Quorum sensing in plant-associated bacteria

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N-acyl homoserine lactone (AHL)-mediated quorum sensing by bacteria regulates traits that are involved in symbiotic, pathogenic and surface-associated relationships between microbial populations and their plant hosts. Recent advances demonstrate deviations from the classic LuxR/LuxI paradigm, which was first developed in *Vibrio*. For example, LuxR homologs can repress as well as activate gene expression, and non-AHL signals and signal mimics can affect the expression of genes that are controlled by quorum sensing. Many bacteria utilize multiple quorum-sensing systems, and these may be modulated via post-transcriptional and other global regulatory mechanisms. Microbes inhabiting plant surfaces also produce and respond to a diverse mixture of AHL signals. The production of AHL mimics by plants and the identification of AHL degradative pathways suggest that bacteria and plants utilize this method of bacterial communication as a key control point for influencing the outcome of their interactions.

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Abbreviations

ACPs	acyl carrier proteins
AHL	*N*-acyl homoserine lactone
CDF	cell density factor
OHL	*N*-(3-oxohexanoyl)-*L*-homoserine lactone
SAM	*S*-adenosyl-methionine

Introduction

The production of small molecular weight signals as a mechanism of cell–cell communication among bacteria is well recognized. Regulation of bacterial gene expression in response to *N*-acyl-homoserine lactone (AHL) signals is the best characterized example of such a system, and has been the subject of several excellent recent reviews [1•,2•,3,4••]. This form of communication has been referred to as 'quorum sensing' as the level of signal required for gene induction occurs only when the appropriate density or 'quorum' of signal producers is present. Owing to space limitations, we focus here on quorum sensing in three representative groups of plant-associated bacteria: symbiotic rhizobia, phytopathogenic *Erwinia* and related species, and plant-associated pseudomonads. We provide a brief overview of quorum sensing, and discuss recent advances in our understanding of gene regulation by quorum sensing and the potential roles of these regulatory schemes in microbe–microbe and plant–microbe interactions in each of these model systems. Lastly, we mention recent discoveries of AHL-degrading enzymes, plant AHL mimics and signal eavesdroppers, and discuss how they influence our perception of plant-associated bacterial communities and our ability to manipulate them.

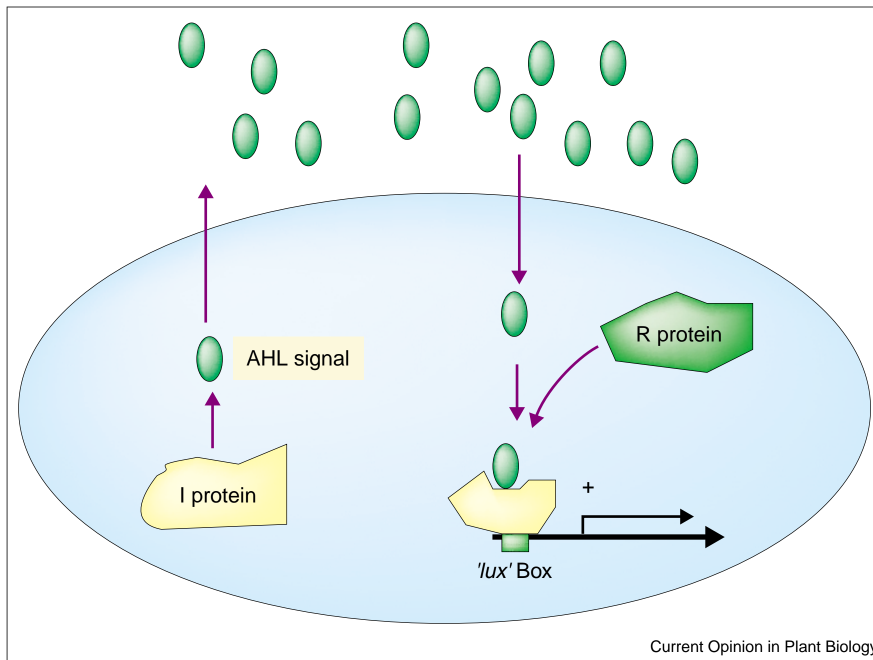
A model for AHL-mediated gene regulation

The basic model for AHL-mediated gene regulation involves a transcriptional regulator (i.e. an R protein) and an AHL synthase (i.e. an I protein) (Figure 1). This model is based upon studies in which the bacterial population size determined the pattern of AHL-mediated gene expression. The R protein can recognize specific promoter sequences and stimulate gene expression only when complexed with an AHL signal. Typically, the AHL synthase is expressed at low levels and, at low cell densities, insufficient intracellular AHL exists to activate the R protein. As the bacterial population increases, AHLs accumulate until sufficient intracellular AHL is present to ensure that some of it binds to the R protein, resulting in the binding of the R protein to its target promoter sequences.

R proteins

Two classes of transcriptional regulators known as R proteins exist. One requires an AHL signal to bind to DNA whereas the second only binds DNA in the absence of AHL signal. The only known example of the latter class is EsaR in the wilt pathogen *Pantoea stewartii* [5]. The carboxy-terminal regions of all known R proteins contain a conserved helix-turn-helix motif that is required for binding to a 20-basepair palindromic DNA sequence (a '*lux*' box), which is located approximately 40 bases upstream of the transcriptional start site. The carboxy-terminal region also contains a second domain that is involved in the multimerization of R proteins that are required for DNA binding. Most R proteins bind DNA as dimers. However, CarR of *Erwinia carotovora* forms higher-order multimers upon AHL binding [6•]. Upon *lux*-box binding, R proteins are hypothesized to stimulate transcription by interacting with the carboxyl terminus of the RNA polymerase α subunit. The amino-terminus of the R protein is involved in two aspects that are critical for the function of R proteins. First, in the absence of bound AHL, it inhibits '*lux*'-box binding. Second, it contains the AHL-binding site that was recently shown to be required for proper folding of *Agrobacterium tumefaciens* TraR and its resistance to proteolytic degradation [7•].

Figure 1



A simplified scheme illustrating some key control features of AHL-mediated autoinduction by I and R proteins. Bacterial cells (shown in blue) contain an I protein that is responsible for the synthesis of freely diffusible signals (green ovals). At high cell density, the signal accumulates intracellularly and interacts with the R protein. This interaction induces a conformational change in the R protein, which alters the affinity of the R protein for specific DNA sequences, known as '*lux*' boxes, that are located within the promoters of the AHL-regulated genes.

I proteins

The I protein is responsible for AHL synthesis. Multiple classes of AHL-synthases have been identified. The first is the LuxI-type that utilizes the cellular metabolites *S*-adenosyl-methionine (SAM) and specific cellular acetylated acyl carrier proteins (ACPs) to form the AHL. The second class of AHL-synthesizing enzymes includes AinS and LuxLM from the marine bacterium *Vibrio harveyi*, and utilizes SAM plus either acylated ACPs or acyl-CoAs as substrates. Recently, *P. fluorescens* F113 was shown to synthesize AHLs using HdtS, a novel acyl-transferase enzyme [8].

The structures of AHLs are highly conserved, consisting of a homoserine lactone ring connected to a fatty acyl sidechain. AHLs vary with respect to the length of the *N*-acyl chains (4–14 carbons), and the chains are either saturated or unsaturated. In addition, the *N*-acyl chain contains either a hydroxy or an oxo group at the third carbon. Most AHLs are believed to diffuse across the cell wall, with the exception of long-chain AHLs (such as the C12 AHL produced by *P. aeruginosa*) that utilize an efflux pump for translocation across the cell wall [9].

Examples of quorum-sensing in plant-associated bacteria

Quorum sensing in rhizobia

The establishment of a rhizobium–legume symbiosis involves a complex exchange of signals between rhizobia and the plant. Successful nodulation results in the formation of root nodules in which bacteria reside and fix atmospheric nitrogen. The bacteria inside the infected plant cells are termed bacteroids and exist in a membrane-bound compartment, the symbiosome. Each infected cell is filled with symbiosomes, resulting in a large bacterial population. Under these circumstances, it is likely that the symbiotic process is subject to quorum regulation.

Although the current data do not support a critical role for AHL signaling in nodulation, signaling does play a role in determining the amount of nodulation that occurs. *Rhizobium* species are known to produce a wide variety of AHLs. For example, in *Rhizobium leguminosarum*, the synthesis of seven AHLs (including a unique C7 AHL) is directed by four LuxI homologs (i.e. CinI, RhiI, TraI and RaiI) [10]. Quorum sensing in *R. leguminosarum* affects nodulation through the RhiR-mediated regulation of the *rhiABC* operon, which is involved in rhizosphere growth. Mutations to *rhiR* or *rhiA* further reduced nodulation in mutant strains that were defective in the synthesis of the lipo-chitin Nod signal. By contrast, *RhiI* mutants had increased nodulation. These effects are subtle and likely reflect AHL-associated differences in growth or metabolism, rather than direct influences of AHLs on nodulation.

Quorum sensing has also been shown to play a role in the development of nodules. The *cinRI* loci in *R. leguminosarum* controls the production of the rhizosphere-expressed RhiABC. The *cin* system in *Rhizobium etli* is required for proper nodule development [11]. CinI is expressed in the infection thread and differentiating bacteroids. *CinI* mutations result in decreased nitrogen fixation, accompanied by atypical bacteroid growth and morphology. Again, these phenotypes are likely general growth effects and not intimately involved in specific nodulation processes.

Although AHL signaling does not appear to play a key role in nodulation, a unique quorum signal was shown recently to directly control nodulation-related gene expression in *Bradyrhizobium japonicum*, a symbiont of soybean [12••,13]. This signal is unidentified but does not appear to be related to AHLs. The accumulation of the signal, known as the cell density factor (CDF), in culture supernatant repressed *nod* gene expression through the action of NolA and NodD2. The induction of NolA expression by CDF requires a two-component regulatory system, involving NwsAB as well as CDF, which likely recognizes the extracellular signal. Although critical for initiating the nodulation processes, induction of the rhizobial *nod* genes by the plant neither occurs nor is required in the latter stages of nodulation. Soybean nodules infected with a *B. japonicum* NolA mutant, unlike those infected with wildtype *B. japonicum*, demonstrated significant *nodY*::*GUS* expression. These results indicate that CDF controls *nod* gene expression *in planta*.

Understanding the relationship between CDF and nodulation may lead to the improved efficacy of *B. japonicum* inoculant on soybean. The efficiency of soybean nodulation depends on the levels of bacterial inoculum applied to the host plant. Loh *et al.* [12••] demonstrated that production of the CDF signal in *B. japonicum* cultures significantly reduced inoculant efficiency on soybean. Significant levels of CDF were found in commercial inoculants, which are grown to high cell density before packaging. Future attempts to modulate CDF production could lead to improvements in inoculation performance.

Quorum sensing in *Erwinia* and related species

Several *Erwinia* species and bacteria from related genera produce AHLs that are typified by *N*-(3-oxohexanoyl)-*L*-homoserine lactone (OHL). The most-studied systems are those operating in *Erwinia carotovora* ssp. *carotovora* (Ecc), *Erwinia chrysanthemi* and *Erwinia* (*Pantoea*) *stewartii*, which have been the subject of recent reviews [4••]. The seminal studies of Salmond and Palva, as well as the subsequent work of others, have firmly established that the production of OHL by *E. carotovora* subspecies is required for their pathogenicity and synthesis of exoenzymes, harpins and antibiotics [14].

A notable new discovery is the regulatory mechanism involving ExpR in Ecc strains. Andersson *et al.* [15] showed that multiple copies of *expR* in strain SCC3193 inhibited exoenzyme production. Consistent with these results, over-production of ExpR in Ecc strain 71 not only abolished exoenzyme production (Chatterjee *et al.*, unpublished data) but also led to very high levels of *rsmA* transcripts, which encode an RNA-binding protein that promotes RNA decay. These latter results are interesting as several lines of evidence now point to the possibility that the OHL effect in Ecc is actually mediated via post-transcriptional regulation. The selection of exoprotein-producing mutants from an OHL-non-producing parent yields primarily *rsmA* mutants. Furthermore, OHL⁻ mutants over-produce RsmA [16], and this apparently accounts for the lack of exoprotein production by OHL-deficient bacteria.

Quorum sensing in plant-associated *Pseudomonas* species

Quorum sensing in *Pseudomonas* has been best characterized in human pathogens and related genera. Nevertheless, plant-associated pseudomonads are known to utilize one or more quorum-sensing systems to regulate multiple traits, some of which affect their persistence and viability on plant surfaces (reviewed in [4••]). For example, in *Pseudomonas aureofaciens* strain 30-84, a root-associated biological control agent that is effective against take-all disease of wheat, two separate quorum-sensing systems (PhzR/PhzI and CsaR/CsaI) have been identified. These systems regulate the production of antibiotics and secondary metabolites, exoprotease activity, and cell-surface features that contribute to their persistence on plants and effectiveness as biological control agents [17,18]. Quorum sensing in the plant pathogen *Pseudomonas syringae* pv. *syringae* also regulates traits that are involved in colony morphology and viability on plants [19].

Independent genetic studies of *Pseudomonas* species with multiple quorum-sensing systems have revealed that promoters that are controlled by quorum sensing differ in their specificity to R and I proteins. For example, in *Pseudomonas aeruginosa*, quorum-sensing controlled promoters were shown either to be specific to LasR or to respond to both LasR and RhlR [20]. Certain bases in the promoter elements determined specificity [20]. In *Pseudomonas aureofaciens* strain 30-84, PhzR and CsaR differ in their ability to regulate specific genes and to recognize AHL signals synthesized by PhzI and CsaI [18]. In these systems, regulation by quorum-sensing is typically under the control of a GacS–GacA two-component signal transduction system but may be regulated by other systems as well [19,21,22].

Microbial populations inhabiting the rhizosphere and plant surfaces produce and respond to a diverse mixture of AHL quorum signals that are produced by different bacterial species [23–25]. For example, when applied to plant roots, roughly 8% of isolates from wheat plants restored phenazine gene expression to a *phzI* mutant of strain 30-84 [23]. Recently, a wheat rhizosphere population was identified that produced signals that interfered with phenazine gene expression, indicating that both 'positive' and 'negative' signaling occurs within the microbial community (L Pierson, 10th International Society for Molecular Plant Microbe Interactions, July 10–14 2001, Madison, WI). Regulation by quorum-sensing has also been implicated in the formation of biofilms by various pseudomonads [4••,26]. As cross communication does occur on plant surfaces, it is interesting to hypothesize that the expression of traits that are regulated by quorum sensing, such as those involved in biofilm

formation and persistence on plants, are subject to community interactions. Differences in the specificity of quorum-sensing promoters for different R proteins and different signals, and hierarchical regulation among regulatory systems, may enable cells to fine-tune their response to the cafeteria of signals available within a shared niche.

Recent discoveries affecting AHL-mediated regulation

Role of AHL-degrading enzymes

Recently, two bacteria were isolated from soil that are able to degrade AHL signals [27,28••]. One bacterium, *Variovax paradoxus* strain VAI-C, has an aminoacylase activity that degrades AHLs and releases homoserine lactone. The second bacterium, *Bacillus* sp. 240B1, contains the gene *aiiA*. This gene encodes an AHL lactonase that degrades the *Erwinia* signal *N*-(3-oxohexanoyl)-*L*-homoserine lactone into *N*-(3-oxohexanyl)-*L*-homoserine. When *aiiA* was introduced into the soft-rotting pathogen *E. carotovora* strain SCG1, it caused a reduction in the amount of AHL released by the strain, a reduction in the level of several extracellular enzymes (including pectolytic enzymes), and attenuated virulence when inoculated onto eggplant and potatoes. The expression of *aiiA* in transgenic tobacco plants resulted in decreased symptoms due to reduced tissue maceration by *E. carotovora* [29••]. There was a strong correlation between disease resistance and AHL-lactonase expression in the plant tissues. It is hypothesized that the degradation of AHL signals in the bacterium when inoculated onto the transgenic plants slowed the expression of virulence genes sufficiently to allow the plant host defenses to block further infection and the spread of the pathogen. This form of 'quorum quenching' may be useful in the control of specific pathogenic bacteria.

AHL-mimics

Although AHL signaling has been studied primarily in the context of bacterial communication, it may be a more common language than originally envisioned. For example, the marine eukaryote *Delisea pulchra* secretes furanone signals that interfere with AHL signaling among bacterial populations [30]. Recent reports [31,32] have revealed that halogenated furanones inhibit quorum-sensing phenotypes such as biofilm formation in *Pseudomonas aeruginosa*, and carbepenem antibiotic and virulence factor production in *E. carotovora*. These mimic molecules are structurally similar to AHLs and may antagonize AHL-type behaviors by binding to the AHL receptor (e.g. LuxR [33,34]).

AHL mimic molecules have also been identified among higher plants. Teplitski *et al.* [35••] demonstrated that various plants (e.g. pea, soybean, rice and *Medicago truncatula*) secreted compounds that affect bacterial AHL-signaling. The varied responses of AHL reporters to the different plant extracts suggested that the plants produced several distinct compounds. Thus, plants may have adapted AHL mimics to communicate with specific bacteria. This strategy could be adopted to protect plants from pathogens. For example, Mae *et al.* [36••] demonstrated that transgenic tobacco expressing *E. cartovora expI* had enhanced resistance to *E. caratovora* invasion. One explanation for this finding is that the production of the *Erwinia* AHL signal by the plant resulted in premature *vir* gene expression, which activated host defense responses.

Signal eavesdroppers

Recently, it was discovered that some microorganisms produce LuxR homologs but lack the ability to produce AHLs. For example, SdiA in the human pathogen *Salmonella enterica* 'recognizes' AHL signals that are produced by other microorganisms [37••]. This may enable non-AHL producers to eavesdrop on communications among other microbes sharing the same niche and regulate the expression of specific genes by the density of signals produced by the microbial community, perhaps without having to produce signals themselves. Thus, our perception of what constitutes a 'quorum' on a plant surface may need to be refined to include the diversity of microbes capable of producing signals and those capable of monitoring signals in a shared niche.

Conclusions

Many plant-associated bacteria, whether involved in symbiotic, pathogenic or commensal relationships with plants, utilize quorum sensing to regulate the expression of a diverse array of genes, some of which are involved in interactions between microbes and/or between microbes and plants. Recent studies of quorum-sensing systems demonstrate that deviations from the classic LuxR/LuxI paradigm are common. It is becoming increasingly clear that bacterial communication occurs on plant surfaces, and participants likely include the community of signal producers, signal eavesdroppers, and the plant host. The production of AHL mimics by plants and AHL-degrading enzymes by bacteria suggests that bacteria and plants have evolved to utilize AHL signaling as a key 'control point' (DA Phillips, H Ferris, DR Cook, DR Strong, unpublished) for influencing the outcome of interactions between microbes and plants. Bacterial communication via AHLs also shows promise as a control point that can be manipulated to alter the outcome of microbe–microbe and microbe–plant interactions. Although an enormous amount of information on quorum sensing is being compiled for single bacterial species, only by considering multiple model systems can the variety of the roles that quorum sensing plays in plant–microbe interactions be fully understood.

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