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BOTANICAL BRIEFING

Altering Plant–Microbe Interaction Through Artificially Manipulating Bacterial Quorum Sensing

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Many bacteria regulate diverse physiological processes in concert with their population size. Bacterial cell-to-cell communication utilizes small diffusible signal molecules, which the bacteria both produce and perceive. The bacteria couple gene expression to cell density by eliciting a response only when the signalling molecules reach a critical threshold (a point at which the population is said to be 'quorate'). The population as a whole is thus able to modify its behaviour as a single unit. Amongst Gram-negative bacteria, the quorum sensing signals most commonly used are *N*-acylhomoserine lactones (AHLs). It is now apparent that AHLs are used for regulating diverse behaviours in epiphytic, rhizosphere-inhabiting and plant pathogenic bacteria and that plants may produce their own metabolites that interfere with this signalling. Transgenic plants that produce high levels of AHLs or which can degrade bacterial-produced AHLs have been made. These plants have dramatically altered susceptibilities to infection by pathogenic *Erwinia* species. In addition, such plants will prove useful tools in determining the roles of AHL-regulated density-dependent behaviour in growth promoting, biological control and pathogenic plant-associated bacterial species.

Key words: *N*-Acylhomoserine lactones, AHLs, *Erwinia*, potato, rhizosphere.

INTRODUCTION

Microbial success critically depends on the ability to perceive and respond rapidly to changes in the local environment. For any individual bacterium, one of the most important of these environmental factors will be the number and growth status of its fellows within its immediate vicinity. Such information may allow the bacterium to anticipate future availability of nutrients or build up of toxins. More importantly, it enables the individuals within the population to coordinate ecological strategies that would not be successful if attempted by a small number of bacteria acting independently. The ability to monitor the local population density is dependent on a cell-to-cell communication system that employs small diffusible signalling molecules. This phenomenon has been termed 'quorum sensing' since initiation of a concerted population response depends on the population reaching a minimal population unit or 'quorum' [reviewed in Withers *et al*. (2001) and Whitehead *et al*. (2001)]. Examples of such density-dependent multicellular behaviour in prokaryotes include diverse processes such as bioluminescence, sporulation, swarming, antibiotic biosynthesis, plasmid conjugal transfer and the production of virulence determinants in animal, fish and plant pathogens (Swift *et al*., 1996). A number of quorum signalling molecules have been identified in both Gram-negative and Gram-positive species. In Gram-negative bacteria, one of the most widespread and best understood families of signal molecules is the *N*-acylhomoserine lactones (AHLs) which vary predominantly in the presence or absence of an acyl chain C3 substituent (oxo- or hydroxy-) and length of the *N*-acyl side chain (four to 14 carbons). Examples of some of the known AHLs together with density-dependent behaviour that they have been shown to regulate are shown in Table 1.

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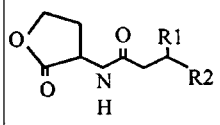
AHL BIOSYNTHESIS AND PERCEPTION

Several bacterial species produce the same AHL, though in each it may be used to regulate different biological processes. Thus LuxI of the marine bacterium *Vibrio fischeri*, synthesizes *N*-(3-oxohexanoyl)-L-homoserine lactone (3-oxo-C6-HSL), which regulates bioluminescence in a cell-density-dependent manner, while CarI of *Erwinia carotovora* also produces 3-oxo-C6-HSL, which, in this bacterium, is responsible for the induction of the secreted plant cell wall-degrading exoenzymes and of the antibiotic carbapenem (Bainton *et al*., 1992; Jones *et al*., 1993). The *cviI* gene of the soil bacterium *Chromobacterium violaceum* encodes the enzyme for *N*-hexanoyl-L-homoserine lactone (C6-HSL) synthesis, which is structurally very similar to 3-oxo-C6-HSL and which induces production of the purple pigment violacein as well as antifungal chitinases (McClean *et al*., 1997; Chernin *et al*., 1998). Inactivation or deletion of *luxI*, *carI* or *cviI* results in loss of density-dependent bioluminescence, virulence or violacein production, respectively. The relevant operons can, however, be induced by the addition of an exogenous supply of an appropriate AHL to the mutant bacteria. Utilizing such operons, reporter strains of *Escherichia coli* and *C. violaceum* have been developed that indicate the presence of particular AHLs through light or pigment production (McClean *et al*., 1997; Winson *et al*., 1998).

TABLE 1. *Examples of a number of AHLs and some of the density-dependent behaviours that they are known to regulate.*

Structure	R		AHL molecule	Bacteria	Behaviour
	R1	R2			
	H	H	C4-HSL	*S. liquefaciens*	Cell motility/swarming
	OH	H	3-hydroxy-C4-HSL	*V. harveyi*	Bioluminescence
	H		C6-HSL	*C. violaceum*	Pigments/antibiotics/chitinase
	O		3-oxo-C6-HSL	*E. carotovora* *P. aureofaciens*	Pathogenicity/antibiotics Biocontrol activity/antibiotics
	H		C7-HSL	*R. leguminosarum*	?
	O		3-oxo-C8-HSL	*A. tumefaciens*	Conjugation
	OH		3-hydroxy-C14:1-HSL	*R. leguminosarum*	Growth inhibition/rhizosphere genes

AHL biosynthetic enzymes fall into three groups. The LuxI type was the first to be discovered and appears to be the most common. These enzymes generate the homoserine lactone moiety from *S*-adenosyl methionine and derive the acyl side chain from the appropriately charged acyl-acyl carrier protein (acyl-ACP) or acyl-coenzyme A (acyl-CoA). Many of these enzymes can produce more than one AHL by utilizing alternative acyl-ACP or acyl-CoA side chain precursors. A second group of AHL biosynthetic enzymes, the LuxM type, has no significant homology to the LuxI type but appears to catalyse AHL synthesis from the same substrates (Hanzelka *et al*., 1999). A third class of AHL synthase, HdtS, has been identified from the biocontrol strain *Pseudomonas fluorescens* F113 (Laue *et al*., 2000). HdtS has no homology to either LuxI or LuxM. Many bacteria utilize more than one AHL biosynthetic enzyme and in some cases the synthases used may belong to different classes (Hanzelka *et al*., 1999). It is not clear why three different classes of enzymes should have evolved to perform the same role; perhaps possession of different AHL synthases may afford some protection against competitor or host species developing inhibitory molecules that target the synthase.

AHLS AND *ERWINIA CAROTOVORA* INFECTIONS

AHLs were initially identified over two decades ago as 'autoinducers' of bioluminescence in certain marine bacteria (Eberhard *et al*., 1981; Cao and Meighen, 1989). The finding 10 years later that production of carbapenem antibiotic by the terrestrial plant pathogen *Erwinia carotovora* was also regulated by identical self-produced AHLs was the first indication of how widespread this form of signalling would prove to be (Bainton *et al*., 1992).

As well as antibiotic production, AHLs were found to be responsible for the induction of a glycine-rich protein (Harpin) that strongly elicits the plant hypersensitive reaction (Mukherjee *et al*., 1997). Even more importantly, AHLs provide global control of exoenzyme production by *E. carotovora*. *E. carotovora carI* mutants appear to be completely avirulent in a tobacco test system; they can neither macerate plant tissue nor multiply *in planta* because they lack pectin lyase, pectate lyase, polygalacturonase, cellulase and protease (Jones *et al*., 1993; Pirhonen *et al*., 1993). It is pertinent to ask how the expression of these exoenzymes only at high cell density in wild-type cells may contribute to the success of *Erwinia* species as plant pathogens. An explanation has been proposed by Pirhonen *et al*. (1993): under aerobic conditions, a successful *E. carotovora* infection requires a relatively high inoculum and the progression of the disease is then a competition between bacterial multiplication and the development of plant resistance (Perombelon and Kelman, 1980). Thus, production of macerating enzymes at low cell densities would not give rise to a successful infection but would result in induction of the local and systemic plant defence response, which in turn would hamper subsequent infections. Such resistance to *E. carotovora* infection is seen when the plant defence response is artificially induced by application of salicylic acid (Palva *et al*., 1994).

If an infecting bacterium were to encounter AHL levels that indicated that it was part of a far larger population than it actually was, it might be induced to mount a pathogenic attack prematurely. The course of the ensuing infection might be drastically altered, with the plant being able to mount a successful defence to a weak attack. To test this theory, plants were engineered to express the *yenI* gene of *Yersinia enterocolitica* (Fray *et al*., 1999). In *Y. enterocolitica*, and in *E. coli* expressing the *yenI* gene product, C6-HSL and 3-oxo-C6-HSL are produced in a 1 : 1 ratio; both AHLs are naturally produced by a number of *E. carotovora* species (Nasser *et al*., 1998; Fig. 1). Initially, transgenic tobacco plants were made to test the feasibility of synthesizing bacterial AHL signalling molecules *in planta*. The predicted AHLs were made at biologically active levels in these plants, but only when YenI was targeted to the chloroplasts (Fray *et al*., 1999). This requirement for plastid

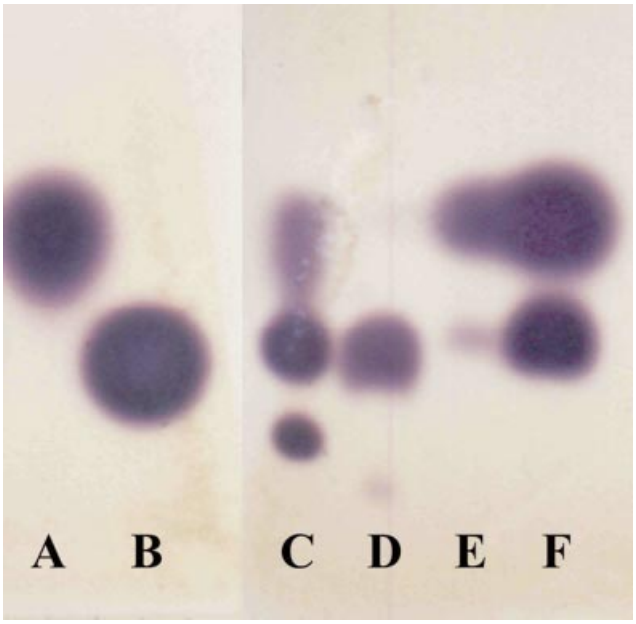


FIG. 1. Thin layer chromatogram showing the range of AHLs produced by various *Erwinia carotovora* species. After chromatography, AHLs were located by overlaying the chromatogram with agar seeded with the *Chromobacterium violaceum* strain mutant for *cviI* (the gene required for AHL synthesis). The presence of AHLs is indicated by complementation of the mutation and restoration of the production of the purple pigment, violacein. A, 3-oxo-C6-HSL standard; B, C6-HSL standard; C–F, spent bacterial culture supernatants of *Erwinia carotovora* subsp. *carotovora* SCRI 193 (C); *Erwinia carotovora* subsp. *atroseptica* SCRI 1043 (D); *Erwinia carotovora* subsp. *atroseptica* SCRI 1039 (E); and *Erwinia chrysanthemi* SCRI 1043 (F). Most strains produce both 3-oxo-C6-HSL and C6-HSL as well as additional AHLs in some cases. The sensitivity of the *C. violaceum* reporter strain varies according to the AHL being detected, thus the intensity of pigment is not a direct indicator of the relative abundance of each AHL.

location is probably due to the availability in this organelle of the immediate precursors for AHL synthesis. The presence of suitable precursors probably reflects the prokaryotic origin of plastids. By using HPLC-mass spectrometry, the AHLs synthesized were confirmed as being 3-oxo-C6-HSL and C6-HSL and these were present at levels of 0·41 μg and 0·35 μg g^{-1} f. wt, respectively, approximating to the 1 : 1 ratio produced by YenI in *Y. enterocolitica*. In bacteria, AHLs with an acyl side chain of six carbons or less have been shown to diffuse freely across the bacterial membrane (though this is not the case for AHLs with a 12 carbon side chain; Pearson *et al*., 1999). Consistent with this, the 3-oxo-C6-HSL and C6-HSL produced in the chloroplasts appear to diffuse freely across the plastid and plasma membranes and can be detected at the plant cell surface by bacterial AHL biosensors.

AHL-producing tobacco plants were able to restore pathogenicity to the avirulent *E. carotovora* CarI mutant (Fig. 2). However, tobacco is not a normal host for *E. carotovora* and, in order for an infection to occur, the inoculum level used was already in excess of the quorum-sensing threshold. To test whether small inocula of wild-type *E. carotovora* could be induced to attempt a pathogenic attack prematurely on AHL-producing plants, and whether such an infection would result in increased resistance, YenI-expressing potato lines were made. These plants were infected using stem stab inoculations with a range of titres of wild-type *E. carotovora atroseptica*. Surprisingly, the plants proved to be susceptible at inoculum levels as low as 10^2, levels that in an untransformed plant did not cause disease symptoms (Fray *et al.*, unpubl. res.). This raises the question of why *Erwinia* does not normally attempt an infection at these lower cell densities if such an infection is likely to be successful. There are two possible explanations:

FIG. 2. AHL-producing transgenic tobacco plants restore pathogenicity to an avirulent AHL-deficient *Erwinia carotovora* subsp. *carotovora* mutant (PNP22). The photograph shows the leaves 4 d after infection. 1, Wild-type tobacco inoculated with wild-type *Erwinia carotovora*; 2, Wild-type tobacco inoculated with AHL-negative *Erwinia carotovora* mutant PNP22; 3, AHL-producing tobacco line inoculated with *Erwinia carotovora* PNP22.

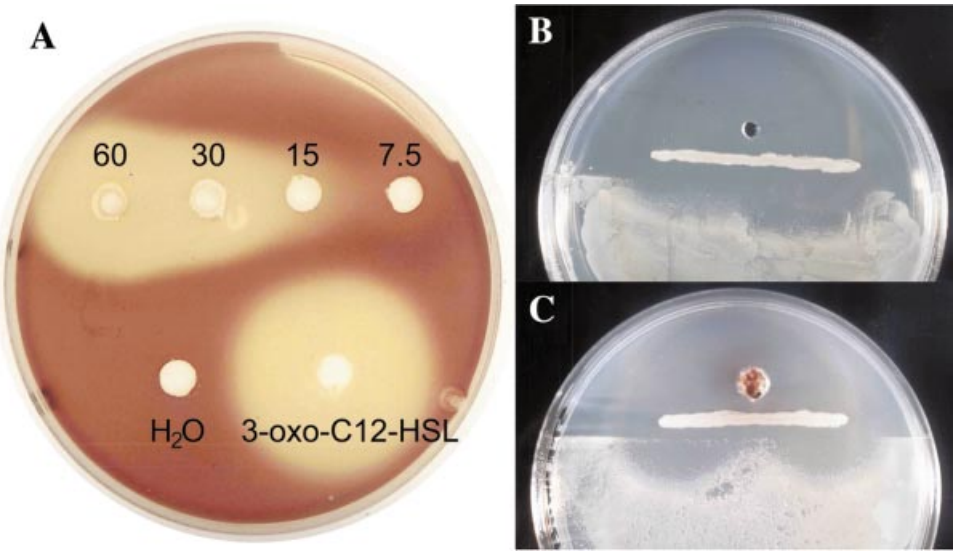


FIG. 3. Inhibition of quorum sensing responses. A, In a reverse assay, 3-oxo-C6-HSL was added to a top agar containing the *Chromobacterium violaceum CviI*⁻ biosensor strain. Inhibition of quorum sensing by the long chain 3-oxo-C12-HSL, or by dilutions of a crude grape extract (60–7·5 µl made up to 60 µl with H_20), results in inhibition of violacein production but not bacterial growth. B and C, Other plant extracts also inhibit AHL responses. Antibiotic production by wild-type *Erwinia carotovora* is revealed by inhibition of growth in a lawn of an *Escherchia coli* strain sensitive to carbabenem. In B, the well contains water whilst in C it contains a crude strawberry extract. Inhibition of antibiotic production (an AHL response) is revealed by growth of the *E. coli* lawn adjacent to the well containing the fruit extract.

it might be a reflection of the strategies employed by either the bacteria or the plant.

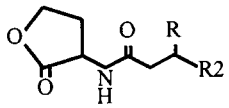
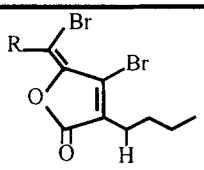
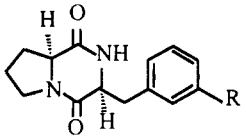
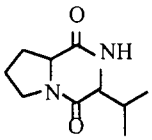
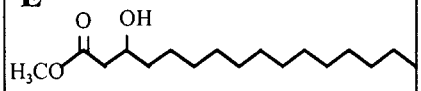
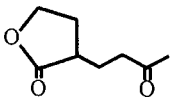
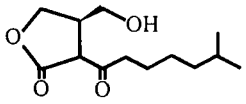
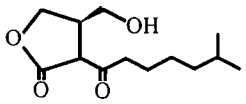
The bacterial strategy

The quorum sensing threshold might be set by the bacteria at a level that is above that needed for a successful infection in potato, but this threshold might still represent the level that maximizes the bacteria's chances of success. *E. carotovora atrosceptica* infects plant species other than potato: in these species an inoculum of 10^2 may not be sufficient to overcome the plant's defence responses. Without a mechanism for identifying each potential host plant and modifying its behaviour according to host plant species, setting a higher threshold for the expression of pathogenicity-associated genes may represent the best evolutionary strategy. Furthermore, other bacterial species will also be present in the rhizosphere or on the plant surface, some of which are likely to be making identical or similar AHLs. This could result in cross-talk between species and an over-estimation by *Erwinia* of its own population size. Indeed, such cross-talk between species in the rhizosphere has been demonstrated by Wood *et al.* (1997). Setting a higher threshold for activation might be one mechanism to compensate for this.

The plant strategy

Plants may have evolved strategies to interfere with the bacteria's AHL signalling system to prevent them from initiating a pathogenic attack. Such interference 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. Examples of compounds with signal inhibiting properties are known and there is evidence for the production of AHL inhibitory molecules by bacteria, algae and plants. In the simplest case, an AHL produced by one bacterial species may be antagonistic to the activity of an AHL used by a second species. This is seen in the case of *Chromobacterium violaceum* where the cognate AHL contains an acyl side chain of six carbons. The presence of this molecule or closely related analogues induces the production of the purple pigment violacein. However, related molecules with acyl side chains of ten or more carbons do not activate violacein production and actually inhibit the normal response to the cognate molecule (McClean *et al.*, 1997; Fig. 3). More complex blocking molecules are produced by the Australian marine alga, *Delisea pulchra*. This macroalga produces halogenated furanones which have some structural similarity to AHLs (Table 2). It appears that *D. pulchra* uses these AHL blockers *in vivo* to inhibit bacterial cell swarming and attachment responses, thus preventing 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 extracts from a number of fruit, including grape and

TABLE 2. *Quorum sensing-related signal molecules*

Structure	R	Molecule name	Producing organism
A 	See Table 1	*N*-acyl homoserine lactones	Many Gram-negative bacteria
B 	Br or H	Halogenated furanones	*Delisea pulchra*
C 	OH	Cyclo(L-Pro-L-Tyr)	*Alteraria alternata*
	H	Cyclo(L-Phe-L-Pro)	*Alteraria alternata* *Pseudomonas fluorescens*
D 		Cyclo(L-Pro-L-Val)	*Alteraria alternata*
E 		3-hydroxy-palmitic acid methyl ester	*Ralastonia solanacearum*
F 		Diffusible factor "DF" (Proposed structure)	*Xanthamonas campestris* pv. *campestris*
G 		A-factor	*Streptomcyes griseus*
H 		IM-2	*Streptomyces viridochromogenes*

A, The basic *N*-acyl homoserine lactone structure (see also Table 1). B, Halogenated furanones produced by a marine alga block bacterial AHL responses. C and D, Some cyclic dipeptides produced by both bacteria and fungi can activate or repress the bacterial AHL receptor. E, The volatile quorum-sensing molecule produced during infections by *Ralastonia solanacearum*. F, Proposed structure for one of the quorum-sensing molecules used by the plant pathogen *Xanthomonas campestris*. G and H, Examples of butyrolactone signal molecules produced by some soil inhabiting *Streptomyces* species.

strawberry (Fig. 3). Bacterial phenotypes controlled through quorum sensing are frequently regulated by additional environmental cues. In some cases, population-density signals can be modulated or overridden by factors such as oxygen tension, nutrient starvation, iron limitation or catabolite repression. 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. The evidence for AHL-inhibitory molecules in potato plants is not as clear as that for certain fruit or for pea seedling exudates. However, if these plants have adopted a strategy of limiting pathogenic attacks by blocking quorum sensing molecules, then engineering them to make AHLs would run counter to the existing defence mechanism and might render such plants more susceptible to infection.

ALTERNATIVE APPROACHES FOR INTERFERING WITH AHL SIGNALLING IN PLANT-ASSOCIATED MICROBES

Supplying transgenic plants with the ability to block or degrade AHL signals may provide an alternative approach for engineering resistance to *E. carotovora* species. There is no evidence that the long chain AHLs function as inhibitors of shorter chain AHL-induced responses in systems other than *Chromobacterium violaceum*. Even if long chain AHLs could suppress exoenzyme production by *E. carotovora* species, problems would remain with their use as some have been shown to have immune modulatory effects in mice and human leukocyte immunoassays (Telford *et al*., 1998). Engineering plants to produce inhibitory halogenated furanones might be possible, although the genes directing their biosynthesis have yet to be cloned from *Delisea pulchra*. Enhancing and extending the natural inhibitory activity apparent in some plant tissues may prove to be a more attractive approach. This is currently hampered by the fact that the nature of the inhibitory molecule(s) is unknown.

A bacterial isolate, *Bacillus* sp. 240B1, from soil was identified as having AHL degrading activity. The gene (*aiiA*) for this AHL degrading activity was cloned and shown to contain motifs conserved in several groups of metallohydrolases (Dong *et al*., 2000). When expressed in a heterologous *E. carotovora* system, AiiA inactivated the endogenous AHLs and reduced bacterial virulence. Recently, transgenic tobacco and potato plants expressing the *aiiA* gene have been made (Dong *et al*., 2001). These plants show considerable resistance to *E. carotovora* pv. *carotovora* infections even at very high bacterial inocula. Even where local disease symptoms were exhibited, the plants appeared to mount a defence response and recover. This appears to be a very promising approach to preventing AHL signalling in plant-associated bacteria. The same authors showed that AiiA is a lactonase, destroying AHL activity by opening the heterocyclic ring. An AHL-degrading activity has also been found in the soil bacterium *Varivorax paradoxus* (Leadbetter and Greenberg, 2000). In this case the enzyme is an AHL-acylase, cleaving the acyl side chain off the homoserine lactone moiety. The gene encoding this enzyme has yet to be cloned but, if it is identified, a transgenic plant approach might also prove effective.

As an alternative to the GM plant approach, a bacterial strain that efficiently degrades AHLs and that is capable of colonizing the rhizosphere might prove an effective biological control agent against certain plant pathogenic bacteria.

AHLS AND OTHER PLANT-ASSOCIATED BACTERIA—DO WE WANT TO BLOCK QUORUM SENSING?

Many Gram-negative bacteria found in association with plants have been shown to produce AHL signal molecules (Cha *et al*., 1998). The list includes epiphytic, pathogenic, rhizosphere-inhabiting and nitrogen-fixing symbionts. For many of these species, the genes under AHL regulation and the ecological roles of quorum sensing have not been studied in detail but, in the case of *Pseudomonas* spp., AHL production was found to be more common among plant-associated species than among soil-borne species (Elasri *et al*., 2001).

Some plant pathogenic bacteria have AHL sensing systems where the link with pathogenicity is indirect. In the plant pathogen *Agrobacterium tumefaciens*, conjugal transfer of the Ti plasmid between bacteria is induced by AHLs. However, AHLs form part of a more complex regulatory circuit that is triggered by opines produced by the transformed host plant tissue (reviewed in Farrand, 1998). For octopine-type Ti plasmids, a luxR homologue (TraR) forms part of an octopine inducible operon that includes enzymes required for octopine catabolism. TraR responds to the AHL 3-oxo-C8-HSL by inducing the genes required for plasmid transfer; in addition, it also up-regulates the synthesis of 3-oxo-C8-HSL itself. The situation is similar for nopaline-type Ti plasmids, but, in this case, agrocinipines act on a repressor to relieve the repression of TraR and to allow AHL-induced plasmid transfer when an appropriate cell density has been reached. Thus, *A. tumefaciens* genetically modifies plants to produce opines which have a direct effect upon AHL production and perception by *A. tumefaciens* itself. However, whilst AHLs contribute to the maintenance (or spread) of the Ti plasmid within the *A. tumefaciens* population, they do not appear to be directly required for plant infection.

For other plant pathogens, a link between AHLs and pathogenicity is not apparent. *Pseudomonas syringae* B728a makes AHLs, but disruption of the genes responsible had no effect on pathogenicity or swarming (Kinscherf and Willis, 1999). In contrast, it is apparent that AHL responses play a direct role in the colonization and behaviour of many bacteria beneficial to plants.

Rhizobium leguminosarum produces a number of different AHL molecules (Lithgow *et al*., 2000) using four different AHL biosynthetic genes (two on the symbiotic plasmid, one on a second large plasmid and one on the chromosome). The largest AHL contains an acyl side chain of 14 carbons, which is unusual both for the length of the acyl side chain and for the fact that it is not fully saturated. This AHL was originally identified as a 'small bacteriocin' because of its growth inhibitory effects on several strains of *R. leguminosarum* (Hirsch, 1979; Wijffelman *et al*., 1983), though Gray *et al*. (1996) subsequently concluded that it induced exponentially growing cells to go into stationary phase rather than causing cell death. As well as the effect on growth, this large AHL was also found to induce expression of the rhizosphere-expressed *rhiABC* operon and to promote plasmid transfer. A complex regulatory circuit operates in this species, with the three other AHL synthesizing loci being regulated by this long chain AHL (Lithgow *et al*., 2000). A role for AHL signalling in nodulation by *R. leguminosarum* is implied by the finding that mutating an AHL receptor decreased nodulation (Cubo *et al*., 1992). The related species *R. etli* CNPAF512 produces at least seven AHLs; disruption of a gene responsible for the production of two of these resulted in a doubling in the number of nitrogen-fixing nodules (Rosemeyer *et al*., 1998). This latter

finding is intriguing in the light of the discovery that some legumes appear to secrete AHL antagonists from their roots (Teplitski *et al*., 2000).

Pseudomonas aureofaciens 30–84 (a soil-borne bacterium that colonizes the wheat rhizosphere) inhibits the fungus *Gaeumannomyces graminis* var. *tritici*, the causative agent of take-all disease of wheat (Wood and Pierson, 1996), and has thus been used as a biological control agent. *P. aureofaciens* 30–84 synthesizes three phenazine antibiotics which are responsible (at least in part) for this antifungal activity. Expression of the phenazine biosynthetic operon is controlled by C6-HSL, which is synthesized by the *phzI* gene product (Wood and Pierson, 1996). Disruption of *phzI* abolishes bio-control activity, but this can be restored *in situ* by co-inoculation with a C6-HSL producing strain that supplies the signalling molecule in *trans* (Wood *et al*., 1997). Restoration of bio-control activity to *phzI*$^-$ strains can also be conferred by AHL-synthesizing plant tissues (Fray *et al*., 1999). AHLs are likely to have a role in promoting active secondary metabolite production in many bio-control strains of pseudomonads (Whitehead *et al*., 2001), as has also been shown for *Pseudomonas chlororaphis* PCL1391 (which inhibits *Fusarium oxysporum* infections of tomato; Chin-A-Woeng *et al*., 2001).

It is possible that gross disruption of AHL-based communication in the rhizosphere may adversely affect the colonization or behaviour of a number of important growth-promoting or bio-control species (Zhang and Pierson, 2001). The disadvantages of this disruption may outweigh any potential gains from reduced pathogenicity of other bacteria.

OTHER BACTERIAL QUORUM SENSING SYSTEMS OF PLANT- AND RHIZOSPHERE-ASSOCIATED BACTERIA

Apart from AHLs, a number of other molecules that effect AHL quorum sensing or that act as quorum sensing molecules in their own right are produced by plant- and rhizosphere-associated bacteria.

Several species of *Pseudomonas*, including biological control strains of *P. fluorescens*, produce cyclic dipeptides (diketopiperazines; Table 2). Though there is no obvious similarity between these molecules and AHLs, they appear to be capable of directly activating or antagonizing the receptors normally used by bacteria for AHL perception (Holden *et al*., 1999). Such compounds are not confined to bacteria; several of these active cyclic dipeptides are also produced by the fungal plant pathogen *Alteneria alternata* and two, cyclo(L-Pro-L-Tyr) and cyclo(L-Pro-L-Phe), act as host-specific phytotoxins against spotted knapweed (*Centaurea maculosa*; Stierle *et al*., 1988). The physiological/ecological role of these molecules in relation to quorum sensing (if any) remains to be established.

The plant pathogen *Ralstonia solanacearum* causes wilting and death in several hundred plant species including economically important crops such as potato, tomato and banana. During infection, *R. solanacearum* integrates a number of self- and plant-derived signals. The first intercellular signal produced by the bacteria is 3-hydroxy-palmitic acid-methyl ester (Table 2), which is volatile and acts as a quorum sensing molecule. This acts through a phosphorelay to derepress a transcriptional activator, PhcA, which is itself induced by a plant-derived signal. PhcA then acts to induce the production of extracellular polysaccharides and various other virulence factors and AHLs, though the role of these AHLs in signalling during the infection process is unclear at the present time (Denny, 1999).

Xanthomonas campestris pv. *campestris*, the causative agent of black rot in crucifers, also regulates extracellular polysaccharide production via diffusible intercellular signal molecules. These molecules are not fully characterized, but the lactone structure shown in Table 2 was proposed for one of these (Chun *et al*., 1997).

Gram-negative bacteria are not alone in exhibiting density-dependent behaviour. Among the *Streptomyces*, small diffusible butyrolactone-containing (BL) autoinducers direct the late log phase-dependent production of antibiotics and novel pigments and may also induce differentiation and spore production (Table 2; Horinouchi and Beppu, 1992). As with AHL synthesis, BL production is inhibited by cerulenin (which irreversibly inhibits β-ketoacyl-acyl carrier protein synthetase) suggesting that, like AHLs, one of the precursors is derived from fatty acid biosynthesis; the other precursor of BLs is probably a glycerol derivative.

Becker *et al*. (1997) presented evidence that BL-mediated communication between *Streptomyces* species contributed to pathogen inhibition in a soil naturally suppressive to *S. scabies*, the causative agent of potato scab disease. However, they concluded that there was no cross-talk between the AHL and BL quorum sensing systems.

CONCLUSIONS

Quorum sensing is an example of multicellular behaviour in prokaryotes and regulates diverse physiological processes in beneficial and pathogenic bacteria. AHLs are one of the most widely used quorum sensing molecules among Gram-negative bacteria and the ability now exists to engineer plants to give false information to bacteria that use this form of signalling. This can be achieved either through AHL production *in planta* or by engineering plants with the ability to degrade AHL molecules. The latter approach is particularly promising as a mechanism to confer disease resistance to *Erwinia* species. However, the role of AHL signalling in a number of important plant-associated bacteria remains to be clarified. The degree of communication within and between mixed bacterial communities and the role that naturally produced plant metabolites may have upon this communication are likely to be fruitful areas for research in the coming years.

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