hydroxy-4-quinolone, known as the *Pseudomonas* quinolone signal (PQS). The structures of the major signaling molecules of *P. aeruginosa* have been represented in Fig. 1. Transcriptome analyses have shown that quinolone signaling directly or indirectly controls the expression of at least 90 genes. The acyl-HSL and PQS signaling systems influence each other; the *las* system activates synthesis of PQS, which in turn activates *rhlI* expression. In addition, LasR, RhlR, and QscR influence expression of genes that can potentially alter intracellular levels of the PQS biosynthesis precursor anthranilate. Together these quorum-sensing systems regulate hundreds of *P. aeruginosa* genes. Different elements of the *P. aeruginosa* quorum-sensing circuit also influence each other at multiple levels; for example, LasR-3-oxo-C12-HSL activates *rhlR* and *rhlI* transcription, and QscR influences expression of a subset of *las*- and *rhl*-controlled genes. In fact, the regulons of LasR, RhlR, and QscR are partially overlapping (Chugani and Greenberg 2010).

The primary system is the Las system, which encodes the proteins LasI and LasR. The LasI protein catalyzes the production of the AHL molecule *N*-3-oxododecanoyl-L-homoserine lactone (3-oxo-C12-HSL). The 3-oxo-C12-HSL molecule docks with the DNA-binding transcription regulator LasR, which allows LasR to bind to the promoters of QS-regulated genes to control virulence factor such as *lasB* (elastase), *lasA* (staphylolysin), *aprA* (alkaline protease),

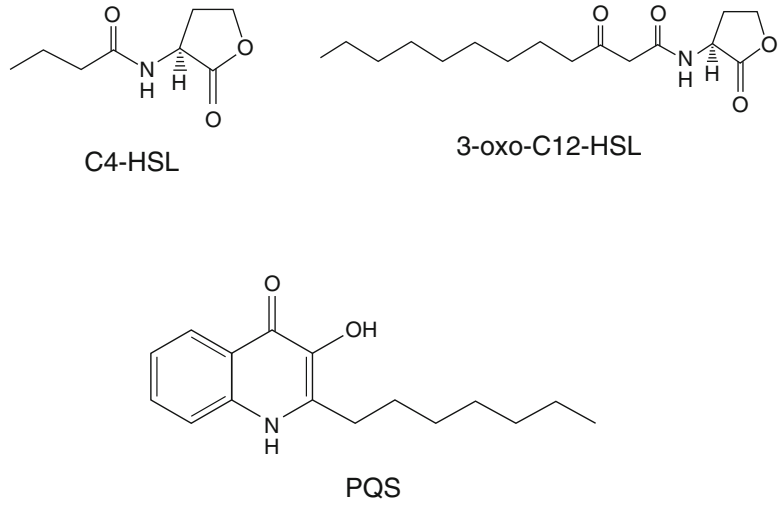


**Fig. 1** Structures of the *P. aeruginosa* major quorum-sensing signal molecules C4-HSL, 3-oxo-C12-HSL, and PQS

*toxA* (exotoxin A), *hcnABC* (hydrogen cyanide synthase), and *lasI*. The Las circuit induces a positive feedback loop to produce more AHL and also induces a secondary QS circuit, the Rhl system. The Rhl system consists of RhlI, which synthesizes *N*-butyryl-L-homoserine lactone (C4-HSL), and the receptor RhlR. As with the Las system, C4-HSL accumulates to a sufficient concentration and binds to RhlR. The Rhl system induces expression of *rhlAB* (rhamnolipid synthesis genes), *rhlI*, *lasB*, *rpoS* (the stationary-phase sigma factor), *lecA* (type 1 lectin), *lecB* (type II lectin), *hcnABC*, and genes involved in pyocyanin production (Winzer et al. 2000).

The additional *P. aeruginosa* gene that codes for a homologue of LasR and RhlR, QscR, is an orphan quorum-sensing signal receptor. QscR mutants are hypervirulent, and a number of genes controlled by the other AHL-based QS systems are repressed by QscR. There are several possible mechanisms for QscR repression of LasR- or RhlR-activated genes. The QscR protein forms homomultimers and also heteromultimers with LasR and RhlR. The heteromultimer formation could interfere with the activity of LasR and RhlR. QscR might also bind to the AHLs and compete with LasR and RhlR for these signals. QscR can also function by direct binding as a homomultimer to specific promoters and function in an acyl-HSL-independent manner, or it could utilize the signal produced by LasI or RhlI. It has been shown that QscR does not bind to LasR-dependent promoters. QscR can repress the activation of selected LasR- and RhlR-dependent quorum-sensing responsive genes. This could be the result of competition for signal, competition for binding sites on the regulatory DNA, or heterodimer formation (Chugani et al. 2001). Thus, it is clear that the *qscR* gene codes for an orphan AHL transcription factor. Unlike *lasR* and *rhlR*, which are linked to *lasI* and *rhlI*, genes that code for the production of acyl-HSL signals, there is no *I* gene linked to *qscR*. However, the DNA-binding activity of QscR is dependent on the presence of a long-chain acyl-HSL. The 3-oxo-C12-HSL produced by LasI and to which LasR responds is an effective ligand for QscR. Like LasR, QscR requires 3-oxo-C12-HSL to fold into an active conformation, but unlike LasR signal binding to QscR is not irreversible. However, binding of purified QscR to DNA is dependent on added acyl-HSL. That two 3-oxo-C12-HSL-responsive transcription factors differ fundamentally in their ability to exist in the absence of the signal. This would allow for a very rapid response of the QscR regulon to sudden decreases in environmental levels of 3-oxo-C12-HSL where the LasR regulon may respond more slowly. Like other transcriptional activators in the LuxR family that have been studied, QscR requires the presence of an acyl-HSL in the culture growth medium for folding in an active state. However,

unlike LasR, which also responds to 3-oxo-C12-HSL, purified QscR requires exogenous addition of 3-oxo-C12-HSL for binding to target DNA. QscR has a broader signal specificity than does LasR, and QscR may even respond to 3-oxo-C10-HSL, C10, and C12 better than it does to 3-Oxo-C12 HSL. This suggests that QscR might respond to signals produced by other bacteria that coexist with *P. aeruginosa*. This also shows the possibility that *qscR* and the genes surrounding it may be relatively recent acquisitions in the *P. aeruginosa* genome (Lequette et al. 2006). The structure of the transcription factor, QscR, bound to N-3-oxododecanoyl-homoserine lactone has been elucidated at a resolution of 2.55 Å. The ligand-bound QscR is a dimer with a unique symmetric "cross-subunit" arrangement containing multiple dimerization interfaces involving both domains of each subunit. The QscR dimer appears poised to bind DNA. QscR recognizes 3-oxo-C12-HSL in almost exactly the same way as LasR. However, QscR shows greater promiscuity in its response to AHLs than does LasR in *P. aeruginosa*. Although the binding pocket surface areas, packing densities, and pocket volumes are nearly identical in QscR and LasR, different interactions involving the 3-oxo position of the acyl chain may be responsible for the more relaxed specificity of QscR relative to LasR (Lintz et al. 2011).

The *Pseudomonas* quinolone signal (PQS) is a third *P. aeruginosa* QS signal that is dependent on the balanced production of 3-oxo-C12-HSL and C4-HSL. The PQS molecule (2-heptyl-3-hydroxy-4-quinolone) plays a significant role in the transcription of Rhl-dependent *P. aeruginosa* virulence genes encoding the production of pyocyanin and rhamnolipid. PQS production is intimately linked to the QS hierarchy, with its production and bioactivity requiring both the *las* and *rhl* QS systems. Additionally, PQS was recently shown to be solubilized by rhamnolipids, the production of which is controlled by the Rhl system, which may be important for the activity of PQS as an extracellular signal. LasR has been shown to regulate PQS production, and the provision of exogenous PQS induces expression of *lasB* (coding for elastase), *rhlI*, and *rhlR* implying that PQS activity constitutes a regulatory link between the *las* and *rhl* quorum-sensing systems. It is possible that PQS upregulates the *rhl* quorum-sensing system in late stationary-phase cultures. The structural genes required for PQS have been identified (*pqsABCDH*) along with a transcriptional regulator (*pqsR*) and a response effector (*pqsE*). The transcription of *pqsH* is regulated by the *las* QS system, linking QS and PQS regulation. Mutations in the PQS genes result in a loss of PQS synthesis and a corresponding loss of pyocyanin production. A mutation in the *pqsE* gene also results in a loss of pyocyanin even though PQS synthesis remains intact. This suggests that *pqsE* is not required for PQS biosynthesis and may have a role in the cellular response to PQS (Gallagher et al. 2002). PQS is synthesized via a "head-to-head" condensation of anthranilate and β-keto dodecanoate and requires the products of the *pqsA*, *pqsB*, *pqsC*, and *pqsD* genes, which also generate over 50 other 2-alkyl-4-quinolones (AHQs) including 2-heptyl-4(1*H*)-quinolone (HHQ). The function of the last gene in the *pqs* operon (*pqsE*) is not known, but while *pqsE* mutants produce parental levels of AHQs, they do not exhibit any PQS-associated phenotypes; consequently, PqsE is considered to facilitate the response to PQS. The immediate precursor of PQS is HHQ, and its conversion to PQS depends on the action of PqsH, a putative monooxygenase that is LasR regulated so linking the AHL and AHQ regulatory systems. Expression of the *pqsABCDE* operon and hence AHQ production are controlled by the LysR-type regulator PqsR (MvfR), which binds directly to the *pqsA* promoter. As PqsR binding is enhanced in the presence of PQS, it implies that PQS acts as a PqsR coinducer. The *pqsR* gene is itself positively regulated by *lasR* and negatively regulated by *rhlR*, establishing a further link between AHL-dependent quorum sensing and AHQ biosynthesis and hence AHQ signaling. Among the many different AHQs produced by *P. aeruginosa*, two of the major compounds are PQS and its precursor, HHQ, although similar concentrations of 2-nonyl-4-quinolone (HNQ), 2-nonenyl-4-quinolone, and 2-heptyl-4-quinolone-*N*-oxide (HQNO) have been

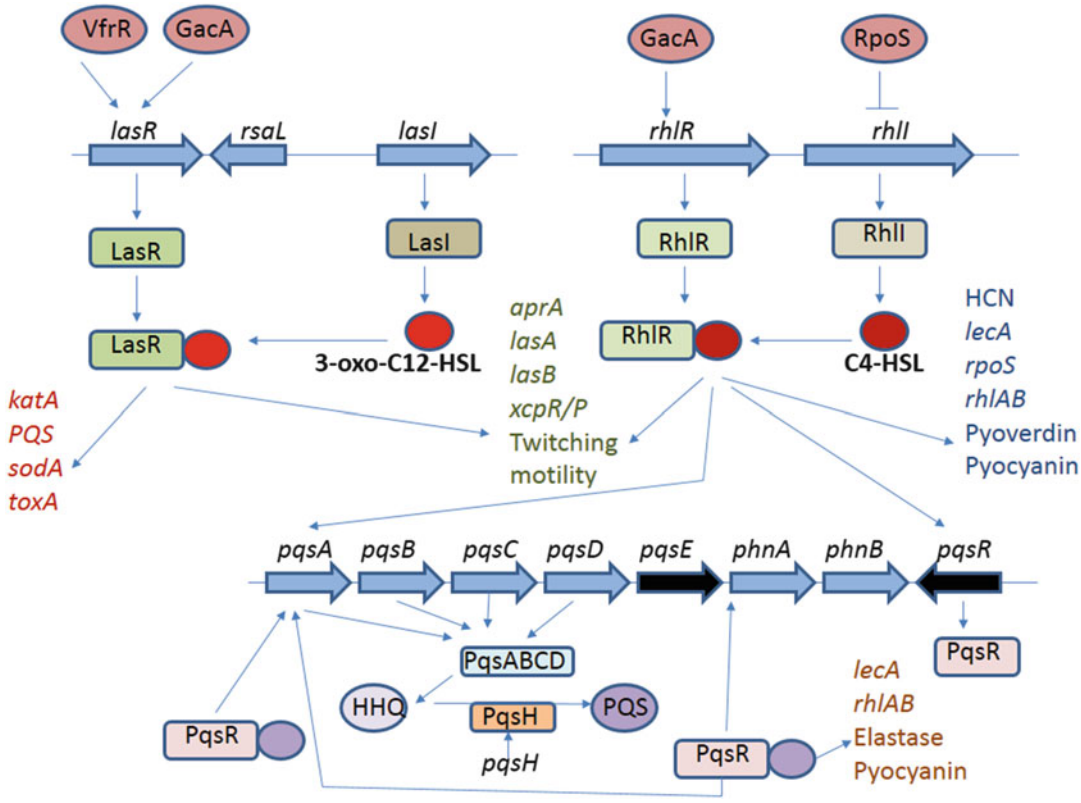


**Fig. 2** Regulation of the LasI/R, RhlI/R, and PQS quorum-sensing systems in *Pseudomonas aeruginosa*

reported to be present in culture supernatants (Diggle et al. 2007). PQS regulates the production of virulence determinants including elastase, rhamnolipids, the galactophilic lectin, LecA, and pyocyanin and influences biofilm development. Thus, PQS signaling plays an important role in *P. aeruginosa* pathogenesis. In contrast to the AHLs, when supplied exogenously, PQS overcomes the cell population density-dependent production of *P. aeruginosa* exoproducts (McKnight et al. 2000; Dubern and Diggle 2008). PQS is also produced in the lungs of cystic fibrosis patients infected with *P. aeruginosa* and is required for virulence in eukaryotes. It can also induce apoptosis and decrease viability of eukaryotic cells (Wade et al. 2005).

The QS signaling systems of *P. aeruginosa* are complex in nature and are well regulated in a hierarchical cascade targeting the expression of genes required for growth, survival, virulence, and biofilm formation (Fig. 2).

## Quorum-Sensing Regulatory Network in *P. aeruginosa*

Quorum sensing controls a significant proportion of the virulence factors used by *P. aeruginosa* for establishing infection. Additional genes can also influence the QS response. The QS regulator (QscR) represses 3-oxo-C12-HSL-regulated virulence factors and prevents the premature activation of QS cascade within a host and in environments where it is not required. This inhibitory effect is controlled by the global activator protein GacA. RsaL, the product of a gene found between *lasI* and *lasR*, negatively regulates the Las QS circuit. The product of the *vrf* gene is a cAMP receptor homologue and is required for the transcription of *lasR*. RsmA avoids early activation and downregulates production of QS-regulated phenotypes – protease, elastase, and

staphylolytic activities – and the production of a cytotoxic lectin, hydrogen cyanide, and pyocyanin. Overexpression of RsmA results in reduction in the expression of the AHL synthase genes *lasI* and *rhlI*. The RpoN is a negative transcriptional regulator of the *lasIR* and *rhlIR* but positively regulates the expression of *rhlI* in minimal media (Willcox et al. 2008). The catabolite repressor homologue Vfr directly induces *lasR* transcription. The stringent response protein RelA, which synthesizes guanosine tetraphosphate (ppGpp) under amino acid starvation conditions on overexpression, causes early activation of several QS-controlled processes and *lasR* and *rhlR* expression. The GacA/GacS two-component regulatory system posttranscriptionally regulates QS through RsmZ and RsmA. In the absence of RsmZ, RsmA represses the synthesis of acyl-HSL signals. RsmA also regulates the production of virulence factors. The anaerobic regulator ANR activates expression of the quorum-controlled hydrogen cyanide biosynthetic genes *hcnABC*; ANR appears to be an important factor in the co-regulation of quorum-controlled genes under oxygen-limiting conditions. The *rsaL* gene, which is directly activated by LasR-3OC12-HSL, encodes an 11-kDa protein which inhibits QS by repressing *lasI*. The stationary-phase sigma factor RpoS can affect the expression of 40 % of QS-regulated genes. The transcriptional regulator VqsR, which is activated by LasR-3-oxo-C12-HSL, is required for AHL production and the expression of QS-controlled genes. Further, the genes required for the synthesis of a direct precursor of PQS (*pqsABCD* and *phnAB*) are activated by the transcriptional regulator MvfR, and it itself is under the control of LasR-3-oxo-C12-HSL. MvfR regulates *rhl*-dependent genes without affecting the production of AHLs or the expression of *lasR* or *rhlR*. MvfR/PQS and *rhl* QS are parallel pathways that converge at the promoters of their target genes. All these suggest that QS in *P. aeruginosa* is highly complex and the QS gene expression is integrated in a highly interconnected network of other regulatory systems (Schuster and Greenberg 2006; Williams and Camara 2009).

## Quorum Sensing in *Pseudomonas fluorescens*

A quorum-sensing system has been found in *P. fluorescens* strain NCIMB 10586 (EI-Sayed et al. 2001). Two genes, *mupR* and *mupI*, have been cloned and sequenced and the gene products are identical to LasR/LuxR and LasI/LuxI, respectively. *mupR* encodes a predicted protein of 234 amino acids with a molecular mass of 26 kDa, while mupI produces a 191 amino acid protein with a molecular mass of 21 kDa. The *mupR/mupI* quorum sensing has been found to regulate the mupirocin biosynthetic gene cluster in *P. fluorescens* NCIMB 10586. Although the QS molecule has not been identified in *P. fluorescens* NCIMB 10586, several QS signals have been reported to be present in different *P. fluorescens* strains. *P. fluorescens* F113 produces at least three different AHLs, N-(3-hydroxy-7-cistetradecenoyl)homoserine lactone (3 OH, C14:1-HSL), N-decanoyl homoserine lactone (C10-HSL), and N-hexanoyl homoserine lactone (C6-HSL). C10-HSL has not been previously found as a naturally occurring AHL (Laue et al. 2000). A gene in *P. fluorescens* F113, termed *hdtS*, was capable of directing synthesis of all three AHLs. The HdtS, a 33-KDa protein, does not belong to the known AHL synthase families and is related to the lysophosphatidic acid acyltransferase family. It was concluded that HdtS is from a third protein family capable of AHL biosynthesis. The three AHLs identified in *P. fluorescens* F113 have yet been identified in any other *P. fluorescens* strains (Laue et al. 2000).

Many AHLs have been identified in *P. fluorescens* 2–79 such as N-(3-hydroxyhexanoyl)-L-HSL, N-(3-hydroxyoctanoyl)-LHSL and N-(3-hydroxydecanoyl)-L-HSL, N-octanoyl-L-HSL, and N-hexanoyl-HSL (Shaw et al. 1997). In *P. fluorescens* NCIMB 10586, a compound characterized as cyclo(L-Phe-L-Pro), a diketopiperazine (DKP), was identified. This shows the complexity of the QS system and the existence of cross-talk among bacterial signaling systems in *P. fluorescens* (Cui 2004).

A food isolate *P. fluorescens* strain 395 was capable of producing C4-HSL and 3-oxo-C8-HSL. It was also demonstrated that the alkaline metalloprotease gene in *P. fluorescens* is regulated by the AHL-based quorum-sensing system at a transcriptional level during the late exponential growth phase (Liu et al. 2007).

## Quorum Sensing in *Pseudomonas syringae*

Plant pathogen *P. syringae* pv. *syringae* strain B728a possesses an AHL QS system called AhlI/R which produces and responds to 3-oxo-C6-HSL. The two regulators are independently involved in the positive regulation of *ahlI* expression. The GacA/GacS system regulates AHL QS in *P. syringae* through regulation of *ahlI* expression. A TetR family transcriptional regulator designated as AefR positively regulates *ahlI* expression independently from GacA/GacS. The expression of *ahlI* is restored in *gacA* and *aefR* mutants on exogenous addition of 3-oxo-C6-AHL, implying that *ahlI* expression is responsive to AHL through a positive feedback mechanism as in other AHL QS systems. This also indicates that neither regulator is acting through a direct interaction at the promoter sequence. The GacA/GacS system present at the top of the regulatory cascade system affects AHL accumulation in *P. syringae* pv. *tomato* strain DC3000 (Chatterjee et al. 2003; Quinones et al. 2004).

## Quorum Sensing in *Pseudomonas putida*

The AHL QS system designated PpuI/R has been identified in two *P. putida* plant-beneficial rhizobacteria. The *ppuI/R* genes of *P. putida* have between them a repressor gene called *rsaL*, which negatively controls *ppuI* and *lasI* gene expression. In *P. putida*, *ppuI* expression is under strong negative transcriptional regulation by RsaL. It plays a major role in keeping the AHL system at very low expression levels, by competing for *ppuI* promoter binding with PpuR/3-oxo-$C_{12}$-HSL. *ppuI* expression in *P. putida* is positively regulated by the GacA/GacS two-component system and undergoes cross-regulation with the stationary-phase RpoS sigma factor (Bertani and Venturi 2004).

## Quorum Sensing in *Pseudomonas aureofaciens* and *Pseudomonas chlororaphis*

The biological control bacterium *Pseudomonas chlororaphis (aureofaciens)* strain 30–84 employs two quorum-sensing (QS) systems: PhzR/PhzI regulates the production of the antibiotics phenazine-1-carboxylic acid, 2-hydroxy-phenazine-1-carboxylic acid, and 2-hydroxy-phenazine, whereas CsaR/CsaI regulates various aspects of the cell surface. The PhzR–PhzI quorum-sensing system regulates phenazine production in a cell density-dependent manner. The *phzR* gene encodes a transcriptional regulator of the phenazine operon, and *phzI* encodes an AHL synthase that directs the synthesis of the signal hexanoyl homoserine lactone (HHL). Upon binding HHL, PhzR becomes activated and induces transcription of the phenazine genes. The GacS–GacA two-component signal transduction system is also involved in controlling phenazine production by regulating transcription of *phzI* and other regulatory elements. The second QS regulatory system, termed CsaR–CsaI (for "cell surface alterations"), is only marginally involved in phenazine regulation. The primary function of the CsaR–CsaI system is the regulation of exoprotease production in conjunction with the PhzR–PhzI system and also the regulation of cell surface properties. CsaI and CsaR are similar to RhlI and RhlR of *P. aeruginosa*. However, *rhlI* and *rhlR* are separated by 181 bp and *rhlI* has its own promoter, whereas *csaR* and *csaI* are separated by only 30 bp, and *csaI* has an RBS but no promoter, implying that *csaI* expression is dependent on *csaR*. The PhzR–PhzI and CsaR–CsaI appear to function independently and do not exist in a hierarchical relationship. However, the AHL produced by PhzI can interact with CsaR and the AHL produced

by CsaI can activate PhzR. The two systems are cooperatively involved in the regulation of exoprotease production and colonization of the wheat rhizosphere. The CsaI/CsaR system is also under positive regulation by GacA/GacS (Zhang and Pierson 2001).

In the rhizobacterium *P. chlororaphis* PCL1391, the expression of the antifungal metabolite phenazine-1-carboxamide (PCN) biosynthetic gene cluster is population density dependent and is regulated by the quorum-sensing genes phzI and phzR via synthesis of the autoinducer N-hexanoyl-L-homoserine lactone (C6-HSL). A mutation in the psrA gene (*Pseudomonas* sigma regulator), the gene product of which is a member of the TetR/AcrR family of transcriptional regulators, resulted in increased production of autoinducer molecules and PCN. PsrA also negatively regulates an as yet unidentified AHL QS system(s) in *P. chlororaphis*. PsrA in *P. chlororaphis* is well connected with the RpoS stationary-phase sigma factor, as it positively regulates *rpoS* expression (Chin et al. 2001, 2005). The *psrA* gene is itself positively regulated by the two-component GacA/GacS system in *P. chlororaphis* (Bertani and Venturi 2004; Chin et al. 2005).

## Quorum Sensing in *Pseudomonas corrugata* and *Pseudomonas mediterranea*

*Pseudomonas corrugata* and *Pseudomonas mediterranea* are two closely related phytopathogenic bacteria. *P. corrugata* CFBP 5454 has an *N*-acyl-homoserine lactone (AHL) QS system PcoI/PcoR which produces C6-HSL, 3-oxo-C6-HSL, and C8-HSL and is involved in virulence and regulates lipodepsipeptides LDP production at high population densities. *P. mediterranea* also produces LDPs as well as possessing an AHL-dependent QS system, designated PmeI/PmeR, which is highly homologous to the PcoI/PcoR system of *P. corrugata* producing and responding to $C_6$-AHL. Downstream of *pmeI* revealed the presence of a homologue of the *rfiA* gene of *P. corrugata* which encodes a transcriptional regulator involved in bacterial virulence. As in other Gram-negative bacteria, the production of AHL signal molecules occurs in a cell density-dependent fashion and requires the expression of the AHL synthase gene, *pcoI*, and the *pcoR* regulator gene. The protein RfiA in *P. corrugate* is an important and novel transcriptional regulator, directly linked to QS by co-transcription with *pcoI* (Licciardello et al. 2009). RfiA and PcoR are required for full virulence in tomato. Mutation of either *pcoR* or *rfiA* drastically reduces virulence in tomato. PmeI and PcoI and PmeR and PcoR have high homology to LuxI and LuxR family proteins of different pathovars of *P. syringae* and other oxidase-negative *Pseudomonas* plant-associated bacterial species other than to oxidase-positive species such as *P. fluorescens* and *P. putida* (Licciardello et al. 2012).

## AI-2-Mediated QS in *P. aeruginosa*

*P. aeruginosa* is unique from the other bacteria because it is does not make its own signaling molecule autoinducer-2 (AI-2). However, there is an increase in the expression of its virulence factor in response to AI-2 produced by other microflora. Although the *P. aeruginosa* QS circuit is AHL signaling based, it is capable of sensing AI-2 and is therefore susceptible to AI-2-mediated QS inhibition. *P. aeruginosa* was able to sense and respond to AI-2 produced by the normal microflora of cystic fibrosis patients, which led to increased virulence factor expression and infection (Duan et al. 2003; Roy et al. 2011).

## Additional AHLs and Signaling Molecules in *Pseudomonas*

Apart from the two main AHLs of *P. aeruginosa* (C4-HSL and 3-oxo-C12-HSL), low concentrations of other distinct AHLs, such as 3-oxo-C14-HSL and 3-oxo-C10-HSL, have been detected in *P. aeruginosa*. These AHLs may be synthesized due to the LasI synthase coupling the wrong acyl carrier protein (ACP) to *S*-adenosylmethionine

(SAM) or from the action of a different type of AHL synthase. The two other unrelated AHL synthase families that have been reported are the LuxM synthase family similar to *Vibrio* spp. and the HdtS synthase homologous to those of *P. fluorescens*. The other signaling molecules are diketopiperazines (DKPs), a novel family of cyclic dipeptides, identified from the culture supernatants of various bacteria. These can interfere with the quorum-sensing systems of various bacteria by binding to the LuxR family of receptors and either activating or antagonizing AHL signals. The DKPs present in the supernatants of pseudomonads are cyclo(ΔAla-L-Val) and cyclo(L-Pro-L-Tyr) in *P. aeruginosa* and cyclo(L-Phe-L-Pro) in *P. fluorescens* and *P. alcaligenes* (Sio et al. 2012).

## Interkingdom Signaling in *P. aeruginosa*

The 3-oxo-C12-HSL and PQS of *P. aeruginosa* are capable of modulating inflammatory and immune responses in mammals. The QS signal 3-oxo-C12-HSL exerts immune-suppressive or anti-inflammatory effects at concentrations below 10 μM, whereas pro-inflammatory or pro-apoptotic effects are found at much higher concentrations. It attenuates LPS-induced inflammation required for the establishment of chronic *P. aeruginosa* infection. The host environment modulates QS in *P. aeruginosa* either through nonenzymatic or enzymatic destruction of AHLs through lactonolysis. The mammalian paraoxonases (PON1, PON2, PON3) are a unique family of calcium-dependent hydrolases and are known to possess enzymatic activities toward a broad range of substrates including the AHLs (Williams and Camara 2009).

## Quorum Quenching in *P. aeruginosa*

The functional analysis of putative acylase genes in the *P. aeruginosa* PAO1 genome, the PA2385 gene, revealed the presence of an acylase that removes the fatty acid side chain from the homoserine lactone (HSL) nucleus of AHL-dependent QS signal molecules. The posttranslational processing of the acylase and the hydrolysis reaction type are similar to those of the beta-lactam acylases, implying that the PA2385 protein is a member of the *N*-terminal nucleophile hydrolase superfamily. The purified acylase was capable of degrading AHLs with side chains ranging in length from 11 to 14 carbons at physiologically relevant low concentrations. The substituent at the 3′ position of the side chain did not affect activity, indicating broad-range AHL quorum-quenching activity. Of the two main AHL signal molecules of *P. aeruginosa* PAO1, *N*-butanoyl-L-homoserine lactone (C4-HSL) and *N*-(3-oxododecanoyl)-L-homoserine lactone (3-oxo-C12-HSL), only 3-oxo-C12-HSL is degraded by the enzyme. The purified protein completely inhibited the accumulation of 3-oxo-C12-HSL and production of the signal molecule 2-heptyl-3-hydroxy-4(1*H*)-quinolone and reduced production of the virulence factors elastase and pyocyanin in *P. aeruginosa* PAO1 cultures. Similar results were observed on overexpressing the PA2385 gene in *P. aeruginosa*. The AHL acylase has in situ quorum-quenching activity, and it enables *P. aeruginosa* PAO1 to modulate its own quorum-sensing-dependent pathogenic potential (Sio et al. 2012).

## Conclusion

*P. aeruginosa* is the most common nosocomial pathogen and is associated with chronic lung disease in cystic fibrosis (CF) patients. The major virulence signaling systems in *P. aeruginosa* are the AHL systems Las and Rhl, which together control the expression of multiple virulence factors in response to cell density. The third group of signaling system, the PQS, connects virulence factor production with adaptation and survival as a strategy to eliminate competition when survival depends on iron availability. Similarly, a number of regulators and more than one AHL-based QS system have been

identified in plant growth promoting *P. aureofaciens*/*P. chlororaphis*, root colonizing *P. fluorescens*, and the plant pathogen *Pseudomonas syringae.* Understanding the molecular mechanisms of regulation of QS and the complete signaling integration network in *Pseudomonas* will help in identifying the various drug targets to interrupt pathogenic activities by designing novel antimicrobials.

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