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Microreview

Integration of environmental and host-derived signals with quorum sensing during plant–microbe interactions

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Summary

Many plant-associated microbes use secreted autoinducer molecules, including *N*-acylhomoserine lactones (AHLs), to regulate diverse behaviours in association with their population density (quorum sensing). Often, these responses are affected by environmental conditions, including the presence of other AHL-producing bacterial species. In addition, plant-derived metabolites, including products that arise as a direct result of the bacterial infection, may profoundly influence AHL-regulated behaviours. These plant products can interact directly and indirectly with the quorum-sensing network and can profoundly affect the quorum-sensing behaviour. Local conditions on a microscopic scale may affect signal molecule longevity, stability and accumulation, and this could be used to give information in addition to cell density. Furthermore, in many Gram-negative bacteria, AHL signalling is subservient to an additional two-component signalling system dependent upon homologues of GacS and GacA. The signal(s) to which GacS responds are not known, but recent research suggests that a self-produced ligand may be being detected. This review will focus on two well-studied examples of AHL-regulated plant-associated behaviour, *Erwinia carotovora* and *Agrobacterium tumefaciens*, to illustrate the complexity of such signalling networks.

Introduction

Many bacteria produce small signal molecules that are secreted into the local environment and trigger specific behavioural responses in the producing population when they exceed a critical concentration threshold. Under laboratory conditions, this threshold is normally reached when the bacterial culture producing the autoinducer (AI) signal molecule reaches a critical density. At this point, the population is said to be 'quorate', and a synchronized change in behaviour can be triggered. For this reason, the production of and response to such autoinducers is frequently termed quorum sensing. Among Gram-negative bacteria, the best studied and possibly most common group of autoinducer signals are *N*-acylhomoserine lactones (AHLs). In plant-associated bacteria, AHLs are found in pathogenic, symbiotic and biological control strains, and they regulate a diverse range of phenotypes including diverse pathogenicity determinants, conjugation, rhizosphere competence and the production of antifungal metabolites (Table 1). A higher proportion of AHL-producing bacteria is found in the immediate vicinity of plant roots (the rhizosphere) than in bulk soil, suggesting a general role in rhizosphere colonization (Elasri *et al*., 2001) and competence, possibly through regulating mechanisms such as exopolysaccharide production, attachment and biofilm formation (von Bodman *et al*., 1998; Denny, 1999; De Kievit *et al*., 2001; Marketon *et al*., 2003). Culture-based experiments show that AHL sensing is generally integrated into much larger control networks with many positive and negative inputs. In addition, it is becoming apparent that some plants may be able to interfere with, and possibly respond to, the bacterial AHL signalling system (Teplitski *et al*., 2000; Bauer and Robinson, 2002; Mathesius *et al*., 2003). The challenge now is to test the current models in naturally occurring plant–bacterial interactions to determine the key regulators in the AHL signalling network during the plant–microbe interaction. This is important because, under such conditions, it is possible that AI-based signalling systems might be conveying alternative or additional information to population size (Redfield, 2002) and, although such signalling mechanisms allow the co-ordination of multicellular behaviour, this may not always be the primary function.

This review will discuss recent research and highlight the importance of plant-derived compounds in contributing to AHL 'quorum-sensing' responses. As examples of this, it will focus on the role of AHL signalling in *Erwinia*

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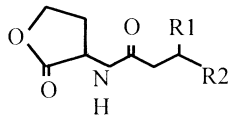
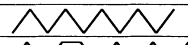
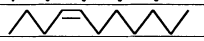
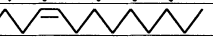
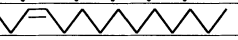
Table 1. Some of the AHLs found in plant-associated bacteria and the known phenotypes that are regulated.

Structure	R1	R2	AHL	Bacteria	Behavior
	H	H	C4-HSL	*Serratia liqufaciens* a	Antibiotic production Swarming
	H		C6-HSL	*P. chlororaphis* PCL1391b *C. violaceum* c *P. aureofaciens* 30-84 d	Phenazine biosynthesis Violacein production Phenazine, HCN biosynthesis
	O		oxo-C6-HSL	*E. carotovora* e	Exoenzyme production, Carbapenem production
	H		C7-HSL	*R. leguminosarum* f	?
	H		C8-HSL	*B. cepacia* g *A. tumefaciens* h *R. leguminosarum* f	Polygalactouronase production Ti plasmid transfer Rhizosphere competance
	O		oxo-C12-HSL	*P. aeruginosa* i	Biofilm formation
	OH		HO-C14:1-HSL	*R. leguminosarum* f	Growth inhibition
	H		C16:1-HSL	*Sinorhizobium meliloti* j	Exopolysaccharide production
	H		C18:1-HSL	*Sinorhizobium meliloti* j	?

a. Eberl *et al.* (1996).
b. Chin-A-Woeng *et al.* (2001).
c. McClean *et al.* (1997).
d. Chancey *et al.* (1999).
e. Bainton *et al.* (1992).
f. Lithgow *et al.* (2000).
g. Aguilar *et al.* (2003).
h. Zhang *et al.* (1993).
i. Pearson *et al.* (1994).
j. Marketon *et al.* (2003).
?, No function yet discovered.

carotovora and *Agrobacterium tumefaciens*, a necrotrophic and a biotrophic plant pathogen respectively.

The AHL signalling molecules and the *lux* paradigm

N-acylhomoserine lactones consist of a homoserine lactone moiety (derived from amino acid metabolism via S-adenosyl methionine) linked to a variable acyl side-chain [derived from fatty acid metabolism via the appropriately charged acyl–acyl carrier protein (acyl-ACP) or acyl–coenzyme A (acyl-CoA)] (Hanzelka and Greenberg, 1996; Schaefer *et al*., 1996). Reflecting its origin as an intermediate in fatty acid metabolism, the acyl side-chain variations generally consist of the presence or absence of an acyl chain C3 substituent (carbonyl, hydroxyl or fully reduced) and the length of the chain, typically 4–12 carbons (Hoang *et al*., 2002; Watson *et al*., 2002). In some *Rhizobium* species, acyl chain lengths up to 18 carbons have been reported, and unsaturated bonds may be present (Lithgow *et al*., 2000; Marketon *et al*., 2002). AHLs with an acyl chain length of six or fewer carbons are frequently referred to as short-chain AHLs, whereas those with chain lengths of 10 or more carbons are known as long-chain AHLs. Several bacterial species produce the same AHL although, in each, it may be used to regulate different biological processes (Fig. 1).

AHLs were first identified in the early 1980s as autoinducers of bioluminescence in the marine bacterium *Vibrio fischeri* (Eberhard *et al*., 1981), and this system has become the archetype for AHL-mediated quorum sensing. *V. fischeri* can be found at either low cell densities free-living in sea water or high densities as a symbiont in specialized organs (light organ) of certain fish and squid (Ruby and Lee, 1998). Significant light production only occurs in the light organ (or in laboratory cultures) where the bacteria are present at a high density. This is because of the requirement for the build-up of the AHL signalling molecule *N*-(3-oxohehanoyl)-L-homoserine lactone (3-oxo-C6HSL), which is synthesized by the LuxI gene product. LuxI is initially expressed at a low level, and the small amounts of 3-oxo-C6HSL AI produced diffuse out of the cell and into the surrounding environment. In the case of

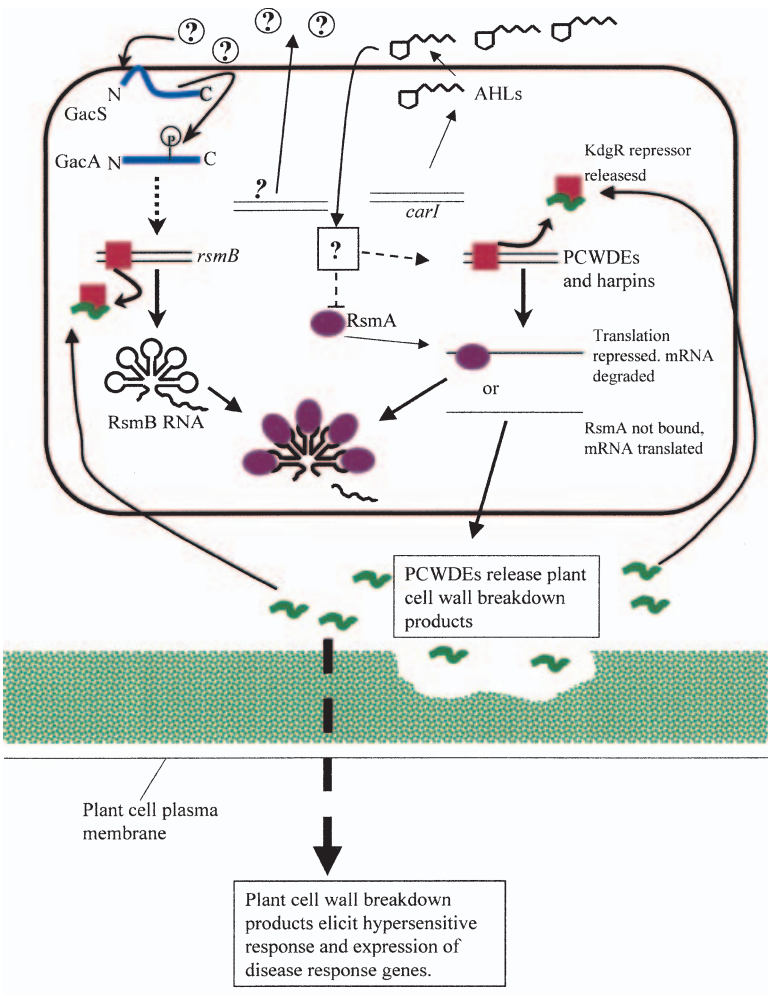


Fig. 1. Regulation of pathogenicity determinants in *E. carotovora*. Transcriptional repression of some exoenzymes and of *rsmB* is brought about by binding of KdgR (red squares) near promoter sequences. Translational repression and RNA turnover of pathogenicity-associated gene transcripts is brought about by binding of RsmA (purple ovals) to sequences within target mRNAs. AHLs activate the expression of pathogenicity determinants in a cell density-dependent manner by a mechanism that is unclear, but which may act antagonistically to RsmA. Secreted plant cell wall-degrading enzymes (PCWDEs) act on plant tissues to release metabolites that enter the bacterium and prevent KdgR repressor binding. Translational repression is removed by the action of *rsmB*, which may sequester RsmA. Transcription of *rsmB* is brought about by a two-component sensor kinase, GacS/GacA (also known as ExpS/ExpA) that responds to an unidentified signal.

the free-living bacterium, most of the autoinducer will never be encountered again. However, in the confines of the host's light organ, where there are many bacteria producing the signal molecule and where diffusion is restricted, 3-oxo-C6HSL levels rise (Boettcher and Ruby, 1995). Above a threshold level, 3-oxo-C6HSL binds to a transcriptional regulator (LuxR), which dimerizes and binds to a 20 bp palindromic sequence (the *lux* box) located upstream of the genes required for light production (Egland and Greenberg, 1999). Bound LuxR stimulates transcription of the *lux* operon and bioluminescence results. In the case of *V. fischeri*, transcription of *luxI* is also stimulated, so the levels of signal molecule rise still higher as a result of positive feedback (Shadel and Baldwin, 1991). It is generally argued that linking light production to cell density has evolved because it prevents the energetically expensive process of bioluminescence from operating under conditions in which it is of no benefit.

The finding in the early 1990s that similar autoinducer molecules regulated the production of antibiotic and pathogenicity determinants by the terrestrial plant pathogen *E. carotovora* was the first indication of how widespread this form of signalling would prove to be (Bainton *et al.*, 1992). However, in *E. carotovora*, the mechanism by which the AHL responses are regulated deviates from the *lux* model.

Erwinia carotovora

Erwinia carotovora is a Gram-negative bacterial phytopathogen that is the causative agent of plant soft rots and the potato disease blackleg (stem rot). Two subspecies are recognized, *Erwinia carotovora* spp. *carotovora* (*Ecc*) and *Erwinia carotovora* spp. *atroseptica* (*Eca*); *Eca* has a narrower host range, being primarily a pathogen of potato (reviewed by Perombelon, 2002; Toth *et al.*, 2003). Different laboratories have favoured various isolates of *Ecc* or *Eca* but, despite subtle differences, a unified gene regulatory network is emerging.

In *Ecc* strain ATCC39048, the AHL signal molecule(s), which usually consist(s) of 3-oxo-C6-HSL and/or C6-HSL, is generated by the action of the LuxI homologue, CarI

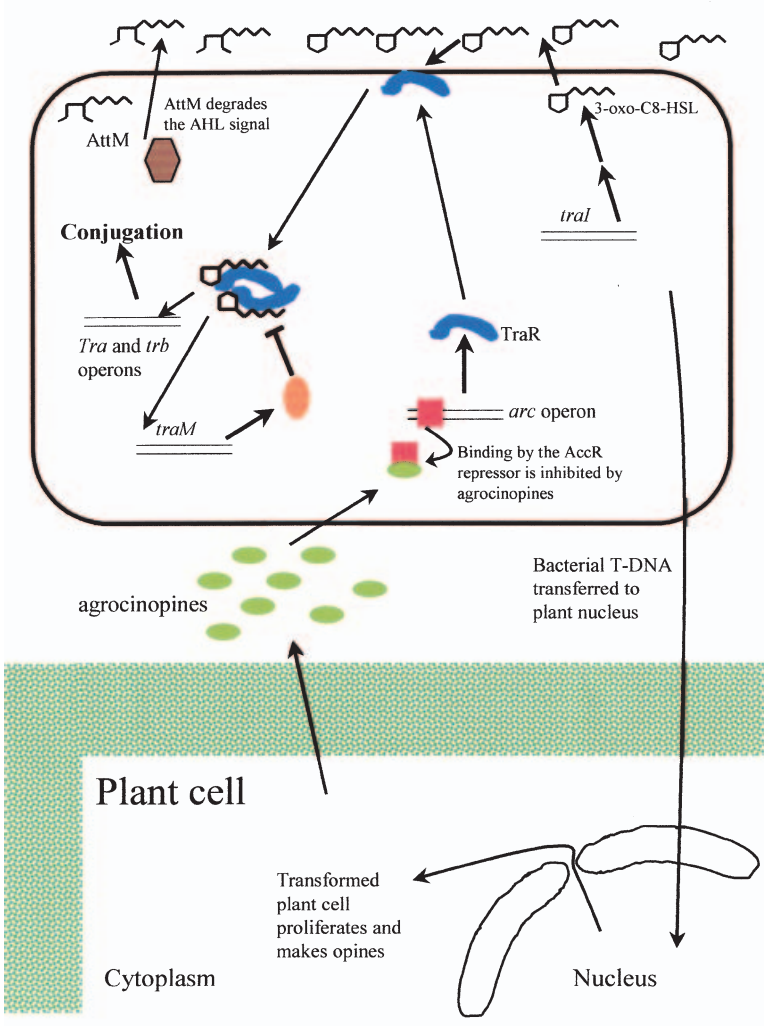


Fig. 2. Regulation of conjugation in nopaline-type *A. tumefaciens* C58. Bacterial cells produce 3-oxo-C8-HSL but can only respond to this signal molecule if the AHL receptor, TraR, is expressed. The *traR* gene is located within the *arc* operon, the other genes of which encode enzymes for agrocinopine catabolism. The transcriptional repressor AccR (red squares) binds the *arc* promoter but is released in the presence of agrocinopines derived from the transformed tumour cells. TraR binds AHL and forms a dimmer complex capable of activating transcription of the *tra* and *trb* operons. The system is attenuated by the antiactivator, TraM, and by AHL-lactonase(s). In octopine-type Agrobacterial strains, transcription of *traR* is activated by octopine interacting with OccR, a transcriptional activator.

(also known as ExpI). The LuxR homologue, CarR (McGowan *et al*., 1995), forms dimers and binds upstream of the operon encoding the enzymes for carbapenem synthesis. CarR dimers can bind to DNA in the absence of signal molecule, but binding of the autoinducer to CarR is required for transcriptional activation of these genes – possibly by inducing the formation of CarR–AHL multimers (Welch *et al*., 2000). This system results in carbapenem production only at high cell densities, when the concentration of 3-oxo-C6HSL reaches 0.1 μg ml^{-1}. It has been proposed that the production of antibiotics at high cell densities has evolved as a mechanism for niche protection, excluding opportunistic bacteria from taking advantage of the nutrient-rich environment produced during an *Erwinia* infection (Axelrood *et al*., 1988). The regulation of antibiotic production by AHLs thus resembles the classic *lux* model. However, AHLs are also required to induce the production of secreted plant cell wall-degrading exoenzymes (PCWDEs) as well as for the expression of harpin genes and components of a type III secretion system. The type III system contributes to the pathogenicity of *E. carotovora*, particularly in the initial stages of infection (Rantakari *et al*., 2001). By analogy with non-macerating plant pathogenic pseudomonads (Deslandes *et al*., 2003; Jin *et al*., 2003), this system may secrete virulence determinant(s) directly into the plant cell. However, what the secreted substrates are and how they contribute to virulence is currently not known.

The PCWDEs and harpins are the major virulence determinants and, as a consequence, *carI*$^-$ mutants are essentially non-pathogenic. However, although there is a dependence upon the AHL signal, this is only one of several plant and environmentally derived signals that must be integrated to regulate pathogenicity.

Exoenzyme production is unaltered in *Ecc* harbouring a disrupted *carR* gene, indicating that this is not the AHL receptor activator driving PCWDE production. A second AHL receptor-like protein is encoded by *expR* and is transcribed convergently with *carI*. However, *expR*$^-$ mutants have normal (cited by Whitehead *et al*., 2002) or slightly increased (Andersson *et al*., 2000) PCWDEs levels, depending on the *Ecc* strain studied. Conversely, overexpression of ExpR results in reduced PCWDE levels and a concomitant increase in the levels of a small RNA-binding protein, RsmA. Thus, there is either an as yet unidentified AHL receptor activator, or AHLs regulate exoenzyme production via an alternative, indirect mechanism. This latter possibility has been raised by Chatterjee *et al*. (2002), who suggested that AHLs might act to remove a post-transcriptional block on exoenzyme gene expression. The model suggests that RsmA (regulator of secondary metabolism) binds to exoenzyme (and possibly *carI*) mRNAs and promotes their degradation, and that AHL signalling acts to remove this inhibitory activity. This model is supported by the fact that *carI*$^-$ mutants (which do not produce AHLs and PCWDEs) can be restored to PCWDE production by a second mutation that inactivates *rsmA* (Chatterjee *et al*., 1995) (Fig. 1). In addition, *rsmA* expression in *E. carotovora* is stimulated by 3-oxo-C6-HSL deficiency (Chatterjee *et al*., 2002). RsmA has homologues in many Gram-negative bacteria (Heeb *et al*., 2002) and is closely related to CsrA (carbon storage regulator) of *Escherichia coli*, which has been shown to bind to specific mRNAs and to promote their degradation (Liu and Romeo, 1997). Like the *E. coli* system, RsmA activity can be blocked by the expression of a non-coding RNA transcript from *rsmB* (Liu *et al*., 1998). This transcript is initially 479 nucleotides in length and contains nine putative RsmA-binding motifs (Liu *et al*., 1998). These multiple binding sites may titrate out RsmA, allowing unbound mRNAs encoding PCWDEs to be translated. Interaction with RsmA appears to induce cleavage and release of a short 259 nucleotide RNA (rsmB′) that may also suppress RsmA expression by an unknown mechanism. Transcription of *rsmB* is regulated via a two-component sensor kinase/response regulator system consisting of ExpS and ExpA respectively (Eriksson *et al*., 1998; Cui *et al*., 2000). ExpS and ExpA appear to be equivalent to GacS and GacA (also known as LemA and GacA) found in a number of Gram-negative bacteria (Heeb and Haas, 2001). In addition to *E. carotovora*, homologues of this sensor kinase two-component system regulate pathogenicity in a number of pseudomonad plant pathogens including *Pseudomonas marginalis* CY091, *P. syringae*, *P. viridiflava* and *P. aeruginosa* (reviewed by Heeb and Haas, 2001). Production of secondary metabolites with antifungal activity by the rhizosphere-colonizing biological control strains *P. aureofaciens* 30-84 and *P. chlororaphis* PCL1391 is also dependent upon a LemA/GacA-regulated mechanism. In this case, GacA/GacS controls an AHL signalling system (Chancey *et al*., 1999; Chin-A-Woeng *et al*., 2001; G. V. Bloemberg, personal communication). However, GacA/GacS also regulates biological control activity in *P. fluorescens* strain CHA0, which does not appear to use AHL signalling (Bull *et al*., 2001). The ligand or stimulus to which GacS responds is unknown, but a dichloromethane-extractable low-molecular-weight compound(s) that activates GacS/GacA-dependent expression of a reporter construct has been isolated from late log phase cultures of *P. fluorescens* CHA0 (Zuber *et al*., 2003). This activator was not an AHL; thus, it appears that a second autoinducer (possibly quorum sensing) system is active in many Gram-negative bacteria. It is not yet known whether all bacteria that use GacS/GacA respond to the same signal molecule or if, like AHL signalling, different bacteria produce different but related molecules. It will be interesting to determine whether *E. carotovora* cultures produce similar compounds capable

of cross-activating the *P. fluorescens* CHA0 reporter. Identifying the ligand to which this sensor responds remains one of the most exciting challenges in this area.

In *E. carotovora*, a further level of complexity results from the fact that the expression of many of the plant cell wall-degrading enzymes is positively regulated by plant cell wall breakdown products that are generated by the action of the bacterial pectinases on the plant tissues. These products include 5-keto-4-deoxouronate, 2,5-diketo-3-deoxygluconate and 2-keto-3-deoxygluconate (KDG) (Chatterjee *et al*., 1985; Nasser *et al*., 1994). Regulation arises via dissociation of the transcriptional repressor KdgR in the presence of these metabolites (Liu *et al*., 1999). Binding sites for the KdgR repressor exist not only in the operators of many of the PCWDEs but also in *rsmB* and, thus, the initial production of pectinases leads to a further induction of virulence genes at both transcriptional and post-transcriptional levels (Hyytiainen *et al*., 2001) (Fig. 1).

Breakdown products released by the action of the bacterial PCWDEs also act as signalling molecules for the plant itself, indicating the presence of a pathogenic attack and triggering the hypersensitive disease response.

The quorum-sensing model proposes that placing pathogenicity-associated genes under density-dependent control provides a mechanism for avoiding the host plant's defence systems (reviewed by Whitehead *et al*., 2002). Treating plants with purified *Ecc* pectic enzymes leads to the induction of plant defence responses as a result of the release of cell wall-derived oligogalacturonide elicitors (Palva *et al*., 1993). Furthermore, this induction of plant defences by exoenzyme treatment confers increased resistance against subsequent *Ecc* infections. A similar systemic resistance to *Ecc* infection is also seen when the plant defence response is artificially induced by the application of salicylic acid (Palva *et al*., 1994), one of the plant-produced compounds involved in signalling a pathogen attack. A successful *E. carotovora* infection requires a relatively high inoculum (10^7 cells g^{-1} diseased tissue; Perombelon, 2002), and the progression of the disease is then a competition between bacterial multiplication and the development of plant resistance (Perombelon and Kelman, 1980). Thus, the premature production of plant cell wall-degrading enzymes at low cell densities would not give rise to a successful infection, but would result in the induction of the local and systemic plant defence response, which in turn would hamper subsequent infections. According to this model, *Erwinia* uses AHLs to monitor its cell density and only initiates a pathogenic attack when its population density is above a critical level, which ensures a high probability of overcoming host resistance.

In the case of potato, *Erwinia* can be present at relatively high levels as a latent infection, although it is not known whether the bacteria is truly dormant or if there is a slow turnover during this period. Breaking of latency is often associated with the presence of free water and anaerobic conditions. This may assist infection in two ways: there is increased leakage of cell contents, which will increase available nutrients and promote initial bacterial growth, and there is suppression of oxygen-dependent host resistance systems (Perombelon, 2002). These include free radical, phenolic and phytoalexin production as well as lignification and suberization of the cell wall. The cell wall modifications give a surprising level of protection: injection of tubers with cell-free *Erwinia* culture supernatants containing large quantities of PCWDEs causes tissue maceration under anaerobic but not aerobic conditions (Maher and Kelman, 1983). If AHL regulation of exoenzyme production exists as a mechanism for preventing premature infection by a small number of cells from triggering host resistance, then it would be anticipated that exposing such a subquorate group of bacteria to a level of AHLs that gave a false indication of the local population size would result in host resistance (at least in an aerobic infection). To test this, transgenic plants have been made that express bacterial AHL synthases (Fray *et al*., 1999; Mae *et al*., 2001). Mae *et al*. (2001) found that tobacco plants producing 3-oxo-C6HSL were less likely to be infected by an inoculum of *Erwinia carotovora*, although the disease progression was unaffected, and the resistance could be overcome by increasing the inoculum fourfold. However, as tobacco is not a normal host for *E. carotovora*, relatively high concentrations of bacteria were used for infection assays, typically between 2×10^5 and 2×10^6 colony-forming units (cfu) in a volume of 5 μl (a level that might have been predicted to exceed the quorum-sensing threshold). We have carried out similar infection studies in transgenic potato plants modified to produce 3-oxo-C6-HSL and C6-HSL (Toth *et al*., unpubl., cited in Fray 2002). In this system, we find that a successful stem infection can be initiated with as few as 10^2 cfu, a level two orders of magnitude lower than would normally be required. The fact that an infection is possible with such a low titre in the case of potato, yet is blocked by AHLs in the case of tobacco, raises questions regarding the role of quorum sensing during naturally occurring *Erwinia* infections. It is possible that, although the quorum-sensing threshold is apparently set above the level required for a successful infection on potato, on other, more resistant species, a larger initiating inoculum is required. In this case, setting a higher threshold for the expression of pathogenicity-associated genes may represent the best strategy for maximizing potential host range. Alternatively, the preliminary infection may not be dependent upon AHL-induced PCWDEs, but favourable conditions (a suppressed disease response and available nutrients) might allow initial bacterial proliferation. Only as nutrient availability becomes limiting in late log phase would there be

a requirement for PCWDEs to release further nutrients allowing disease progression and causing the appearance of disease symptoms. If this were the case, then AHL signalling would not be a mechanism for avoiding plant defences, but simply a means of increasing nutrient supply and ensuring disease continuation. In an alterative model, Redfield (2002) has argued that most bacterial autoinducer-sensing systems have not evolved for intercellular communication and group co-ordination at all; rather, their primary function is as a means of detecting the extent of diffusion and mixing in the cell's microenvironment. In this scenario, autoinducer levels will rise above threshold levels not only when the producing population is large but also when rates of diffusion away from an individual producing cell are low, for example as a result of physical barriers encountered by a bacterium or microcolony in plant intercellular spaces.

Regardless of what information is communicated, AHL-mediated signalling is clearly important during the infection process. This makes it an attractive target for strategies that aim to limit disease severity. To date, the most effective of these has been the use of a bacterial AHL lactonase enzyme, AiiA, which inactivates AHLs by opening the lactone ring (Dong *et al*., 2000). Transgenic potato plants expressing AiiA showed increased resistance to *Erwinia* infections (Dong *et al*., 2001), and a measure of biological control activity was provided in co-infection assays using bacterial strains capable of degrading the AHL signal (Molina *et al*., 2003; Uroz *et al*., 2003). Given the importance of AHL signalling during pathogenesis, it might not be surprising if plants had evolved strategies to interfere with this form of bacterial signalling. One of the first responses of the plant after exposure to *E. carotovora* or to pectic oligomers is a rapid influx of protons into the cells around the wound site. This has the effect of raising the pH of the apoplastic fluid between cells in the immediate vicinity from <6.4 to >8.2 (Baker *et al*., 1990). AHLs are rapidly degraded after alkalization of bacterial growth medium above pH 8.0, so a similar alkalization around a wound site would be anticipated to promote AHL turnover and may thus slow or halt the production of virulence factors. Other mechanisms by which plants might interfere with AHL-directed PCWDE secretion could include the production of signal mimics, signal blockers or signal-degrading enzymes or the production of compounds that block the activity of the AHL-producing enzymes. The marine algae *Delisea pulchra* produces halogenated furanones, which have some structural similarity to AHLs and appear to act as inhibitors of AHL perception *in vivo* – preventing bacterial cell swarming and attachment responses that lead to the build-up of bacterial biofilms on the algal surfaces (Givskov *et al*., 1996; Gram *et al*., 1996). Teplitski *et al*. (2000) reported AHL inhibitory activities in exudates from pea seedlings. The compounds responsible have not been identified, but they preferentially partition into polar solvents (unlike the AHL molecules themselves). We have also found compounds in a number of plant extracts that have similar partitioning characteristics in aqueous solvents: inhibitory activities were particularly pronounced in fruit (grape and strawberry) extracts, but were not detected in common host plants for *Ecc* such as potato (Fray, 2002). Bacterial phenotypes controlled through quorum sensing are frequently regulated by additional environmental cues. In some cases, AHL responses can be modulated or over-ridden by factors such as oxygen tension, nutrient starvation, iron limitation or catabolite repression (our unpublished results). It is possible that the plant-produced compounds are indirectly altering the bacterial AHL response rather than targeting it directly but, even if this were the case, such compounds could prove to be important in determining the outcome of interactions between higher plants and a diversity of pathogenic and symbiotic bacteria. Some authors have reported evidence of plant-derived AHL mimics capable of weakly activating quorum-sensing responses (Teplitski *et al*., 2000). It is important that a number of different biosensors are used in such experiments as the *E. coli luxABCDE* sensors (Winson *et al*., 1998) that are most commonly used show a considerable level of background light production in late log and early stationary phase and, although less than true AHL-induced activation, this background light production can be strongly affected by subtle variations in media composition or growing conditions.

It is now apparent that AHLs can elicit a range of specific responses in a number of eukaryotes including acting as chemoattractants for the zoospore stage of the green alga *Enteromorpha* (which preferentially settles on AHL-producing bacterial biofilms) (Joint *et al*., 2002). Oxo-C12HSL has been found to have immune modulatory effects in mammals, causing murine immune systems to become less effective against bacteria (Telford *et al*., 1998). Mathesius *et al*. (2003) found extensive alterations in the proteome profiles of *Medicago truncatula* roots exposed to physiologically relevant levels of long-chain AHLs (generally regarded as those AHLs with a carbon chain length of 10 or more), raising the possibility that some plants may be able to respond directly to the signal molecules produced by pathogenic or beneficial plant microbes.

Agrobacterium tumefaciens

Agrobacterium tumefaciens is a Gram-negative bacteria that causes crown gall tumours in plants by transferring a fragment of DNA into the nuclear genome of the host plant. Transformed plant tissues proliferate and produce opines, which serve as carbon and nitrogen sources for the *Agrobacterium*. There is little evidence that AHL-mediated

quorum sensing is used directly to regulate the expression of pathogenicity determinants in the case of *A. tumefaciens*. Here, the main role of AHL signalling found to date is in regulating the initiation of conjugation and the transfer of the tumour-inducing (Ti) virulence plasmid to a Ti-plasmidless saprophytic *A. tumefaciens* recipient in the tumoursphere. In *E. carotovora*, plant-derived cell wall breakdown products that arise as a result of bacterial infection feed back (via KdgR) to upregulate the AHL response. *A. tumefaciens* takes this one step further and can only respond to the AHL signal if an appropriate opine (a plant-derived infection product) is also present (Fuqua and Winans, 1996).

A successful infection by *A. tumefaciens* results in a section of the Ti plasmid (the T-DNA) being transferred and integrated into the host plant's nuclear genome. The T-DNA encodes genes that, when expressed by the host plant, manipulate plant hormone levels and phytohormone sensitivity, resulting in the proliferation of transformed cells that results in the characteristic crown gall tumour. In addition, the T-DNA contains genes that direct the synthesis of opines – unique condensation products of an amino acid with a ketoacid or sugar. Genes for opine catabolism are encoded on the Ti plasmid but outside the transfer DNA (T-DNA).The ability to catabolize opines is rare among other soil bacteria; thus, the induced plant tumour becomes a cell factory supplying the infecting bacteria with large quantities of a unique carbon and nitrogen source (Guyon *et al*., 1993).

Agrobacterial strains and the Ti plasmids that they contain are categorized according to the class of opines whose synthesis they encode. For both octopine and nopaline types, conjugation is induced when TraR (a homologue of LuxR and CarR) binds its cognate AHL (3-oxo-C8-HSL), synthesized by the *traI* gene product (a homologue of LuxI and CarI) (Fig. 2). In the absence of AHLs, TraR is present as insoluble monomers that are rapidly degraded by proteases. In the presence of AHLs, however, the tertiary structure of the protein is altered, and soluble TraR dimers can form (Zhu and Winans, 2001; Vannini *et al*., 2002). TraR dimers are resistant to proteases and are capable of transcription activation after binding to a conserved 18 bp target sequence (the tra box) in the promoters of *tra* and *trb* operons (Zhu and Winans, 1999), the product of which are required for conjugation. It has been suggested that, before AHL binding, the fat-soluble TraR monomers are predominantly found in the *A. tumefaciens* cytoplasmic membrane, whereas after exposure to 3-oxo-C8-HSL, AHL-containing TraR dimers are found in the cytoplasm (Qin *et al*., 2000). It was proposed that a membrane location for the TraR monomer may favour interaction with externally sourced AHLs. (Qin *et al*., 2000). TraR is not, however, constitutively expressed; in both octopine and nopaline strains, *traR* is placed in the same transcriptional units as genes encoding the enzymes required for catabolism of the appropriate opines. Transcription of these opine catabolism genes is induced by octopine or agrocinopines, produced by the transformed tumour cells. In the nopaline-type Ti plasmid pTiC58, *traR* is part of the *arc* operon, a group of five genes required for nopaline catabolism. Expression of the *arc* operon is repressed by AccR, which binds specifically to a sequence in the *arc* promoter in the absence of agrocinopines (an opine produced by the nopaline-type strains). This DNA-binding activity is inhibited by agrocinopines A and B, which thus serve to derepress the *arc* operon, inducing both opine catabolism and TraR expression (Piper *et al*., 1999) (Fig. 2). A similar situation exists for octopine-type Ti plasmids: here, TraR is one of 14 genes in the *occ* operon (the other 13 encode products for opine transport or catabolism). The *occ* operon is positively regulated by OccR, which binds as a tetramer to the *occ* promoter in the presence and absence of octopine. Octopine causes prebound OccR to undergo a conformational change that partially relaxes a high-angle DNA bend and activates transcription (Akakura and Winans, 2002a,b). The *occ* and *arc* operons appear to be unrelated; thus, similar mechanisms for placing *traR* under opine induction have evolved independently on two occasions. This suggests a strong evolutionary pressure to restrict bacterial conjugation to the environment of the transformed tumour cells.

Even after induction by opines and AHL-mediated dimerization, much of the newly synthesized TraR is not free to bind DNA and activate the transcription of *tra* and *trb*. This is because an 11.2 kDa antiactivator protein, TraM, associates with dimerized TraR and prevents DNA binding. Binding of TraM to TraR is independent of AHLs and occurs regardless of whether TraR is free or already bound to DNA (Luo *et al*., 2000). Null mutations in *traM* result in premature expression of *tra* and *trb* and constitutive conjugation even at low population density (Piper and Farrand, 2000). TraM has been proposed to fulfil two roles: to prevent small amounts of TraR from inducing conjugation in the absence of opines or of sufficient AHLs and, secondly, to downregulate *tra* and *trb* transcription, preventing TraR from inducing excessive levels of conjugation. This latter role is consistent with the finding that TraM can displace bound TraR, and that TraR (in the presence of AHL) activates TraM expression (Luo *et al*., 2000). A further method for attenuating AHL responses, and possibly of actually exiting from quorum sensing-dependent conjugation, is conferred by an AHL-lactonase, AttM (homologous to AiiA), that is expressed in stationary phase (Zhang *et al*., 2002) and may serve to remove the AHL signal from the local environment. *A. tumefaciens* C58 actually contains three genes homologous to AiiA, although only two of these have been shown to possess

AHL-lactonase activity (Carlier *et al*., 2003), and only the expression of *attM* has been studied in detail. The expression of *attM* is suppressed by AttJ, which specifically binds to the *attM* promoter, until *A. tumefaciens* cells reach stationary phase. The trigger for derepression of *attM* is not clear, although it does not appear to be triggered by 3-oxo-C8-HSL (Zhang *et al*., 2002).

These layers of regulation suggest that AHL-mediated quorum sensing in *A. tumefaciens* has evolved to give a brief burst of conjugation activity when the bacterial population is at a sufficiently high density, but only when it is in the presence of bacterially transformed plant tissues that produce the cognate opine. The evolutionary advantage of this system remains unclear.

It has been proposed recently that, for many bacterial species, the primary role of secreted autoinducers is not as a means of measuring population size, but rather is used to sense local diffusion rates in the microenvironment surrounding the producing cell (Redfield, 2002). It is certainly true that the number of cells required to produce the threshold level of AHL will be dependent upon local diffusion rates, which could vary dramatically on a microscopic scale. In addition, the diffusion rate may also be affected by the length of the acyl side-chain. AHLs with chain lengths of six carbons or less (short-chain signals) appear to traverse bacterial membranes freely by passive diffusion, whereas more hydrophobic molecules, such as 3-oxo-C12HSL, with acyl chains of 12 carbons require a dedicated export system (Pearson *et al*., 1999). We have made transgenic plants containing plastid-targeted bacterial LuxI homologues that result in AHL production in the chloroplast compartment of the cell. When C6-HSL is made, it is readily detected at the leaf surface (presumably as a result of efficient diffusion across the chloroplast and plant cell plasma membranes) (Fray *et al*., 1999). However, when 3-oxo-C12-HSL is made, almost all the signal molecule is retained within the chloroplast (our unpublished results). If it is the case that short- but not long-chain AHLs can diffuse across plant membranes, then in the confines of plant tissues, the 'quorum-sensing threshold' will be dependent upon the number of AI-producing bacteria as well as the physical properties of the AHL molecule. Many plant-associated bacteria produce both long- and short-chain AHL molecules (Table 1). When confined by plant tissues, the relative levels of the more hydrophobic long-chain AHLs may rise because of the presence of a greater diffusion barrier, or they may decrease if their hydrophobic nature results in preferential sequestration into the host membranes. In either event, the ratio of long- to short-chain AHLs is likely to be altered, and bacteria could use this information to regulate behaviour appropriately. It is curious that the most hydrophobic AHL molecules are produced by species of *Rhizobium* which, during their plant-associated form, are found in multiple small nitrogen-fixing symbiosomes completely surrounded by plant-derived membranes within a host cell. However, it is currently not known whether the differentiated bacteroids within these symbiosomes are producing an AHL signal or whether such communication is reserved for the free-living form.

AHL-mediated signalling is clearly important in many plant–microbe associations. The information communicated by such autoinducers has been questioned but, in most cases, it is likely to include some aspect of population density. However, AHL levels alone are often insufficient to trigger the full range of behavioural changes, and such quorum-sensing systems form part of integrated signalling networks. Inputs from additional autoinducers, the host plant and environmental sources come together to induce the 'quorum-sensing' behaviour.

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References

Aguilar, C., Bertani, I., and Venturi, V. (2003) Quorum-sensing system and stationary-phase sigma factor (*rpoS*) of the onion pathogen *Burkholderia cepacia* genomovar I type strain, ATCC 25416. *Appl Environ Microbiol* **69:** 1739–1747.

Akakura, R., and Winans, S.C. (2002a) Constitutive mutations of the OccR regulatory protein affect DNA bending in response to metabolites released from plant tumors. *J Biol Chem* **277:** 5866–5874.

Akakura, R., and Winans, S.C. (2002b) Mutations in the *occQ* operator that decrease OccR-induced DNA bending do not cause constitutive promoter activity. *J Biol Chem* **277:** 15773–15780.

Andersson, R.A., Eriksson, A.R.B., Heikinheimo, R., Mae, A., Pirhonen, M., Koiv, V., *et al.* (2000) Quorum sensing in the plant pathogen *Erwinia carotovora* subsp *carotovora*: the role of *expR* (Ecc). *Mol Plant–Microbe Interact* **13:** 384–393.

Axelrood, P.E., Rella, M., and Schroth, M.N. (1988) Role of antibiosis in competition of *Erwinia* strains in potato infection courts. *Appl Environ Microbiol* **54:** 1222–1229.

Bainton, N.J., Stead, P., Chhabra, S.R., Bycroft, B.W., Salmond, G.P.C., Stewart, G.S.A.B., and Williams, P. (1992) *N*-(3-oxohexanoyl)-L-homoserine lactone regulates carbapenem antibiotic production in *Erwinia carotovora*. *Biochem J* **288:** 997–1004.

Baker, C.J., Mock, N., Atkinson, M.M., and Hutcheson, S. (1990) Inhibition of the hypersensitive response in tobacco by pectate lyase digests of cell-wall and of polygalacturonic acid. *Physiol Mol Plant Pathol* **37:** 155–167.

Bauer, W.D., and Robinson, J.B. (2002) Disruption of bacterial quorum sensing by other organisms. *Curr Opin Biotechnol* **13:** 234–237.

von Bodman, S.B., Majerczak, D.R., and Coplin, D.L. (1998) A negative regulator mediates quorum-sensing control of exopolysaccharide production in *Pantoea stewartii* subsp. *stewartii*. *Proc Natl Acad Sci USA* **95:** 7687–7692.

Boettcher, K.J., and Ruby, E.G. (1995) Detection and quantification of *Vibrio fischeri* autoinducer from symbiotic squid light organs. *J Bacteriol* **177:** 1053–1058.

Bull, C.T., Duffy, B., Voisard, C., Defago, G., Keel, C., and Haas, D. (2001) Characterization of spontaneous gacS and gacA regulatory mutants of *Pseudomonas fluorescens* biocontrol strain CHA0. *Antonie van Leeuwenhoek Int J G* **79:** 327–336.

Carlier, A., Uroz, S., Smadja, B., Fray, R., Latour, X., Dessaux, Y., and Faure, D. (2003) The Ti plasmid of *Agrobacterium tumefaciens* harbors an *attM*-paralogous gene, *aiiB*, also encoding *N*-acyl homoserine lactonase activity. *Appl Environ Microbiol* **69:** 4989–4993.

Chancey, S.T., Wood, D.W., and Pierson, L.S. (1999) Two-component transcriptional regulation of *N*-acyl-homoserine lactone production in *Pseudomonas aureofaciens*. *Appl Environ Microbiol* **65:** 2294–2299.

Chatterjee, A.K., Thurn, K.K., and Tyrell, D.J. (1985) Isolation and characterization of Tn*5* insertion mutants of *Erwinia chrysanthemi* that are deficient in polygalacturonate catabolic enzymes oligogalacturonate lyase and 3-deoxy-D-glycero-2,5-hexodiulosonate dehydrogenase. *J Bacteriol* **162:** 708–714.

Chatterjee, A., Cui, Y., Liu, Y., Dumenyo, C.K., and Chatterjee, A.K. (1995) Inactivation of *rsmA* leads to overproduction of extracellular pectinases, cellulases, and proteases in *Erwinia carotovora* subsp. *carotovora*. in the absence of the starvation/cell density-sensing signal, *N*-(3-oxohexanoyl)-1-homoserine lactone. *Appl Environ Microbiol* **61:** 1959–1967.

Chatterjee, A., Cui, Y., and Chatterjee, A.K. (2002) RsmA and the quorum-sensing signal, *N*-[3-oxohexanoyl]-1-homoserine lactone, control the levels of *rsmB* RNA in *Erwinia carotovora* subsp. *carotovora* by affecting its stability. *J Bacteriol* **184:** 4089–4095.

Chin-A-Woeng, T.F.C., van den Broek, D., de Voer, G., van der Drift, K.M.G.M., Tuinman, S., Thomas-Oates, J.E., *et al.* (2001) Phenazine-1-carboxamide production in the biocontrol strain *Pseudomonas chlororaphis* PCL1391 is regulated by multiple factors secreted into the growth medium. *Mol Plant–Microbe Interact* **14:** 969–979.

Cui, Y., Chatterjee, A., and Chatterjee, A.K. (2000) Effects of the two-component system comprising GacA and GacS of *Erwinia carotovora* subsp *carotovora* on the production of global regulatory *rsmB* RNA, extracellular enzymes, and Harpin (Ecc). *Mol Plant–Microbe Interact* **14:** 516–526.

De Kievit, T.R., Gillis, R., Marx, S., Brown, C., and Iglewski, B.H. (2001) Quorum-sensing genes in *Pseudomonas aeruginosa* biofilms: their role and expression patterns. *Appl Environ Microbiol* **67:** 1865–1873.

Denny, T.P. (1999) Autoregulator-dependent control of extracellular polysaccharide production in phytopathogenic bacteria. *Eur J Plant Pathol* **105:** 417–430.

Deslandes, L., Olivier, J., Peeters, N., Feng, D.X., Khounlotham, M., Boucher, C., *et al.* (2003) Physical interaction between RRS1-R, a protein conferring resistance to bacterial wilt, and PopP2, a type III effector targeted to the plant nucleus. *Proc Natl Acad Sci USA* **100:** 8024–8029.

Dong, Y.H., Xu, J.L., Li, X.Z., and Zhang, L.H. (2000) AiiA, an enzyme that inactivates the acylhomoserine lactone quorum-sensing signal and attenuates the virulence of *Erwinia carotovora*. *Proc Natl Acad Sci USA* **97:** 3526–3531.

Dong, Y.H., Wang, L.H., Xu, J.L., Zhang, H.B., Zhang, X.F., and Zhang, L.H. (2001) Quenching quorum-sensing-dependent bacterial infection by an *N*-acyl homoserine lactonase. *Nature* **411:** 813–817.

Eberhard, A., Burlingame, A.L., Eberhard, C., Kenyon, G.L., Nealson, K.H., and Oppenheimer, N.J. (1981) Structural identification of autoinducer of *Photobacterium fischeri* luciferase. *Biochemistry* **20:** 2444–2449.

Eberl, L., Winson, M.K., Sternberg, C., Christiansen, G., Chhabra, S.R., Bycroft, B.W., *et al.* (1996) Involvement of *N*-acyl-L-homoserine lactone autoinducers in controlling the multicellular behaviour of *Serratia liquefaciens*. *Mol Microbiol* **20:** 127–136.

Egland, K., and Greenberg, E.P. (1999) Quorum sensing in *Vibrio fischeri*: elements of the luxI promoter. *Mol Microbiol* **31:** 1197–1204.

Elasri, M., Delorme, S., LeManceau, P., Stewart, G., Laue, B., Glickmann, E., *et al.* (2001) Acyl-homoserine lactone production is more common among plant-associated *Pseudomonas* spp. than among soilborne *Pseudomonas* spp. *Appl Environ Microbiol* **67:** 1198–1209.

Eriksson, A.R.B., Andersson, R.A., Pirhonen, M., and Palva, E.T. (1998) Two-component regulators involved in the global control of virulence in *Erwinia carotovora* subsp. *carotovora*. *Mol Plant–Microbe Interact* **11:** 743–752.

Fray, R.G. (2002) Altering plant–microbe interaction through artificially manipulating bacterial quorum sensing. *Ann Bot* **89:** 245–253.

Fray, R.G., Throup, J.P., Wallace, A., Daykin, M., Williams, P., Stewart, G.S.A.B., and Grierson, D. (1999) Plants genetically modified to produce *N*-acylhomoserine lactones communicate with bacteria. *Nature Biotechnol* **17:** 1017–1020.

Fuqua, C., and Winans, S.C. (1996) Localisation of OccR-activated and TraR-activated promoters that express two ABC-type permeases and the *traR* gene of Ti plasmid pTiR10. *Mol Microbiol* **20:** 1199–1210.

Givskov, M., DeNys, R., Manefield, M., Gram, L., Maximilien, R., Eberl, L., *et al.* (1996) Eukaryotic interference with homoserine lactone-mediated prokaryotic signalling. *J Bacteriol* **178:** 6618–6622.

Gram, L., DeNys, R., Maximilien, R., Givskov, M., Steinberg, P., and Kjelleberg, S. (1996) Inhibitory effects of secondary metabolites from the red alga *Delisea pulchra* on swarming motility of *Proteus mirabilis*. *Appl Environ Microbiol* **62:** 4284–4287.

Guyon, P., Petit, A., Tempe, J., and Dessaux, Y. (1993) Transformed plants producing opines specifically promote growth of opine-degrading Agrobacteria. *Mol Plant–Microbe Interact* **6:** 92–98.

Hanzelka, B.L., and Greenberg, E.P. (1996) Quorum sensing in *Vibrio fischeri*: evidence that S-adenosylmethionine is the amino acid substrate for autoinducer synthesis. *J Bacteriol* **178:** 5291–5294.

Heeb, S., and Haas, D. (2001) Regulatory roles of the GacS/GacA two-component system in plant-associated and other Gram-negative bacteria. *Mol Plant–Microbe Interact* **14:** 1351–1363.

Heeb, S., Blumer, C., and Haas, D. (2002) Regulatory RNA as mediator in GacA/RsmA-dependent global control of

exoproduct formation in *Pseudomonas fluorescens* CHA0. *J Bacteriol* **184:** 1046–1056.
Hoang, T.T., Sullivan, S.A., Cusick, J.K., and Schweizer, H.P. (2002) beta-ketoacyl acyl carrier protein reductase (FabG) activity of the fatty acid biosynthetic pathway is a determining factor of 3-oxo-homoserine lactone acyl chain lengths. *Microbiology* **148:** 3849–3856.
Hyytiainen, H., Montesano, M., and Palva, E.T. (2001) Global regulators ExpA (GacA) and KdgR modulate extracellular enzyme gene expression through the RsmA-rsmB system in *Erwinia carotovora* subsp. *carotovora*. *Mol Plant–Microbe Interact* **14:** 931–938.
Jin, Q.L., Thilmony, R., Zwiesler-Vollick, J., and He, S.Y. (2003) Type III protein secretion in *Pseudomonas syringae*. *Microbes Infect* **5:** 301–310.
Joint, I., Tait, K., Callow, M.E., Callow, J.A., Milton, D., Williams, P., and Camara, M. (2002) Cell-to-cell communication across the prokaryote–eukaryote boundary. *Science* **298:** 1207–1207.
Lithgow, J.K., Wilkinson, A., Hardman, A., Rodelas, B., Wisniewski-Dye, F., Williams, P., and Downie, J.A. (2000) The regulatory locus cinRI in *Rhizobium leguminosarum* controls a network of quorum-sensing loci. *Mol Microbiol* **37:** 81–97.
Liu, M.Y., and Romeo, T. (1997) The global regulator CsrA of *Escherichia coli* is a specific mRNA-binding protein. *J Bacteriol* **179:** 4639–4642.
Liu, Y., Cui, Y.Y., Mukherjee, A., and Chatterjee, A.K. (1998) Characterization of a novel RNA regulator of *Erwinia carotovora* ssp. *carotovora* that controls production of extracellular enzymes and secondary metabolites. *Mol Microbiol* **29:** 219–234.
Liu, Y., Jiang, G.Q., Cui, Y.Y., Mukherjee, A., Ma, W.L., and Chatterjee, A.K. (1999) *kdgR* (Ecc) negatively regulates genes for pectinases, cellulase, protease, harpin (Ecc), and a global RNA regulator in *Erwinia carotovora* subsp. carotovora. *J Bacteriol* **181:** 2411–2421.
Luo, Z., Qin, Y., and Farrand, S.K. (2000) The antiactivator TraM interferes with the autoinducer-dependent binding of TraR to DNA by interacting with the C-terminal region of the quorum-sensing activator. *J Biol Chem* **275:** 7713–7722.
McClean, K.H., Winson, M.K., Fish, L., Taylor, A., Chhabra, S.R., Camara, M., *et al.* (1997) Quorum sensing and *Chromobacterium violaceum*: exploitation of violacein production and inhibition for the detection of *N*-acylhomoserine lactones. *Microbiology* **143:** 3703–3711.
McGowan, S., Sebaihia, M., Jones, S., Yu, B., Bainton, N., Chan, P.F., *et al.* (1995) Carbapenem antibiotic production in *Erwinia caratovora* is regulated by *carR* a homolog of the *luxR* transcriptional activator. *Microbiology* **141:** 541–550.
Mae, A., Montesano, M., Koiv, V., and Palva, E.T. (2001) Transgenic plants producing the bacterial pheromone *N*-acyl-homoserine lactone exhibit enhanced resistance to the bacterial phytopathogen *Erwinia carotovora*. *Mol Plant–Microbe Interact* **14:** 1035–1042.
Maher, E.A., and Kelman, A. (1983) Oxygen status of potato-tuber tissue in relation to maceration by pectic enzymes of *Erwinia carotovora*. *Phytopathology* **73:** 536–539.
Marketon, M.M., Gronquist, M.R., Eberhard, A., and González, J.E. (2002) Characterization of the *Sinorhizobium meliloti sinR/sinI* locus and the production of novel *N*-acyl homoserine lactones. *J Bacteriol* **184:** 5686–5695.
Marketon, M.M., Glenn, S.A., Eberhard, A., and Gonzalez, J.E. (2003) Quorum sensing controls exopolysaccharide production in *Sinorhizobium meliloti*. *J Bacteriol* **185:** 325–331.
Mathesius, M., Mukders, S., Gao, M., Teplittski, M., Caetano-Anolles, G., Rolfe, B.G., and Bauer, W. (2003) Extensive and specific responses of a eukaryote to bacterial quorum-sensing. *Proc Natl Acad Sci USA* **100:** 1444–1449.
Molina, L., Constantinescu, F., Michel, L., Reimmann, C., Duffy, B., and Defago, G. (2003) Degradation of pathogen quorum-sensing molecules by soil bacteria: a preventive and curative biological control mechanism. *FEMS Microbiol Ecol* **45:** 71–81.
Nasser, W., Reverchon, S., Condemine, G., and Robertaudouy, J. (1994) Specific interactions of *Erwinia chrysanthemi* KdgR repressor with different operators of genes involved in pectinolysis. *J Mol Biol* **236:** 427–440.
Palva, T.K., Holmstrom, K.O., Heino, P., and Palva, E.T. (1993) Induction of plant defense response by exoenzymes of Erwinia carotovora subsp. carotovora. *Mol Plant–Microbe Interact* **6:** 190–196.
Palva, T.K., Hurtig, M., Saindrenan, P., and Palva, E.T. (1994) Salicylic acid induced resistance to *Erwinia carotovora* subsp. *carotovora* in tobacco. *Mol Plant–Microbe Interact* **7:** 356–363.
Pearson, J.P., Gray, K.M., Passador, L., Tucker, K.D., Eberhard, A., Iglewski, B.H., and Greenberg, E.P. (1994) Structure of the autoinducer required for expression of *Pseudomonas aeruginosa* virulence genes. *Proc Natl Acad Sci USA* **91:** 197–201.
Pearson, J.P., Van Delden, C., and Iglewski, B.H. (1999) Active efflux and diffusion are involved in transport of *Pseudomonas aeruginosa* cell-to-cell signals. *J Bacteriol* **181:** 1203–1210.
Perombelon, M.C.M. (2002) Potato diseases caused by soft rot erwinias: an overview of pathogenesis. *Plant Pathol* **51:** 1–12.
Perombelon, M.C.M., and Kelman, A. (1980) Ecology of the soft rot erwinias. *Annu Rev Phytopathol* **18:** 361–387.
Piper, K.R., and Farrand, S.K. (2000) Quorum sensing but not autoinduction of Ti plasmid conjugal transfer requires control by the opine regulon and the antiactivator TraM. *J Bacteriol* **182:** 1080–1088.
Piper, K.R., Beck von Bodman, S., Hwang, I., and Farrand, S.K. (1999) Hierarchical gene regulatory systems arising from fortuitous gene associations: regulating quorum-sensing by the opine regulon of *Agrobacterium*. *Mol Microbiol* **32:** 1077–1089.
Qin, Y.P., Luo, Z.Q., Smyth, A.J., Gao, P., von Bodman, S.B., and Farrand, S.K. (2000) Quorum-sensing signal binding results in dimerization of TraR and its release from membranes into the cytoplasm. *EMBO J* **19:** 5212–5221.
Rantakari, A., Virtaharju, O., Vahamiko, S., Taira, S., Palva, E.T., Saarilahti, H.T., and Romantschuk, M. (2001) Type III secretion contributes to the pathogenesis of the soft-rot pathogen *Erwinia carotovora*: partial characterization of the *hrp* gene cluster. *Mol Plant–Microbe Interact* **14:** 962–968.
Redfield, R.J. (2002) Is quorum sensing a side effect of diffusion sensing? *Trends Microbiol* **10:** 365–369.
Ruby, E.G., and Lee, K.-H. (1998) The *Vibrio fischeri–Euprymna scolopes* light organ association: current ecological paradigms. *Appl Environ Microbiol* **64:** 805–812.
Schaefer, A.L., Val, D.L., Hanzelka, B.L., Cronan, J.E., and

Greenberg, E.P. (1996) Generation of cell-to-cell signals in quorum sensing: acyl homoserine lactone synthase activity of a purified *Vibrio fischeri* LuxI protein. *Proc Natl Acad Sci USA* **93:** 9505–9509.

Shadel, G.S., and Baldwin, T.O. (1991) The *Vibrio Fischeri* LuxR Protein is capable of bi-directional stimulation of transcription and both positive and negative regulation of the luxR gene. *J Bacteriol* **173:** 568–574.

Telford, G., Wheeler, D., Williams, P., Tomkins, P.T., Appleby, P., Sewell, H., *et al.* (1998) The *Pseudomonas aeruginosa* quorum-sensing signal molecule N-(3-oxododecanoyl)-1-homoserine lactone has immunomodulatory activity. *Infect Immun* **66:** 36–42.

Teplitski, M., Robinson, J.B., and Bauer, W.D. (2000) Plants secrete substances that mimic bacterial *N*-acyl homoserine lactone signal activities and affect population density-dependent behaviours in associated bacteria. *Mol Plant–Microbe Interact* **13:** 637–648.

Toth, I.K., Bell, K.S., Holeva, M.C., and Birch, P.R.J. (2003) Soft rot erwiniae: from genes to genomes. *Mol Plant Pathol* **4:** 17–30.

Uroz, S., D'Angelo-Picard, C., Carlier, A., Elasri, M., Sicot, C., Petit, A., *et al.* (2003) Novel bacteria degrading *N*-acylhomoserine lactones and their use as quenchers of quorum-sensing-regulated functions of plant-pathogenic bacteria. *Microbiology* **149:** 1981–1989.

Vannini, A., Volpari, C., Gargolgi, C., Muraglia, E., Cortese, R., De Francesco, R., *et al.* (2002) The crystal structure of the quorum sensing protein TraR bound to its autoinducer and target DNA. *EMBO J* **21:** 4393–4401.

Watson, W.T., Minogue, T.D., Val, D.L., von Bodman, S.B., and Churchill, M.E.A. (2002) Structural basis and specificity of acyl-homoserine lactone signal production in bacterial quorum sensing. *Mol Cell* **9:** 685–694.

Welch, M., Todd, D.E., Whitehead, N.A., McGowan, S.J., Bycroft, B.W., and Salmond, G.P.C. (2000) N-acyl homoserine lactone binding to the CarR receptor determines quorum-sensing specificity in *Erwinia*. *EMBO J* **19:** 631–641.

Whitehead, N.A., Byers, J.T., Commander, P., Corbett, M.J., Coulthurst, S.J., Everson, L., *et al.* (2002) The regulation of virulence in phytopathogenic *Erwinia* species: quorum sensing, antibiotics and ecological considerations. *Antonie van Leeuwenhoek Int J G* **81:** 223–231.

Winson, M.K., Swift, S., Fish, L., Throup, J.P., Jorgensen, F., Chhabra, S.R., *et al.* (1998) Construction and analysis of luxCDABE-based plasmid sensors for investigating *N*-acyl homoserine lactone-mediated quorum sensing. *FEMS Microbiol Lett* **163:** 185–192.

Zhang, H.B., Wang, L.H., and Zhang, L.H. (2002) Genetic control of quorum-sensing signal turnover in *Agrobacterium tumefaciens*. *Proc Natl Acad Sci USA* **99:** 4638–4643.

Zhang, L.-H., Murphy, P.J., Kerr, A., and Tate, M.E. (1993) *Agrobacterium* conjugation and gene regulation by *N*-acyl-1-homoserine lactones. *Nature* **362:** 446–447.

Zhu, J., and Winans, C. (1999) Autoinducer binding by the quorum-sensing regulator TraR increases affinity for target promoters in vitro and decreases TraR turnover rates in whole cells. *Proc Natl Acad Sci USA* **96:** 4832–4837.

Zhu, J., and Winans, C. (2001) The quorum sensing transcriptional regulator TraR requires its cognate signalling ligand for protein folding, protease resistance and dimerization. *Proc Natl Acad Sci USA* **98:** 1507–1512.

Zuber, S., Carruthers, F., Keel, C., Mattart, A., Blumer, C., Pessi, G., *et al.* (2003) GacS sensor domains pertinent to the regulation of exoproduct formation and to the biocontrol potential of *Pseudomonas fluorescens* CHAO. *Mol Plant–Microbe Interact* **16:** 634–644.