4-29-2003

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Annu. Rev. Phytopathol. 2003. 41:455–82
doi: 10.1146/annurev.phyto.41.052002.095652

First published online as a Review in Advance on April 29, 2003

QUORUM SENSING IN PLANT-PATHOGENIC BACTERIA

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Key Words autoinducer, homoserine lactone, cell-cell communication, bacterial signaling, virulence

■ **Abstract** Quorum sensing (QS) allows bacteria to assess their local population density and/or physical confinement via the secretion and detection of small, diffusible signal molecules. This review describes how phytopathogenic bacteria have incorporated QS mechanisms into complex regulatory cascades that control genes for pathogenicity and colonization of host surfaces. Traits regulated by QS include the production of extracellular polysaccharides, degradative enzymes, antibiotics, siderophores, and pigments, as well as Hrp protein secretion, Ti plasmid transfer, motility, biofilm formation, and epiphytic fitness. Since QS regulatory systems are often required for pathogenesis, interference with QS signaling may offer a means of controlling bacterial diseases of plants. Several bacterial pathogens of plants that have been intensively studied and have revealed information of both fundamental and practical importance are reviewed here: *Agrobacterium tumefaciens*, *Pantoea stewartii*, *Erwinia carotovora*, *Ralstonia solanacearum*, *Pseudomonas syringae*, *Pseudomonas aeruginosa*, and *Xanthomonas campestris*.

INTRODUCTION

The term quorum sensing (QS) describes a well-understood mechanism of bacterial cell-cell communication and conveys the concept that certain traits are only expressed when bacteria are crowded together [reviewed in (61)]. This allows them to act in a coordinated manner and reinforces the notion that individual bacteria benefit from co-operative group behavior to survive, compete, and persist in nature or to colonize a particular host. QS involves the exchange of low molecular weight, diffusible signal molecules between members of a localized population. If signal production by the population is greater than its loss by diffusion or inactivation, the signal accumulates to a threshold level and activates cognate receptor proteins.

These in turn may trigger widespread changes in gene expression in members of the population. A key requirement for quorum sensing is, therefore, growth of cells in close proximity, as in a biofilm or when confined in an enclosed, diffusion-limited environment. Either condition allows localized signal build-up to occur.

The paradigm for QS, historically called autoinduction (106), is the signal-mediated activation of bioluminescence *lux* genes in the symbiotic bacterium *Vibrio fischeri*, which produces light when colonizing the light organs of certain marine animals. The autoinducer (AI) signal for *V. fischeri* QS is *N*-(3-oxo-hexanoyl)-L-homoserine lactone (3-oxoC6HL), which is made by the LuxI synthase from S-adenosyl methionine and 3-oxo-hexanoyl-acyl carrier protein (102, 110, 145). This signal molecule moves freely across bacterial membranes. Inside the bacterium, 3-oxoC6HL interacts with its receptor, LuxR, to form an active complex with increased affinity for a palindromic *lux* box element found in the promoter regions of genes in the *lux* regulon. The *luxI* and *luxR* genes themselves are part of this regulon and are therefore autoregulated by a dual positive feedback mechanism [reviewed in (135)]. The remaining genes of the *lux* regulon encode enzymes required for luminescence [reviewed in (7, 56, 143)].

N-acyl-homoserine lactones (AHLs), such as 3-oxoC6HL, are the most commonly reported type of quorum sensing signals. Over 50 species of Proteobacteria, including plant-associated species, produce AHLs (45, 57, 147, 158). Other bacterial groups use different types of compounds to regulate population density-dependent behaviors. These include the furanosyl borate diester AI-2 signal of *V. harveyi* (20), γ-butyrolactone in *Streptomyces* (159), oligopeptides in various gram-positive species (44, 78, 107), cyclic dipeptides in several gram-negative species (67), and bradyoxetin in *Bradyrhizobium japonicum* (87). The plant pathogenic bacteria *Ralstonia solanacearum* and *Xanthomonas campestris* use unique fatty acid and butyrolactone derivatives as QS signals to regulate production of pathogenicity factors (6, 21, 49). *Salmonella*, which does not synthesize AHLs, nevertheless has a receptor for them (SdiA), enabling it to detect and respond to nearby AHL-producing bacteria (98). Thus, the ability to "listen" to the quorum sensing signals of other species may constitute an important aspect of interpopulation communication and community structuring in natural environments (30, 116, 129, 134). However, the mere presence of QS signal synthase orthologs and/or detection of QS signal activity in heterologous reporter systems is not sufficient to define a bona fide QS system. An authentic QS signaling system should also feature a cognate signal receptor/regulator and target genes expressed in a population density-dependent manner (154, 156).

A wide range of behaviors is affected by AHL-mediated QS regulation, including bioluminescence, swarming motility, biofilm formation, cell division, stress survival, horizontal DNA transfer, and the synthesis of colonization and virulence factors, such as extracellular polysaccharides (EPS), surfactants, antibiotics, and extracellular enzymes (57, 147, 158). Of particular interest to plant pathologists is the fact that the expression of pathogenicity factors in a variety of plant pathogens is critically dependent on QS (31, 117, 147). In *Pseudomonas aeruginosa*, one of the

best-studied model systems for QS, AHL perception directly or indirectly affects the expression of over 200 genes (150), suggesting that such regulation could be important for many cryptic as well as observed behaviors. Although the basic mechanisms of AHL-mediated QS are generally well understood in vitro, the dynamics of signal sensing and regulation in nature are more difficult to define, and new levels of complexity are now surfacing. For example, different bacteria produce different AHLs, and a given species may produce more than one AHL. The acyl side chains of known AHL molecules vary in length (4–18 carbons), can contain double bonds, or are frequently substituted with a carbonyl or hydroxyl group at the C3 position (54, 147). In addition, QS regulation may be quite strain-specific, with different strains making substantially different sets of AHLs, or no detectable AHLs at all (15, 46). A bacterium may have two or more AHL receptors, each responding to different AHLs (112, 157, 163), and the effects of one AHL can be dominant over another (112, 130). The amount of AHL synthesized is often, but not always, subject to positive feedback regulation via the cognate AHL receptor (61). Moreover, the amounts and kinds of AHLs actually produced by bacteria can depend markedly on environmental conditions (14, 160). Finally, the ability of cells to respond to a "threshold" concentration of an AHL may depend on various modulatory factors (35, 53, 69), global physiological regulators such as RpoS (81, 104, 151, 155), RsmA (18), and Crp (43), or environmental sensing systems, such as GacS/GacA (163). These complexities make it very difficult to predict from laboratory studies how QS regulation actually works for bacteria in natural environments.

In this review, we focus primarily on QS regulation mediated by AHLs and fatty acids in several plant pathogenic bacteria to provide a perspective of how traits governed by QS contribute to bacterial fitness and pathogenesis. In addition, we consider the ability of host plants to influence AHL-mediated QS in plant-associated bacteria. Finally, we discuss the biological relevance of bacterial QS in natural habitats, including plant hosts. For reviews and recent articles on the role of quorum sensing in beneficial or symbiotic plant microbe-relationships see (88, 115, 137, 147, 152, 157).

QUORUM SENSING IN PLANT PATHOGENIC BACTERIA

QS regulation has a significant role in the biology of plant-microbe interactions (117) and several studies on plant pathogenic bacteria have contributed at a fundamental level to our present understanding of QS mechanisms. For example, the first X-ray crystal structures for key QS proteins resulted from research on EsaI, a LuxI homolog from *Pantoea stewartii* subsp. *stewartii* (145), and TraR, a LuxR homolog from *Agrobacterium tumefaciens* (142, 162).

Crown Gall

A. tumefaciens causes crown gall tumors in plants by transferring T-DNA from its tumor-inducing (Ti) plasmid into the chromosome of a plant cell (164). The

resulting tumors produce metabolites called opines, which serve as a novel source of nutrients for the pathogen (34). Both AHLs and a subclass of opines, the conjugal opines, act as chemical signals to induce the transfer of the resident Ti plasmid to plasmidless agrobacteria frequently cohabiting the gall (47). QS control is mediated by TraR, a LuxR-type AHL receptor that responds most strongly to *N*-3-oxo-octanoyl-HL (3-oxoC8HL) (161) for its activity. This AHL is synthesized by the LuxI homolog TraI (60, 68). Transfer of the nopaline-type Ti plasmid, pTiC58, depends on the phosphodiester opines, agrocinopines A and B (47), whereas the transfer of octopine-type Ti plasmids requires octopine (1, 164). The agrocinopines interact with the AccR repressor to derepress both the coregulated agrocinopine catabolic locus (*acc*) and the linked, divergently transcribed *arc* (agrocinopine regulation of conjugation) operon that contains $traR_{nop}$ (118, 119). In the octopine strains, octopine interacts with the OccR transcription factor to activate an operon comprised of the octopine catabolic genes (*occ*), an ABC-type transport system and $traR_{oct}$ (58, 60). The TraR regulon of both types of plasmids includes two linked *tra* operons, a separate *trb* operon, and the *rep* operon for the replication of the Ti plasmid (1, 48, 83, 84). Together, the *tra* and *trb* operons encode the structural components for Ti plasmid conjugal transfer. In each system, *traR* is part of a separate transcription unit near the *tra* gene cluster, whereas *traI* is the first gene in the *trb* operon (59, 61, 68) (Figure 1). Interestingly, the genome sequence for *A.*

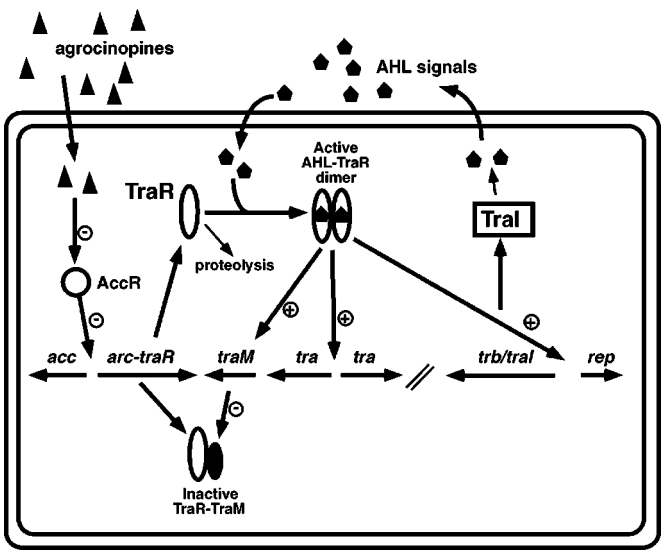

Figure 1 Quorum sensing regulation of the Ti plasmid conjugal transfer genes in *A. tumefaciens*. Opines (*triangles*) induce synthesis of the TraR response regulator (*open ovals*) via AccR (*circle*). In the presence of high concentrations of AHL signals (*pentagons*), produced by the TraI synthase (*rectangle*), TraR activates the *tra* regulon. TraM (*closed oval*) titrates the activity of TraR at high AHL concentrations.

tumefaciens strain C58 reveals no additional *luxI* homologue genes, but features a number of genes with motif structures of the LuxR family of transcription factors (see website for the Laboratory for Bioinformatics, Institute of Computing, University of Campinas, Brazil, http://cancer.lbi.ic.unicamp.br/agroC58/).

Considerable progress has been made toward understanding the molecular and structural basis of QS control of Ti plasmid conjugal transfer. It is believed that nascent TraR protein requires the AHL cofactor to serve as a scaffold for proper protein folding, because AHL does not associate with prefolded TraR (166). This is consistent with the fact that the AHL ligand is deeply embedded within the N-terminal signal-accepting domain of TraR (142, 162). In the absence of AHL, TraR is subject to rapid proteolytic degradation (166). In the nopaline strain C58, ApoTraR appears to be plasma membrane-associated and dissociates from the membrane when complexed with AHL (126). AHL-TraR dimerizes and positions the C-terminal helix-turn-helix domain to bind to the major groove of the 18-bp *tra*-box recognition site. *tra*-boxes are found immediately upstream of the *traAFB*, *traCDG*, *traI-trb*, and *rep* operons (126, 142, 162, 165). TraR also activates the *traI-trb* operon, thereby creating a positive feed-back loop for *traI* (60, 68). Active TraR also stimulates the expression of the *traM* gene, which encodes a small anti-activator with affinity for the C-terminal domain of TraR (53, 69). TraM is thought to "titrate" TraR to prevent Ti plasmid transfer until TraR levels exceed those of TraM (119, 136). TraR also controls the expression of the Ti plasmid's *rep* gene system and consequently increases its copy number at high population densities (53, 83) (Figure 1). In addition, the conjugation of certain octopine-type Ti plasmids is suppressed by mannopine opines (16, 108). This is due to the inhibition of TraR activity by TrlR, which appears to be a C-terminally truncated form of TraR that forms a nonproductive TrlR/TraR heterodimer (16, 108). QS regulation in *A. tumefaciens* is also governed by an active signal-turnover process mediated by the *attM*-encoded AHL lactonase, which has structural and functional similarity to the AiiA lactonase of *Bacillus cereus* (38, 39). The concentration of 3-oxo-C8HL declines rapidly at the onset of stationary phase, as does the frequency of conjugal Ti transfer. Interestingly, *attM* is part of a previously characterized locus involved in bacterial attachment to plant cells (94).

Ti plasmids use both opines and QS to regulate Ti plasmid conjugation and replication. This suggests that the opine-dependent regulation of conjugation provides a major biological advantage to either the bacterium or the Ti plasmid, or both. It is attractive to speculate that in young tumors the colonizing agrobacteria have sufficient nutrients and consequently favor rapid growth over Ti plasmid maintenance. In this regard, it is well known that crown galls contain a significant proportion of agrobacteria that are avirulent as a result of plasmid loss or rearrangement, and that such strains seem to have a definite growth advantage (11). However, when nutrients become limiting and the bacterial population densities are higher, the QS-dependent activation of conjugation may allow the bacteria to regain the plasmid. This mechanism would confer an advantage not only to the individual recipient cell, but to the species overall, because it would increase the potential of migratory cells to initiate new infections. The higher plasmid copy

number would also increase the expression of the opine catabolic genes and enable more efficient use of the available opines (83).

Stewart's Wilt of Corn

P. stewartii subsp. *stewartii* (*Pnss*; synon. *Erwinia stewartii*) causes Stewart's bacterial wilt and leaf blight of sweet corn and maize. This disease is transmitted by the corn flea beetle, *Chaetocnema pulicaria*. Infested beetles feed on emerging corn seedlings and introduce the pathogen into the xylem and intercellular spaces of the leaves. Once in the plant, *Pnss* grows in the apoplast of young leaves causing "water-soaked" lesions and colonizes the xylem vessels leading to subsequent wilting (12). Vascular occlusion is due to the accumulation of large amounts of EPS on pit membranes (12). The biosynthetic pathway for EPS synthesis is encoded by the *cps* gene cluster (24), which is related in organization and function to typical group I capsule (M-antigen) biosynthetic gene clusters in other enteric bacteria. Mutations in the *cpsA-M* locus lead to loss of wilting and systemic movement (24), thus establishing EPS as a primary virulence factor. The *cps* genes of *Pnss*, *Escherichia coli*, and *Erwinia amylovora* are similarly regulated by the Rcs (regulator of capsule synthesis) two-component signal transduction system (64, 74, 121). The plasma-membrane spanning sensory protein RcsC detects environmental signals, possibly desiccation and/or osmolarity (133), and signal perception results in phosphorylation and activation of the RcsB response regulator (64). An accessory protein, RcsA, is also needed for full induction of the *cps* genes, presumably by forming a more effective RcsA/RcsB activation complex (64). EPS synthesis in *Pnss* strain DC283 is regulated in part by the EsaI/EsaR QS system. The AHL synthase, EsaI, catalyzes the production of 3-oxoC_6HL and minor amounts of 3-oxoC_8HL (9). Interestingly, the *esaI* gene is constitutively expressed and not subject to EsaR-mediated autoregulation. Mutations in the *esaI* gene have pleiotropic effects, eliminating AHL production, EPS synthesis, and virulence, whereas mutations in the *esaR* gene lead to constitutive, growth-independent hypermucoidy (9). In contrast, the wild-type strain produces EPS in a population density-dependent manner, with measurable levels detected primarily at population densities $>10^8$ cells/ml (10).

The fact that *esaR* mutants are fully induced for EPS synthesis at low cell density indicates that EsaR acts as a negative regulator of the *cps* genes in the absence of AHL (10). Genetic studies and in vitro DNA binding experiments using purified EsaR and a synthetic *lux* box-like DNA fragment both showed that EsaR functions as an autorepressor of the *esaR* gene (101, 126). It is not yet known how EsaR mediates repression of the *cps* genes, because experiments to test the most logical regulatory scenarios have been inconclusive. Specifically, EsaR failed to bind to DNA within the *cspA* promoter region, which lacks a potential *lux*-box (T. Minogue & S. von Bodman, unpublished); it has no effect on RcsA/B binding at the *cpsA* promoter in competition DNA binding experiments (F. Bernhard, personal communication); and it did not appreciably affect expression of any of the *rcs* genes.

Recently, M. Merighi & D. Coplin (unpublished) found that the Hrp regulon in *Pnss* is also partially controlled by the EsaI/EsaR QS system. The Hrp type III secretion system is required for growth of *Pnss* in the intercellular spaces of leaves and xylem and is most likely responsible for the water-soaking symptoms on young corn leaves (23, 52). The *hrp* secretion and *wts* effector genes are controlled by a regulatory cascade consisting of the HrpX/HrpY two-component signal transduction system, the NtrC-like transcriptional enhancer HrpS, and the HrpL alternative sigma factor (97). These function as a cascade where HrpY activates *hrpS*, HrpS activates *hrpL*, and HrpL controls expression of the *hrp* and *wts* genes. An *esaI* mutant was found to be greatly reduced in water-soaking ability on corn seedlings and unable to elicit a hypersensitive response (HR) in tobacco. This mutant phenotype was corrected by ectopic overexpression of *hrpS*, but not *hrpY*. In addition, the expression of a *hrpJ-uidA* reporter gene fusion was shown to be cell-density dependent and activated when cultures were grown to about 3×10^8 CFU/ml. Moreover, the expression of several *hrp* secretion and effector genes was reduced by 100–1000-fold in an *esaI* mutant background, but was normal in an *esaI/esaR* deletion mutant. The *hrpS* and *hrpL* promoters were similarly regulated, but expression of the *hrpXY* promoter was not affected. These findings suggest that EsaR mediates the effect of the *esaI* mutation and acts, either directly or indirectly, as a repressor of *hrpS*.

When inoculated on susceptible sweet corn seedlings, the *esaI* mutant strain fails to induce wilting and causes only very weak water-soaking (10). This was expected because this strain has a functional copy of EsaR that represses EPS synthesis and Hrp-mediated water-soaking. In contrast, seedlings inoculated with the wild-type strain are killed. Somewhat surprisingly, the *esaR* mutant and the *esaI/esaR* double deletion mutant are significantly less virulent than the wild-type strain, even though they produce excessive amounts of EPS (10) and express *hrp* genes (M. Merighi & D. Coplin, unpublished). These findings clearly indicate that QS control of pathogenicity genes is important. The reduced virulence of these mutants emphasizes that early production of some virulence factors, such as EPS, may be counterproductive unless they are made at the proper time and location during the infection. Recent studies by von Bodman and coworkers suggest that EPS synthesis may be part of a multistep host invasion process. In vitro attachment assays with the wild type and QS mutants indicated that EPS synthesis interferes with the ability of *Pnss* to attach to plastic surfaces (80). Specifically, wild-type strain DC283 appeared to attach at a low level, perhaps only transiently; the non-mucoid *esaI* and *cps* mutant strains attached firmly in substantially greater numbers than the wild type; and the hypermucoid *esaI/esaR* double mutant lost its ability to adhere to a surface. The same attachment deficiency could be artificially induced in the *esaI* mutant strain through exogenous addition of AHL, the degree of attachment being inversely proportional to amount of AHL supplied (M. Koutsoudis & S. von Bodman, unpublished). In plant assays, similar behavior was observed; the *esaI* mutant remained localized at the site of infection, whereas the wild-type strain moved at an impressive rate through the vascular system (100). Interestingly, the

hypermucoid *esaI/esaR* double mutant initially remained at the site of inoculation, but eventually dispersed within the plant tissue, although at a much reduced and more variable rate than the wild-type strain. These data suggest that invasion by the *esaI* mutant strain may be impaired after the initial attachment stage. In contrast, invasion of the plant by the hypermucoid Δ*esaR/esaI* strain was delayed and more variable. The electron microscopy studies of the *Pnss* host invasion process reported by E.J. Braun (12) 20 years ago highlight some of the same events and seem quite consistent with the step-wise colonization process outlined above.

The related pantoea, *Erwinia herbicola* pv. *gypsophilae* (synon. *Pantoea agglomerans*), which causes galls on gypsophila, produces C_4HL (I. Barash, personal communication), but the closely related fireblight pathogen, *E. amylovora*, which shares many mechanisms of virulence with *P. stewartii*, has not been reported to make any AHLs in culture.

Soft Rot Erwinias

QS in *E. carotovora* subsp. *carotovora* (*Ecc*; synon. *Pectobacterium*) controls the population density-dependent expression of pathogenicity factors, such as extracellular enzymes and the Hrp secretion system, as well as carbapenem antibiotic production [reviewed in (88, 147, 148)]. The primary AHL, 3-oxoC_6HL, is produced by the LuxI-like signal synthase CarI (also called ExpI). Mutants defective in *carI* do not produce carbapenem, pectolytic enzymes, endoglucanases, and proteases and fail to secrete harpin. They are, therefore, completely nonpathogenic (5, 17, 27, 72, 120). The Car QS system directly regulates the genes for production of the antibiotic carbapenem, which constitute the *carA-H* biosynthetic operon (96). A *luxR*-like gene, *carR*, is located 150 bp upstream of *carA* (96) and is not linked to *carI*. Mutations in either *carR* or *carI* block carbapenem synthesis (17, 72, 95), while mutations in *carI*, but not in *carR*, affect exoenzyme production. Ligand-free CarR forms a functional dimer that is capable of binding to the *carA* promoter, albeit with low affinity. In the presence of the cognate AHL, however, CarR forms multimeric complexes that bind the target promoter more efficiently, even though a clearly defined *lux* box is lacking (146, 147). Like *A. tumefaciens* TraR, CarR is stabilized by AHL (146). In addition, the *carR* gene is autoregulated. The gene for another AHL receptor, *expR*, is located adjacent to *carI* and convergently transcribed (2, 95). However, no genes directly regulated by ExpR have been identified and *expR* mutations have only minor effects on exoenzyme production.

Studies by Nasser et al. (105) have shown that *E. chrysanthemi* (*Ech*) strain 3937 makes three different AHLs, 3-oxoC_6HL, C_6HL, and C_{10}HL, and that *Ech* has a homologous set of linked *expI* and *expR* genes and a second, unidentified AHL synthase. ExpI is responsible for the synthesis of 3-oxoC_6HL and C_6HL. Disruption of either *expI* or *exp*R has very little effect on the overall production of pectolytic enzymes in vitro, even though the ExpR regulator binds to several different *Ecc* and *Ech pel* promoters in the absence of AHL. It is unclear what

function the ExpI/R QS regulatory pair may have in *Ech*. One possibility is that redundant AHL/response regulator combinations may control exoenzyme synthesis in these two erwinias, thereby accounting for the lack of a dramatic effect of *expR* mutations. *E. carotovora* subsp. *betavasculorum* strain 168 also produces AHLs and contains a set of QS regulatory components designated EcbI and EcbR. Disruption of the *ecbI* gene eliminates AHL production and leads to loss of antibiotic synthesis and reduced pectate lyase activity (25).

In contrast to the relatively simple regulation of carbapenem biosynthesis, the population density-dependent control of extracellular virulence factors in *Ecc* is only one part of a complex regulatory hierarchy in which the components may have different roles at different stages of infection or growth (Figure 2). The effects of QS appear to be mediated in part by the RsmA-*rsmB* (regulator of secondary metabolism) negative regulatory system (17, 29, 79), which is conserved in *E. coli* (71) and many *Erwinia* and *Pseudomonas* spp. (89, 113). The key factor in this pathway is RsmA, a small posttranscriptional regulator that binds to specific mRNAs thereby blocking ribosome binding and promoting RNA decay. Mutants of *rsmA* overproduce degradative enzymes, are hypervirulent, and cause a HR in tobacco. These phenotypes are independent of AHL production, indicating that an *rsmA* mutation is epistatic to *carI*. The *rsmB* gene encodes an untranslated RNA species that complexes with RsmA to neutralize its activity (85). Overproduction of *rsmB* overcomes negative regulation by RsmA.

The RsmA-*rsmB* system is subject to additional upstream regulation (Figure 2). RsmC and KdgR stimulate *rsmA* expression and repress *rsmB* (28, 85), whereas the ExpA/ExpS two-component system (homologous to GacA/GacS) activates *rsmB* and represses *rsmA* (70). In addition to affecting *rsmA* expression, KdgR acts

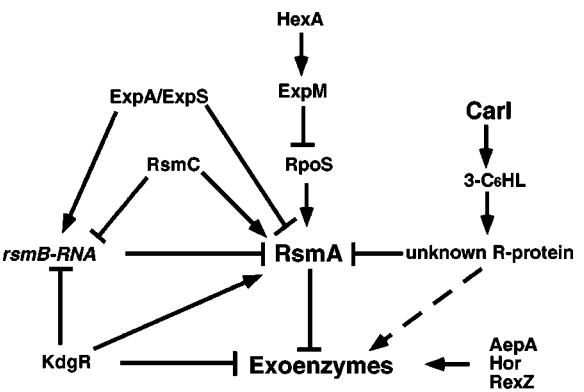

Figure 2 Schematic representation of the complex regulatory network involved in controlling exoenzyme synthesis in *E. carotovora* subsp. *carotovora*. Arrow heads indicate positive regulation and flattened ends denote negative regulation. Modified from (148) according to recent findings of Chatterjee et al. (18).

directly as a general repressor of the genes involved in pectin and oligogalacturonide catabolism (86). Another regulator, HexA (hyperproduction of exoenzymes), negatively regulates levels of *rsmB* and RpoS (103). Since RpoS is required for activation of *rsmA*, one possible function of the Rsm regulatory pathway may be to turn down exoenzyme synthesis during stationary phase or periods of stress.

There is genetic evidence indicating that QS affects the levels of RsmA protein. Chatterjee and associates (17) found that *rsmA* mutants do not require AHL for exoenzyme production, and recently they reported that overproduction of *rsmB* RNA also overrides the need for AHL (18). Moreover, *rsmB* mutants do not produce any exoenzymes, even when *carI* is overexpressed. Levels of *rsmA* transcripts are enhanced in a *carI* mutant, whereas those of *rsmC* are unchanged (18, 79). Likewise, an *rsmA-lacZ* reporter gene fusion is enhanced in a *carI* mutant, whereas the expression of an *rsmB-lacZ* fusion is unaffected (18). It is not clear how AHL represses *rsmA* transcription because a cognate QS response regulator for exoenzyme production has not been identified and the two most likely candidates, *carR* and *expR*, seem to have little effect on *rsmA* expression (2). This suggests that QS control of *rsmA* may be indirect and a third response regulator may directly govern the expression of some virulence genes. The complex upstream regulation of the RsmA system emphasizes that numerous conditions must be met before pathogenicity factors are produced in planta and that QS serves as only one switch among many. It is also significant that different bacteria can use the same regulatory proteins in different ways to accomplish this. For example, the QS systems of *P. aeruginosa* (described below) are also integrated with RsmA regulation, but in this species RsmA instead controls production of AHL (113).

It is easy to envision why *Ecc* would make carbapenem late in infection in order to protect its food supply from other bacteria that might try to colonize the rotted tissue (4). However, the value of QS regulation of pathogenicity genes in soft rotters is less clear, and it may play different roles in necrogens and biotrophs. The popular explanation for delayed production of cell wall-degrading enzymes is that they can release pectic wall fragments that can elicit host defense responses prematurely. The pathogen may therefore need to attain sufficient numbers before risking detection (36, 120). This theory is consistent with an experiment in which *carI* was introduced into transgenic tobacco plants so that they made 3-oxoC_6HL and presumably caused *Ecc* to produce pectic enzymes early in infection (90). The increased resistance of the transgenic plants to soft rot suggests that such early enzyme production might be detrimental to the pathogen.

Epiphytic Survival of *Pseudomonas syringae* Pathovars

AHLs are produced by strains of many phytopathogenic pseudomonads, including *P. corrugata*, *P. savastanoi*, and *P. syringae* pvs. *syringae*, *tabaci*, *angulata*, *tomato*, *coronafaciens*, and *maculicola* (15, 42). The LuxI and LuxR homologs have been identified in some of these bacteria (42, 46, 76, 147). In *P. syringae* pv. *syringae* strain B3A, inactivation of the *ahlI* AHL synthase gene results in loss of AHL production, altered colony morphology, and reduced epiphytic viability

(42). Recent studies on *P. syringae* pv. *syringae* B728a by Lindow and coworkers (personal communication) provide a broader perspective of the role of QS in epiphytes. In this strain, as in several other plant pathogenic bacteria, the *luxI/luxR* homologous genes, *ahlI* and *ahlR*, are convergently transcribed and *ahlI* exhibits autoinduction in response to the AHL signal (46). The AhlI synthase generates primarily 3-oxo-C_6HL and *ahlI* is regulated at two or more levels. First, *ahlI* expression is dependent on the GacA/GacS two-component signal transduction pathway, which is widely distributed among pseudomonads. Second, it is partially dependent upon a novel regulator, AefR, (autoinducer and epiphytic fitness regulator), which is a member of the TetR family of transcription factors. Expression of *ahlI* is severely reduced, but not eliminated, in an *aefR* mutant strain, suggesting that it acts as a positive regulator of *ahlI*, at least at low population densities. However, at high population density, expression of *aefR* is inhibited in an AHL-dependent manner, effectively removing it from the pathway. In this manner, it may act as a "governor" of QS behaviors.

AefR may play a pivotal role in epiphytic fitness (S. Lindow, personal communication). It does this by first inducing certain fitness genes during the initial phases of colonization and then subsequently downregulating these genes at later stages of colonization, when the cells have reached a "quorum." Strain B728a does not exhibit swarming motility at low densities, whereas *aefR* and *ahlR* mutants, which produce less AHL, are highly motile under these conditions. As a result, isolated cells on a leaf surface are likely to be motile, possibly to facilitate discovery of a favorable microhabitat, but become nonmotile when in sedentary aggregates. Both *aefR* and *ahlR* mutants were also deficient in production of EPS and hypersensitive to oxidative stress in culture. Such phenotypes may well account for the greater-stress sensitivity and decreased survival of these mutants on leaves. AHL-deficient mutants of B728a also exhibited reduced virulence when examined at later stages of infection in bean, showing water-soaking symptoms, but not subsequent tissue maceration. Thus, AHL-mediated regulation appears to be important in different ways at each stage of invasion. Preliminary evidence suggests that QS only starts to operate after the bacteria have colonized the plant for a couple of days. By monitoring an *ahlI::gfp* reporter gene fusion in B728a cells on bean leaves, Lindow and associates found that very few bacterial cells expressed the signal synthase gene until two days after inoculation, and then expression increased with time. This correlated well with the size of cell aggregates on the leaf, leading them to propose that enhanced AhlI (and presumably AHL) synthesis is related to the increasing proportion of cell aggregates that have achieved a threshold size at which autoinduction is possible, i.e., a "quorum."

Pseudomonas aeruginosa, a Pathogen of Both Plants and Animals

P. aeruginosa is an environmental generalist that can be isolated from diverse habitats including water, soil, animals, and plants (109, 127). In humans, it is the

leading cause of bronchiopulmonary infections in cystic fibrosis patients and nocosomial infections in burn victims and immune-compromised individuals (55, 112). In many plants, it is an opportunistic pathogen that causes soft rot. *P. aeruginosa* is not only a well-studied model for QS, but it has recently been used in studies to define universal virulence mechanisms across phylogenetic boundaries (127, 138). Infiltration of *P. aeruginosa* into *Arabidopsis* or injection into lettuce leaves causes initial water-soaked lesions and chlorosis, followed by tissue maceration and systemic infection (127). Analysis of various mutants with impaired virulence in mouse, *Arabidopsis*, lettuce, nematodes, and insects identified *gacA*, *gacS*, *lasR*, and *mucD* as genes that are necessary for full virulence in all hosts (127, 138).

P. aeruginosa has two AHL synthase/receptor pairs. The first gene pair, *lasI* and *lasR*, controls the expression of the second, comprised of *rhlI* and *rhlR* (112). The LasI synthase produces 3-oxoC_{12}HL, and RhlI catalyzes the synthesis of C_4HL (111, 147). QS regulation mediated by both LasR and RhlR is ultimately governed by the GacS/GacA two-component signal transduction system. Both LasR and RhlR, along with their cognate AHLs, affect, either directly or indirectly, the expression of over 200 genes (150), and control the expression of an arsenal of extracellular virulence factors and secondary metabolites including elastase, exotoxin A, alkaline protease, chitanase, lectin, rhamnolipid, pyocyanin, phenazine, hydrogen cyanide, superoxide dismutase, and catalase. Some of these factors contribute to the growth of bacteria in planta (127, 138, 147).

Ralstonia solanacearum

R. solanacearum causes a vascular wilt disease of several hundred plant species, including tobacco, tomato, potato, and bananas. The pathogen can survive for long periods as a resident in the soil and then infect plants through wounds and openings formed by lateral root emergence. It must then penetrate the cortex of the root, breach the suberized endodermal barrier, and finally enter the xylem vessels. Once in the vascular system, it will colonize the entire plant, attaining populations in excess of 10^{10} cells/cm length of stem tissue. The early stages of infection may involve attachment, motility, and microcolony formation (73, 140). After the bacteria invade the cortex, pectolytic enzymes disrupt the middle lamella, enabling the bacteria to spread through the tissue and, together with Hrp effectors, the enzymes release nutrients from host cells (33, 131). The plants eventually wilt owing to the accumulation of bacteria and EPS in the xylem.

Most of the traits needed for infection and virulence are regulated by the Phc (phenotype conversion) regulatory system in a population density–dependent manner [reviewed in (32, 33, 131)]. PhcA, a LysR-type transcriptional regulator, is at the center of a complex regulatory hierarchy and its activity is modulated by a unique, volatile signal molecule, 3-OH palmitic acid methyl ester (3-OH PAME) (13, 49). *phcA* mutants do not produce EPS, pectin methyl esterase, and endoglucanase, and they are hypermotile and exhibit increased production of polygalacturonase and siderophore. PhcA directly regulates endoglucanase and pectin methyl

esterase production but interfaces with four other response regulators and two sensors, which affect the remaining Phc-controlled traits. 3-OH PAME is synthesized by the *phcB* encoded S-adenosyl methionine-dependent methyl transferase that converts 3-hydroxypalmitic acid into a methyl ester (13, 50). 3-OH PAME then acts as a signal for an atypical two-component regulatory system that posttranscriptionally modulates the activity of PhcA. This consists of a membrane-bound sensor-kinase, PhcS, that phosphorylates PhcR, an unusual response regulator with a C-terminal kinase domain in place of a DNA-binding domain (22). Mutations that specifically inactivate the PhcR kinase domain create a *trans* dominant allele that constitutively represses the Phc regulon. This suggests that unphosphorylated PhcR serves as a negative regulator of the Phc phenotype at subthreshold concentrations of 3-OH PAME. Subsequent phosphorylation of PhcR in response to the signal ligand then inactivates it. Immunoblot and Northern assays have shown that PhcR posttranscriptionally reduces the amount of PhcA available by an undefined mechanism (131).

QS regulation may be important to *R. solanacearum* as it makes the transition from life in the soil to that of a parasite. Low levels of 3-OH PAME lead to reduced EPS and exoenzyme synthesis, but enhanced motility and siderophore production (22). Conversely, inducing levels of 3-OH PAME promote PhcA activity, resulting in enhanced expression of EPS and exoenzymes and decreased motility and siderophore synthesis. In this manner, the Phc regulatory system serves as master control switch to turn off behaviors suited to free-living survival and to turn on those needed for initial host contact, microcolony/biofilm formation, and pathogenesis.

Although the Phc pathway is present in most *R. solanacearum* strains, it is apparently absent from most other plant pathogens. It was recently reported that a *phcA* ortholog in the nonpathogenic, facultative chemolithoautotroph *Ralstonia eutropha* (synon. *Alcaligenes eutrophus*) fully complements *R. solanacearum phcA* mutants (62). This species also appears to make a form of 3-OH PAME and contain orthologs of *phcB* and *phcS*.

The sequence of the *R. solanacearum* genome reveals two pairs of *luxI/luxR* homologs (http://sequence.toulouse.inra.fr/R.solanacearum.html). One of these, *solI/solR*, has been characterized (50). SolI catalyzes the synthesis of C_6HL and C_8HL. Interestingly, the expression of *solI* and *solR* is controlled by PhcA and RpoS (50, 51). However, inactivation of the *solI/solR* genes does not affect virulence, EPS synthesis, or exoenzyme production and the traits they regulate are not known.

Cell-to-Cell Signaling in *Xanthomonas campestris* pv. *campestris*

X. campestris pv. *campestris* (*Xcc*) is a vascular pathogen that causes black rot of cabbage and other cruciferous plants. The bacterium can multiply as an epiphyte on leaves and then enter the vascular system through hydathodes during wet weather. It multiplies in the xylem, initially causing typical V-shaped lesions and finally a serious vascular wilt. Symptoms are due to blockage of the xylem by bacterial cells

and xanthan gum, which is the major EPS produced by xanthomonads. Extracellular enzymes, such as endoglucanase and polygalacturnonase, and a Hrp secretion system contribute significantly to the ability of *Xcc* to multiply in host tissue. Two QS-like systems involving diffusible signaling factors control traits related to pathogenicity and epiphytic fitness (6, 21, 122). The diffusible signal factor (DSF), a fatty acid derivative, regulates exoenzymes and cyclic glucans in *Xcc* strain 8004 (144), whereas the diffusible factor (DF), a butyrolactone produced by *Xcc* straim B24, regulates xanthomonadin pigment production (21, 121); both signals control EPS synthesis [reviewed in (32, 147)]. Most of the known pathogenicity genes in *Xcc*, including the *gum* genes for xanthan gum synthesis, are coordinately regulated by the Rpf system (regulation of pathogenicity factors). Nine *rpf* genes (*rpfA-I*) are linked within a 22.1-kb region, and transposon-induced mutations within this region abolish pathogenicity, exoenzyme production, and EPS synthesis (6, 139). Among these genes, *rpfC* and *rpfG* encode a two-component regulatory system (40), *rpfA* encodes an aconitase with a possible function in iron homeostasis (153), and *rpfB and rpfF* are autoregulatory genes involved in production of the novel signal molecule DSF (6). RpfB is predicted to function as a long-chain fatty acyl CoA ligase and RpfF has some structural features of enoyl CoA hydratases. The structure of DSF is not known. Early work suggested that DSF is a fatty acid derivative, not an AHL (6), whereas recent preliminary NMR studies are consistent with it containing a butyrolactone ring (M. Dow, personal communication). This would make DSF somewhat similar in structure to AHLs, which are N-substituted γ-butyrolactones. Most *X. campestris* strains produce DSF, but the amount varies with strain and pathovar (6). DSF levels peak in early stationary phase cultures and then disappear. Endoglucanase and protease production parallel DSF levels, but supplementation of log phase cultures with DSF does not cause early activation of protease gene expression or increase DSF synthesis.

The *rpfHCG* operon encodes the putative receptor system for DSF. RpfC is a hybrid two-component regulatory protein with domains typical of both a sensor kinase and response regulator but it features an additional C-terminal histidine phosphotransferase (HPt) receiver domain (132, 139). RpfH is homologous to the *trans*-membrane sensor domain of RpfC and may stabilize RpfC in the cell membrane (132). The response regulator, RpfG, has a typical input domain attached to an HD-GYP domain (132). Proteins with an HD-GYP domain are believed to be phosphodiesterases involved in cyclic diguanylate signaling and typically contain Gly-Gly-Asp-Glu-Phe and Glu-Ala-Leu motifs (3). Consequently, RpfG may indirectly control EPS and exoenzyme synthesis by governing the synthesis or turnover of cyclic di-GMP (26).

The second QS-like system in *Xcc* strain B24 uses a similar diffusible factor (DF) to control pigment (xanthomonadin) and EPS synthesis (122, 124). Non-pigmented *pigB* mutants cannot synthesize DF, make fourfold less EPS, cause fewer lesions on cabbage, and are impaired in epiphytic survival (123). DF has been purified and structural analysis indicates that it may be 2-(2-methyl-3-oxo-butyl)-butyrolactone (21). DF levels plateau in stationary phase. Although similar

in structure, DF and DSF are clearly distinct compounds because they differ in biological activity and chromatographic mobility (124). A subsequent study has shown that *pigB* and DF are needed for epiphytic survival because normal xanthomonadin production is critical for protection against UV light (125). However, the effect of a *pigB* mutation on the rate of hydathode colonization is primarily due to changes in EPS production, rather than to lower epiphytic populations (123).

From surveys of various plant-associated, rhizosphere and soil bacteria, it appears that AHL signaling is very rare in xanthomonads (15, 75), which have come to rely on butyrolactones instead. At present, the only other xanthomonad known to have the Rpf system for production and detection of DSF is *X. oryzae* pv. *oryzae* (19). In this pathovar, strains with mutations in a gene homologous to *rpfF* are weakly virulent, do not make DF, and grow poorly under low iron conditions, but still produce normal levels of EPS and xylanase (19). This implies that *rpfF* is involved in controlling an iron-uptake system in *X. oryzae* pv. *oryzae* and that a defect in this system may cause the decreased virulence of *rpfF* mutants.

Analysis of the genomic sequence of *Xylella fastidiosa* (41), a fastidious vascular pathogen, has revealed homologs of *rpfF, rpfB, rpfC,* and *rpfG* and a set of EPS biosynthetic genes that are similar to those for the production of xanthan gum. In addition to occupying a similar niche and possibly sharing virulence mechanisms, this pathogen may have also retained (or acquired) similar genes for regulating pathogenicity.

HOST PLANT MECHANISMS FOR DEALING WITH QUORUM SENSING

Pathogenic bacteria depend quite significantly on QS regulation to coordinate their colonization and infection of plant hosts. Therefore, it seems relevant to ask if plants can take advantage of this dependency. For example, plants might be able to disrupt QS in pathogens by enzymatically destroying their QS signals or by synthesizing compounds that mimic these QS signals to confuse the pathogen. Alternatively, plants might be able to detect the pathogen's QS signals and use this as information to trigger various defense responses appropriate to the kinds and amounts of signals detected. At present, there are no reports to indicate that plants have the ability to produce compounds that specifically inhibit the synthesis of bacterial QS signals. However, a significant percentage of soil bacteria do have enzymes for degrading or inactivating AHLs (8, 37, 39, 82, 116, 128, 149). The best studied of these enzymes is AiiA, a lactonase from *Bacillus cereus* that opens the lactone ring of all AHLs tested, reducing their potency as signals by about 1000-fold (39). Interestingly, when AiiA was expressed in transgenic tobacco and potato, the plants became highly resistant to infection by *E. carotovora* (38), which depends heavily on 3-oxo-C6-HSL-mediated QS for pathogenicity. Thus, destruction or inactivation of AHL signals in situ could be a useful strategy for engineering disease-resistant plants. However, the limited evidence available

suggests that AHLs are not degraded substantially by plant enzymes, at least in the rhizosphere of soil-grown plants. It was observed that AHLs produced by members of a natural tomato rhizosphere community were able to activate AHL reporter cells on the root surface even when the reporter cells were some distance from the AHL-producing cells (134). Thus it appears that AHLs can diffuse broadly over root surfaces, perhaps in the mucigel layer (134). Such results are consistent with an earlier report of interpopulation AHL signaling in the rhizosphere of field-grown wheat (114). Perhaps nonspecific destruction of QS signals by plant hosts would adversely affect not only pathogens, but valuable symbionts as well, so that more specific mechanisms of disease resistance would be favored by natural selection.

There is now good evidence that higher plants, including pea, rice, tomato, soybean, and *Medicago truncatula*, secrete various compounds that act like (mimic) bacterial QS signals (141). Such "signal-mimic" compounds may provide plants with important tools to disrupt or manipulate QS regulation in associated bacteria (8). The first QS signal-mimic compounds were discovered in a marine red alga, *Delisea pulchra* (63). The active compounds were shown to be a set of halogenated furanones that are structurally similar to AHLs, interact specifically with AHL receptors (91, 92), inhibit AHL-mediated QS in many bacteria (63), interfere with biofilm formation (66), and substantially alter the structure of natural bacterial communities on the algal surface in marine environments (77). Higher plants also produce signal-mimic compounds that can inhibit bacterial responses to an added AHL signal. However, in contrast to the strictly inhibitory activities of the furanone mimics from *Delisea*, many of the signal-mimics from higher plants stimulate AHL-induced QS behaviors (141). The plant compounds have not yet been chemically identified, but most have different solvent-partitioning properties than bacterial AHLs (141). Pea and *M. truncatula* appear to secrete at least a dozen different AHL signal-mimics (141; P. Gao, M. Teplitski, W.D. Bauer, unpublished). *M. truncatula* seedlings have also been reported (93) to produce compounds that inhibit the ability of *Vibrio harveyi* to respond to AI-2, its furanosyl borate diester QS signal (20). A broad range of bacterial species carry a homolog of the AI-2 synthase, LuxS, and produce AI-2 or similar substances that can activate QS-regulated luminescence in *V. harveyi* (99). It is not yet clear whether these species have a receptor for AI-2 and use AI-2 as a QS signal (147). If they do, then it appears that higher plants produce signal-mimic compounds that positively and negatively affect AI-2 as well as AHL-mediated QS systems in bacteria.

The QS signals used by bacterial pathogens in their attack on plant hosts might have the unintended side effect of alerting their host to impending invasion and triggering defense responses. This possibility was suggested by a recent proteomic analysis of the responses of *M. truncatula* to added AHLs. Exposing roots of *M. truncatula* to nanomolar or micromolar concentrations of 3-oxo-C_{12}HL from the opportunistic pathogen, *P. aeruginosa*, or 3-oxo-$C_{16:1}$HL from the N-fixing symbiont, *Sinorhizobium meliloti*, resulted in significant changes in the accumulation of over 150 different proteins of diverse function (93). The concentration of the added AHL, the structure of the AHL, and the length of exposure all affected the

accumulation of these proteins, suggesting that there may be considerable specificity to the responses. Exposure of the plant to AHLs also altered the secretion of AHL and AI-2 signal-mimic compounds by the roots. Thus, host plant detection of bacterial QS signals and the triggering of diverse host responses could be a relevant layer of interaction in many pathogenic and symbiotic associations.

CONCLUDING REMARKS AND PERSPECTIVES

The studies presented in this review illustrate how plant pathogenic bacteria use QS signals to regulate genes for epiphytic fitness, such as motility in *R. solanacearum*, antibiosis in *E. carotovora*, and UV light resistance in *X. campestris*, as well as those for major pathogenicity factors, including EPS in *P. stewartii*, *X. campestris*, and *R. solanacearum*, type III secretion systems in *P. stewartii* and *E. carotovora*, and exoenzyme production in *E. carotovora*, *X. campestris*, and *R. solanacearum*. QS regulation appears to be an important mechanism for making the transition from planktonic cells to aggregates, adjusting to the presence of other bacteria. A good example of this would be *R. solanacearum*, where the 3-OH PAME-dependent Phc pathway turns down motility, siderophore production, and salt tolerance, which are important in the free-living, low virulence state, and switches on production of EPS and cell wall–degrading enzymes, to initiate a high virulence state. In planta, some hosts may provide enough nutrients for initial growth of pathogens, permitting the population and signal buildup required for QS regulation of virulence factors such as *hrp* genes and degradative enzymes. Early expression of such virulence factors may be detrimental to the pathogen. For example, the pectic enzymes secreted by *E. carotovora* could release oligogalacturonide elicitors of plant defense responses before the pathogen has reached the critical mass needed to overwhelm local host barriers and defense responses. Similarly, the premature production of EPS slime by a vascular pathogen, such as *P. stewartii*, could block attachment and subsequent steps of host invasion. At this point, a rationale for QS regulation of the Hrp secretion system is not apparent.

Comparisons of phylogenetic trees constructed from sequences from the LuxI and LuxR protein families with those from 16S rDNA show that QS systems originated very early in evolution of the gram-negative Proteobacteria (65). Functional pairs of LuxI and LuxR-homologs appear to have coevolved together, but in many cases multiple pairs or individual member components seem to have been inherited horizontally (65). Although some traits are directly regulated by a LuxR homolog, e.g., carbenapem synthesis in *E. carotovora*, multifaceted phenotypes such as pathogenicity are indirectly controlled by complex hierarchial signaling cascades that probably arose by the addition of independently acquired regulatory genes. To this end, plant pathogens employ some unique or unusual QS schemes. For example, *P. stewartii* was the first bacterium shown to have a LuxR homolog that functions as a transcriptional repressor. *A. tumefaciens* uses AHL binding to stabilize the nascent receptor protein and has a lactonase for degrading the signal in stationary phase. *R. solanacearum* uses a unique fatty acid ester signal

molecule, and both *R. solanacearum* and *X. campestris* use two-component systems rather than LuxR homologs to mediate responses to their unique QS signals. In *R. solanacearum*, the PhcR response regulator posttranscriptionally affects levels of PhcA and, in *X. campestris*, RpfG somehow affects levels of cyclic di-GMP.

Our knowledge of QS regulation, signal receptors, and synthases in plant pathogens is still very incomplete. For example, additional LuxR-like regulators have been postulated, but not identified, for *P. stewartii*, *E. carotovora*, *R. solanacearum*, and *X. campestris*, and no global view of functions subject to QS regulation is available for any plant pathogen. Genomics and proteomics promise to rapidly expand our perspective of QS regulation in plant pathogenic species, as is occurring in model animal pathogens and plant symbionts. As we acquire this information, we must also begin to study how QS regulation actually works in planta, where diffusion of signals is an open question and where signal-mimics from the host and its responses to QS signals come into play. In the end, the complexities could be daunting. In all likelihood, the avirulent phenotype of QS mutants will rest not only on the coordinated expression of a few crucial genes, but also on the incremental and conditional contributions from many other genes. Which genes are crucial and how their expression is affected by QS regulation remain to be determined. How large does the local population of the pathogen have to be before it becomes "quorate"? Does QS turn genes on and off many times during infection as bacteria alternate between planktonic cells and aggregates or biofilms? When are traits such as EPS synthesis, exoenzyme production, and Hrp secretion expressed during infection and why? Do different QS-dependent genes have different quorate thresholds? Alternatively, does population density and/or confinement merely fulfill a prerequisite condition before other factors, such as specific plant signals or contact, can act?

Whatever mechanisms are involved, it is clear from mutant studies that phytopathogenic bacteria depend on the exchange of external QS signal molecules for normal infection and pathogenesis. Consequently, these signals present attractive targets for control of bacterial diseases through genetic engineering of plants. Research to date has focused on transgenic plants that produce either AHLs to jam pathogen signaling or trick them into revealing their presence too soon or AHL lactonases that degrade the signals. The preliminary finding that these plants are more disease resistant is very promising and again confirms the importance of QS regulation to pathogen attack. However, in terms of reducing plant disease through manipulation of QS, the challenge for future work is to make changes that are specific to particular pathogens, tissues, and developmental windows without also impacting beneficial microbes.

ACKNOWLEDGMENTS

The authors thank Steve Lindow for providing unpublished results and Isaac Barash, Arun Chatterjee, Tim Denny, Max Dow, Clay Fuqua, and Alan Poplawsky, who helped make this review more accurate, complete, and up to date. The authors

acknowledge support for their individual research programs from National Science Foundation grant MCB-0211687 to SVB; USDA NRI grants 35319-12637 to SvB, 2002-35319-11559 to WDB, and 2002-35319-11562 to DLC; USDA Agricultural Experiment Station grant CONSOO712 to SvB; University of Connecticut Research Foundation to SvB; United States-Israel Binational Research and Development Fund grant US-3265-01C to DLC; a Ohio Plant Biotechnology Consortium grant to WDB; and state and federal funds appropriated to the Ohio Agricultural Research and Development Center, The Ohio State University, and the University of Connecticut. This is scientific contribution #1993 of the Storrs Agricultural Experiment Station, Storrs, CT.

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LITERATURE CITED

1. Alt-Morbe J, Stryker JL, Fuqua C, Li PL, Farrand SK, Winans SC. 1996. The conjugal transfer system of *Agrobacterium tumefaciens* octopine-type Ti plasmids is closely related to the transfer system of an IncP plasmid and distantly related to Ti plasmid *vir* genes. *J. Bacteriol.* 178:4248–57
2. Andersson RA, Eriksson AR, Heikinheimo R, Mae A, Pirhonen M, et al. 2000. Quorum sensing in the plant pathogen *Erwinia carotovora* subsp. *carotovora*: the role of *expR(Ecc). Mol. Plant-Microbe Interact.* 13:384–93
3. Ausmees N, Mayer R, Weinhouse H, Volman G, Amikam D, et al. 2001. Genetic data indicate that proteins containing the GGDEF domain possess diguanylate cyclase activity. *FEMS Microbiol. Lett.* 204:163–67
4. Axelrood PE, Rella M, Schroth MN. 1988. Role of antibiosis in competition of *Erwinia* strains in potato infection courts. *Appl. Environ. Microbiol.* 54:1222–29
5. Bainton NJ, Stead P, Chhabra SR, Bycroft BW, Salmond GP, et al. 1992. *N*-(3-oxo-hexanoyl)-L-homoserine lactone regulates carbapenem antibiotic production in *Erwinia carotovora. Biochem. J.* 288:997–1004
6. Barber CE, Tang JL, Feng JX, Pan MQ, Wilson TJ, et al. 1997. A novel regulatory system required for pathogenicity of *Xanthomonas campestris* is mediated by a small diffusible signal molecule. *Mol. Microbiol.* 24:555–66
7. Bassler BL. 2002. Small talk. Cell-to-cell communication in bacteria. *Cell* 109:421–24
8. Bauer WD, Robinson JB. 2002. Disruption of bacterial quorum sensing by other organisms. *Curr. Opin. Biotechol.* 13:234–37
9. Beck von Bodman S, Farrand SK. 1995. Capsular polysaccharide biosynthesis and pathogenicity in *Erwinia stewartii* require induction by an *N*-acylhomoserine lactone autoinducer. *J. Bacteriol.* 177:5000–8
10. Beck von Bodman S, Majerczak DR, Coplin DL. 1998. A negative regulator mediates quorum-sensing control of exopolysaccharide production in *Pantoea stewartii* subsp. *stewartii. Proc. Natl. Acad. Sci. USA* 95:7687–92
11. Belanger C, Canfield ML, Moore LW, Dion P. 1995. Genetic analysis of nonpathogenic *Agrobacterium tumefaciens* mutants arising in crown gall tumors. *J. Bacteriol.* 177:3752–57
12. Braun EJ. 1982. Ultrastructural investigation of resistant and susceptible maize inbreds infected with *Erwinia stewartii. Phytopathology* 72:159–66
13. Brumbley SM, Carney BF, Denny TP.

1993. Phenotype conversion in *Pseudomonas solanacearum* due to spontaneous inactivation of PhcA, a putative LysR transcriptional regulator. *J. Bacteriol.* 175: 5477–87

14. Byers JT, Lucas C, Salmond GP, Welch M. 2002. Nonenzymatic turnover of an *Erwinia carotovora* quorum-sensing signaling molecule. *J. Bacteriol.* 184:1163–71
15. Cha C, Gao P, Chen YC, Shaw PD, Farrand SK. 1998. Production of acyl-homoserine lactone quorum sensing signals by gram-negative plant-associated bacteria. *Mol. Plant-Microbe Interact.* 11:1119–29
16. Chai Y, Zhu J, Winans SC. 2001. TrlR, a defective TraR-like protein of *Agrobacterium tumefaciens*, blocks TraR function in vitro by forming inactive TrlR:TraR dimers. *Mol. Microbiol.* 40:414–21
17. Chatterjee A, Cui Y, Liu Y, Dumenyo CK, Chatterjee AK. 1995. Inactivation of *rsmA* leads to overproduction of extracellular pectinases, cellulases, proteases in *Erwinia carotovora* subsp. *carotovora* in the absence of the starvation/cell density-sensing singal, *N*-(3-oxohexanoyl)-L-homoserine lactone. *Appl. Environ. Microbiol.* 61:1959–67
18. Chatterjee A, Cui YY, Chatterjee AK. 2002. RsmA and the quorum-sensing signal, N-[3-oxohexanoyl]-L-homoserine lactone, control the levels of *rsmB* RNA in *Erwinia carotovora* subsp. *carotovora* by affecting its stability. *J. Bacteriol.* 184:4089–95
19. Chatterjee S, Sonti RV. 2002. *rpfF* mutants of *Xanthomonas oryzae* pv. *oryzae* are deficient for virulence and growth under low iron conditions. *Mol. Plant-Microbe Interact.* 15:463–71
20. Chen X, Schauder S, Potier N, van Drosselaer A, Pelczer I, et al. 2002. Structural identification of a bacterial quorum-sensing signal containing boron. *Nature* 415:545–49
21. Chun W, Cui J, Poplawsky AR. 1997. Purification, characterization and biological role of a pheromone produced by *Xanthomonas campestris* pv. *campestris*. *Physiol. Mol. Plant Pathol.* 51:1–14
22. Clough SJ, Flavier AB, Schell MA, Denny TP. 1997. Differential expression of virulence genes and motility in *Ralstonia* (*Pseudomonas*) *solanacearum* during exponential growth. *Appl. Environ. Microbiol.* 63:844–50
23. Coplin DL, Frederick RD, Majerczak DR. 1992. New pathogenicity loci in *Erwinia stewartii* identified by random Tn5 mutagenesis and molecular cloning. *Mol. Plant-Microbe Interact.* 5:81–88
24. Coplin DL, Majerczak DR. 1990. Extracellular polysaccharide genes in *Erwinia stewartii*: directed mutagenesis and complementation analysis. *Mol. Plant-Microbe Interact.* 3:286–92
25. Costa JM, Loper JE. 1997. EcbI and EcbR: homologs of LuxI and LuxR affecting antibiotic and exoenzyme production by *Erwinia carotovora* subsp. *betavasculorum*. *Can. J. Microbiol.* 43:1164–71
26. Crossman L, Slater H, Williams P, Dow M. 2002. *Cell-cell signaling and pathogenicity gene expression in Xanthomonas campestris*. Presented at Soc. Gen. Microbiol. 150th Ordinary Meet., Warwick, UK
27. Cui Y, Madi L, Mukherjee A, Dumenyo CK, Chatterjee AK. 1996. The RsmA-mutants of *Erwinia carotovora* subsp. *carotovora* strain Ecc71 overexpress $hrpN_{Ecc}$ and elicit a hypersensitive reaction-like response in tobacco leaves. *Mol. Plant-Microbe Interact.* 9:565–73
28. Cui Y, Mukherjee A, Dumenyo CK, Chatterjee AK. 1999. *rsmC* of the soft-rotting bacterium *Erwinia carotovora* subsp. *carotovora* negatively controls extracellular enzyme and harpin(Ecc) production and virulence by modulating levels of regulatory RNA (*rsmB*) and RNA-binding protein (RsmA). *J. Bacteriol.* 181:6042–52
29. Cui YY, Chatterjee A, Liu Y, Dumenyo CK, Chatterjee AK. 1995. Identification

of a global repressor gene, *rsmA*, of *Erwinia carotovora* subsp. *carotovora* that controls extracellular enzymes, *N*-(3-oxohexanoyl)-L-homoserine lactone and pathogenicity of soft-rotting in *Erwinia* spp. *J. Bacteriol.* 177:5108–15

30. Davey ME, O'Toole GA. 2000. Microbial biofilms: from ecology to molecular genetics. *Microbiol. Mol. Biol. Rev.* 64:847–67
31. de Kievit TR, Iglewski BH. 2000. Bacterial quorum sensing in pathogenic relationships. *Infect. Immun.* 68:4839–49
32. Denny TP. 1995. Involvement of bacterial exopolysaccharides in plant pathogenesis. *Annu. Rev. Phytopathol.* 33:173–97
33. Denny TP. 2000. *Ralstonia solanacearum*—a plant pathogen in touch with its host. *Trends Microbiol.* 8
34. Dessaux Y, Petit A, Tempe J. 1992. Opines in *Agrobacterium* biology. In *Molecular Signals in Plant-Microbe Interaction*, ed. DPS Verma, pp. 109–36. Boca Raton, FL: CRC Press
35. Diggle SP, Winzer K, Lazdunski A, Williams P, Camara M. 2002. Advancing the quorum in *Pseudomonas aeruginosa*: MvaT and the regulation of *N*-acylhomoserine lactone production and virulence gene expression. *J. Bacteriol.* 184:2576–86
36. Dixon RA, Harrison MJ, Lamb CJ. 1994. Early events in the activation of plant defence responses. *Annu. Rev. Phytopathol.* 32:479–501
37. Dong YH, Gusti AR, Zhang Q, Xu JL, Zhang LH. 2002. Identification of quorum-quenching *N*-acyl homoserine lactonases from *Bacillus* species. *Appl. Environ. Microbiol.* 68:1754–59
38. Dong YH, Wang LH, Xu JL, Zhang HB, Zhang XF, Zhang LH. 2001. Quenching quorum-sensing-dependent bacterial infection by an *N*-acyl homoserine lactonase. *Nature* 411:813–17
39. Dong YH, Xu JL, Li XZ, Zhang LH. 2000. AiiA, an enzyme that inactivates the acyl-homoserine lactone quorum-sensing signal and attenuates the virulence of *Erwinia carotovora. Proc. Natl. Acad. Sci. USA* 97:3526–31
40. Dow JM, Daniels MJ. 1994. Pathogenicity determinants and global regulation of pathogenicity of *Xanthomonas campestris* pv. *campestris. Curr. Top. Microbiol. Immunol.* 192:29–41
41. Dow JM, Daniels MJ. 2000. *Xylella* genomics and bacterial pathogenicity to plants. *Yeast* 17:263–71
42. Dumenyo CK, Mukherjee A, Chun W, Chatterjee AK. 1998. Genetic and physiological evidence for the production of *N*-acyl homoserine lactones by *Pseudomonas syringae* pv. *syringae* and other fluorescent plant pathogenic *Pseudomonas* species. *Eur. J. Plant Pathol.* 104:569–82
43. Dunlap PV. 1999. Quorum regulation of luminescence in *Vibrio fischeri. J. Mol. Microbiol. Biotechnol.* 1:5–12
44. Dunny GM, Winans SC. 1999. Bacterial life: neither lonely nor boring. In *Cell-Cell Signaling in Bacteria*, ed. GM Dunny, SC Winans, pp. 1–5. Washington, DC: ASM Press
45. Eberl L. 1999. *N*-acyl homoserinelactone-mediated gene regulation in gram-negative bacteria. *Syst. Appl. Microbiol.* 22:493–506
46. Elasri M, Delorme S, Lemanceau P, Stewart G, Laue B, et al. 2001. Acyl-homoserine lactone production is more common among plant-associated *Pseudomonas* spp. than among soilborne *Pseudomonas* spp. *Appl. Environ. Microbiol.* 67:1198–209
47. Farrand SK. 1998. Conjugal plasmids and their transfer. In *The Rhizobiaceae: Molecular Biology of Model Plant-Associated Bacteria*, ed. HP Spaink, A Kondorosi, PJJ Hooykaas, pp. 199–223. Dordrecht: Kluwer
48. Farrand SK, Hwang I, Cook DM. 1996. The *tra* region of the nopaline-type Ti plasmid is a chimera with elements related

to the transfer system of RSF1010, RP4, and F. *J. Bacteriol.* 178:4233–47

49. Flavier AB, Clough SJ, Schell MA, Denny TP. 1997. Identification of 3-hydroxypalmitic acid methyl ester as a novel autoregulator controlling virulence in *Ralstonia solanacearum*. *Mol. Microbiol.* 26:251–59
50. Flavier AB, Ganova-Raeva LM, Schell MA, Denny TP. 1997. Hierarchical autoinduction in *Ralstonia solanacearum*: control of acyl-homoserine lactone production by a novel autoregulatory system responsive to 3-hydroxypalmitic acid methyl ester. *J. Bacteriol.* 179:7089–97
51. Flavier AB, Schell MA, Denny TP. 1998. An RpoS (σ^S) homologue regulates acylhomoserine lactone-dependent autoinduction in *Ralstonia solanacearum*. *Mol. Microbiol.* 28:475–86
52. Frederick RD, Ahmad M, Majerczak DR, Arroyo-Rodríguez AS, Manulis S, Coplin DL. 2001. Genetic organization of the *Pantoea stewartii* subsp. *stewartii hrp* gene cluster and sequence analysis of the *hrpA*, *hrpC*, *hrpN*, and *wtsE* operons. *Mol. Plant-Microbe Interact.* 14:1213–22
53. Fuqua C, Burbea M, Winans SC. 1995. Activity of the *Agrobacterium* Ti plasmid conjugal transfer regulator TraR is inhibited by the product of the *traM* gene. *J. Bacteriol.* 177:1367–73
54. Fuqua C, Eberhard A. 1999. Signal generation in autoinduction systems: synthesis of acylated homoserine lactones by LuxI-type proteins. See Ref. 44, pp. 211–30
55. Fuqua C, Greenberg EP. 1998. Self perception in bacteria: quorum sensing with acylated homoserine lactones. *Curr. Opin. Microbiol.* 1:183–89
56. Fuqua C, Greenberg EP. 2002. Listening in on bacteria: acyl-homoserine lactone signalling. *Nat. Rev. Mol. Cell Biol.* 3:685–95
57. Fuqua C, Parsek MR, Greenberg EP. 2001. Regulation of gene expression by cell-to-cell communication: acyl-homoserine lactone quorum sensing. *Annu. Rev. Genet.* 35:439–68
58. Fuqua C, Winans SC. 1996. Localization of OccR-activated and TraR-activated promoters that express two ABC-type permeases and the *tra* R gene of Ti plasmid pTiR10. *Mol. Microbiol.* 20:1199–210
59. Fuqua C, Winans SC, Greenberg EP. 1996. Census and consensus in bacterial ecosystems: the LuxR-LuxI family of quorum-sensing transcriptional regulators. *Annu. Rev. Microbiol.* 50:727–51
60. Fuqua WC, Winans SC. 1994. A LuxR-LuxI type regulatory system activates *Agrobacterium* Ti plasmid conjugal transfer in the presence of a plant tumor metabolite. *J. Bacteriol.* 176:2796–806
61. Fuqua WC, Winans SC, Greenberg EP. 1994. Quorum sensing in bacteria: the LuxR-LuxI family of cell density-responsive transcriptional regulators. *J. Bacteriol.* 176:269–75
62. Garg RP, Yindeeyoungyeon W, Gilis A, Denny TP, Van Der Lelie D, Schell MA. 2000. Evidence that *Ralstonia eutropha* (*Alcaligenes eutrophus*) contains a functional homologue of the *Ralstonia solanacearum* Phc cell density sensing system. *Mol. Microbiol.* 38:359–67
63. Givskov M, Nys Rd, Manefield M, Gram L, Maximilien R, et al. 1996. Eukaryotic interference with homoserine lactone-mediated prokaryotic signalling. *J. Bacteriol.* 178:6618–22
64. Gottesman S. 1995. Regulation of capsule synthesis: modification on the two-component paradigm by an accessory unstable regulator. In *Two-Component Signal Transduction*, ed. JA Hoch, T Silhavy, pp. 253–62. Washington, DC: ASM Press
65. Gray KM, Garey JR. 2001. The evolution of bacterial LuxI and LuxR quorum sensing regulators. *Microbiology* 147:2379–87
66. Hentzer M, Riedel K, Rasmussen TB, Heydorn A, Andersen JB, et al. 2002. Inhibition of quorum sensing in *Pseudomonas*

aeruginosa biofilm bacteria by a halogenated furanone compound. *Microbiology* 148:87–102

67. Holden MTG, Chhabra SR, de Nys R, Stead P, Bainton NJ, et al. 1999. Quorum-sensing cross talk: isolation and chemical characterization of cyclic dipeptides from *Pseudomonas aeruginosa* and other gram-negative bacteria. *Mol. Microbiol.* 33:1254–66
68. Hwang I, Li PL, Zhang L, Piper KR, Cook DM, et al. 1994. TraI, a LuxI homologue, is responsible for production of conjugation factor, the Ti plasmid *N*-acylhomoserine lactone autoinducer. *Proc. Natl. Acad. Sci. USA* 91:4639–43
69. Hwang I, Smyth AJ, Luo ZQ, Farrand SK. 1999. Modulating quorum sensing by antiactivation: TraM interacts with TraR to inhibit activation of Ti plasmid conjugal transfer genes. *Mol. Microbiol.* 34:282–94
70. Hyytiainen H, Montesano M, Palva ET. 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.* 14:931–38
71. Jackson DW, Suzuki K, Oakford L, Simecka JW, Hart ME, Romeo T. 2002. Biofilm formation and dispersal under the influence of the global regulator CsrA of *Escherichia coli*. *J. Bacteriol.* 184:290–301
72. Jones S, Yu B, Bainton NJ, Birdsall M, Bycroft BW, et al. 1993. The *lux* autoinducer regulates the production of exoenzyme virulence determinants in *Erwinia carotovora* and *Pseudomonas aeruginosa*. *EMBO J.* 12:2477–82
73. Kang Y, Liu H, Genin S, Schell MA, Denny TP. 2002. *Ralstonia solanacearum* requires type 4 pili to adhere to multiple surfaces and for natural transformation and virulence. *Mol. Microbiol.* 46:427–37
74. Kelm O, Kiecker C, Geider K, Bernhard F. 1997. Interaction of the regulator proteins RcsA and RcsB with the promoter of the operon for amylovoran biosynthesis in *Erwinia amylovora*. *Mol. Gen. Genet.* 256:72–83
75. Khmel IA, Veselova MA, Metlitskaya AZ, Klein S, Lipasova VA, et al. 2002. Synthesis of signaling N-acyl-homoserine-lactones participating in quorum sensing regulation in rhizospheric and soil-borne bacteria *Pseudomonas* and *Xanthomonas*. *Russ. J. Genet.* 38:467–69
76. Kinscherf TG, Willis DK. 1999. Swarming by *Pseudomonas syringae* B728a requires *gacS* (*lemA*) and *gacA* but not the acyl-homoserine lactone biosynthetic gene *ahlI*. *J. Bacteriol.* 181:4133–36
77. Kjelleberg S, Steinberg P. 2002. Defenses against bacterial colonisation of marine plants. In *Phyllosphere Microbiology*, ed. SE Lindow, E Poinar, V Elliott, pp. 152–72. St. Paul: APS Press
78. Kleerebezem M, Quadri LE. 2001. Peptide pheromone-dependent regulation of antimicrobial peptide production in Gram-positive bacteria: a case of multicellular behavior. *Peptides* 22:1579–96
79. Kòiv V, Mäe A. 2001. Quorum sensing controls the synthesis of virulence factors by modulating *rsmA* gene expression in *Erwinia carotovora* subsp. *carotovora*. *Mol. Genet. Genomics* 265:287–92
80. Koutsoudis M, Minogue TD, von Bodman SB. 2002. *Quorum sensing regulation of capsular polysaccharide synthesis in Pantoea stewartii subsp. stewartii and its relationship to surface attachment and biofilm formation. Abstr. #64475*. Presented at Gen. Meet. ASM, Salt Lake City
81. Latifi A, Foglino M, Tanaka K, Williams P, Lazdunski A. 1996. A hierarchical quorum-sensing cascade in *Pseudomonas aeruginosa* links the transcriptional activators LasR and RhIR (VsmR) to expression of the stationary-phase sigma factor RpoS. *Mol. Microbiol.* 21:1137–46
82. Leadbetter JR. 2001. Plant microbiology. Quieting the raucous crowd. *Nature* 411:748–49

83. Li P-L, Farrand SK. 2000. The replicator of the nopaline-type Ti plasmid pTiC58 is a member of the *repABC* family and is influenced by the TraR-dependent quorum-sensing regulatory system. *J. Bacteriol.* 182:179–88
84. Li P-L, Hwang I, Miyagi H, True H, Farrand SK. 1999. Essential components of the Ti plasmid *trb* system, a type IV macromolecular transporter. *J. Bacteriol.* 181:5033–41
85. Liu Y, Cui Y, Mukherjee A, Chatterjee AK. 1998. Characterization of a novel RNA regulator of *Erwinia carotovora* subsp. *carotovora* that controls production of extracellular enzymes and secondary metabolites. *Mol. Microbiol.* 29:219–34
86. Liu Y, Jiang G, Cui Y, Mukherjee A, Ma WL, Chatterjee AK. 1999. $kdgR_{Ecc}$ negatively regulates genes for pectinases, cellulase, protease, $Harpin_{Ecc,}$ and a global RNA regulator in *Erwinia carotovora* subsp. *carotovora. J. Bacteriol.* 181:2411–21
87. Loh J, Carlson RW, York WS, Stacey G. 2002. Bradyoxetin, a unique chemical signal involved in symbiotic gene regulation. *Proc. Natl. Acad. Sci. USA* 99:14446–51
88. Loh J, Pierson EA, Pierson LS III, Stacey G, Chatterjee A. 2002. Quorum sensing in plant-associated bacteria. *Curr. Opin. Plant Biol.* 5:1–6
89. Ma W, Cui Y, Liu Y, Dumenyo CK, Mukherjee A, Chatterjee AK. 2001. Molecular characterization of global regulatory RNA species that control pathogenicity factors in *Erwinia amylovora* and *Erwinia herbicola* pv. *gypsophilae. J. Bacteriol.* 183:1870–80
90. Mäe A, Montesano M, Kòiv V, Palva ET. 2001. Transgenic plants producing the bacterial pheromone *N*-acyl-homoserine lactone exhibit enhanced resistance to the bacterial phytopathogen *Erwinia carotovora. Mol. Plant Microbe-Interact.* 14:1035–42
91. Manefield M, de Nys R, Kumar N, Read R, Givskov M, et al. 1999. Evidence that halogenated furanones from *Delisea pulchra* inhibit acylated homoserine lactone (AHL)-mediated gene expression by displacing its receptor protein. *Microbiology* 145:283–91
92. Manefield M, Rasmussen TB, Henzter M, Andersen JB, Steinberg P, et al. 2002. Halogenated furanones inhibit quorum sensing through accelerated LuxR turnover. *Microbiology* 148:1119–27
93. Mathesius U, Mulders S, Gao M, Teplitski M, Caetano-Anolles G, et al. 2003. Extensive and specific responses of a eukaryote to bacterial quorum sensing signals. *Proc. Natl. Acad. Sci. USA* 100:14444–49
94. Matthysse AG, Yarnall H, Boles SB, McMahan S. 2000. A region of the *Agrobacterium tumefaciens* chromosome containing genes required for virulence and attachment to host cells. *Biochim. Biophys. Acta* 1490:208–12
95. McGowan S, Sebaihia M, Jones S, Yu B, Bainton N, et al. 1995. Carbapenem antibiotic production in *Erwinia carotovora* is regulated by CarR, a homologue of the LuxR transcriptional activator. *Microbiology* 141:541–50
96. McGowan SJ, Sebaihia M, Porter LE, Stewart GS, Williams P, et al. 1996. Analysis of bacterial carbapenem antibiotic production genes reveals a novel ß-lactam biosynthesis pathway. *Mol. Microbiol.* 22:415–26
97. Merighi M, Majerczak DR, Stover EH, Coplin DL. 2003. The HrpX/HrpY two-component system activates *hrpS* expression, the first step in the regulatory cascade controlling the Hrp regulon in *Pantoea stewartii* subsp. *stewartii. Mol. Plant-Microbe Interact.* 16:238–48
98. Michael B, Smith JN, Swift S, Heffron F, Ahmer BM. 2001. SdiA of *Salmonella enterica* is a LuxR homolog that detects mixed microbial communities. *J. Bacteriol.* 183:5733–42
99. Miller MB, Bassler BL. 2001. Quorum sensing in bacteria. *Annu. Rev. Microbiol.* 55:165–99

100. Minogue TD, Koutsoudis M, von Bodman SB. 2002. Quorum sensing modulates biofilm formation in *Pantoea stewartii* subsp. *stewartii*. *Phytopathology* 92:S57 (Abstr.)
101. Minogue TD, Wehland M, Berhard F, von Bodman SB. 2002. The autoregulatory role of EsaR, a quorum sensing regulator in *Pantoea stewartii* subsp. *stewartii*: evidence for a repressor function. *Mol. Microbiol.* 44:1625–35
102. More MI, Finger LD, Stryker JL, Fuqua C, Eberhard A, Winans SC. 1996. Enzymatic synthesis of a quorum-sensing autoinducer through use of defined substrates. *Science* 272:1655–58
103. Mukherjee A, Cui Y, Ma W, Liu Y, Chatterjee AK. 2000. *hexA* of *Erwinia carotovora* subsp. *carotovora* strain Ecc71 negatively regulates production of RpoS and *rsmB* RNA, a global regulator of extracellular proteins, plant virulence and the quorum sensing signal, *N*-(3-oxohexanoyl)-L-homoserine lactone. *Environ. Microbiol.* 2:203–15
104. Mukherjee A, Cui Y, Ma W, Liu Y, Ishihama A, et al. 1998. RpoS (sigma-S) controls expression of *rsmA*, a global regulator of secondary metabolites, harpin, and extracellular proteins in *Erwinia carotovora*. *J. Bacteriol.* 180:3620–34
105. Nasser W, Bouillant ML, Salmond G, Reverchon S. 1998. Characterization of the *Erwinia chrysanthemi expI-expR* locus directing the synthesis of two *N*-acylhomoserine lactone signal molecules. *Mol. Microbiol.* 29:1391–405
106. Nealson KH. 1977. Autoinduction of bacterial luciferase. Occurrence, mechanism and significance. *Arch. Microbiol.* 112:73–79
107. Novick RP, Muir TW. 1999. Virulence gene regulation by peptides in staphylococci and other Gram-positive bacteria. *Curr. Opin. Microbiol.* 2:40–45
108. Oger P, Kim KS, Sackett RL, Piper KP, Farrand SK. 1998. Octopine-type Ti plasmids code for a mannopine-inducible dominant-negative allele of *traR*, the quorum-sensing activator that regulates Ti plasmid conjugal transfer. *Mol. Microbiol.* 27:277–88
109. Parsek MR, Greenberg EP. 2000. Acyl-homoserine lactone quorum sensing in gram-negative bacteria: a signaling mechanism involved in associations with higher organisms. *Proc. Natl. Acad. Sci. USA* 97:8789–93
110. Parsek MR, Val DL, Hanzelka BL, Cronan JE, Greenberg EP. 1999. Acyl homoserine-lactone quorum-sensing signal generation. *Proc. Natl. Acad. Sci. USA* 96:4360–65
111. Pearson JP, Passador L, Iglewski BH, Greenberg EP. 1995. A second N-acylhomoserine lactone signal produced by *Pseudomonas aeruginosa*. *Proc. Natl. Acad. Sci. USA* 92:1490–94
112. Pesci EC, Iglewski BH. 1997. The chain of command in *Pseudomonas* quorum sensing. *Trends Microbiol.* 5:132–34
113. Pessi G, Williams F, Hindle Z, Heurlier K, Holden MT, et al. 2001. The global posttranscriptional regulator RsmA modulates production of virulence determinants and *N*-acylhomoserine lactones in *Pseudomonas aeruginosa*. *J. Bacteriol.* 183:6676–83
114. Pierson EA, Wood DW, Cannon JA, Blachere FM, Pierson LS III. 1998. Interpopulation signaling via *N*-acyl-homoserine lactones among bacteria in the wheat rhizosphere. *Mol. Plant-Microbe Interact.* 11:1078–84
115. Pierson LS III. 2000. Expanding the club: engineering plants to talk to bacteria. *Trends Plant Sci.* 5:89–91
116. Pierson LS III, Morello J. 2001. Positive and negative communication among rhizobacteria: effect on patterns of microbial gene expression. *Phytopathology* 91:S160 (Abstr.)
117. Pierson LS III, Wood DW, Beck von Bodman S. 1999. Quorum sensing in other plant-associated bacteria. See Ref. 44, pp. 101–15

118. Piper KR, Beck von Bodman S, Farrand SK. 1993. Conjugation factor of *Agrobacterium tumefaciens* regulates Ti plasmid transfer by autoinduction. *Nature* 362:448–50
119. Piper KR, Beck von Bodman S, Hwang I, Farrand SK. 1999. Hierarchical gene regulatory systems arising from fortuitous gene associations: controlling quorum sensing by the opine regulon in *Agrobacterium*. *Mol. Microbiol.* 32:1077–89
120. Pirhonen M, Flego D, Heikinheimo R, Palva ET. 1993. A small diffusible signal molecule is responsible for the global control of virulence and exoenzyme production in the plant pathogen *Erwinia carotovora*. *EMBO J.* 12:2467–76
121. Poetter K, Coplin DL. 1991. Structural and functional analysis of the *rcsA* gene from *Erwinia stewartii*. *Mol. Gen. Genet.* 229:155–60
122. Poplawsky AR, Chun W. 1997. *pigB* determines a diffusible factor needed for extracellular polysaccharide slime and anthomonadin production in *Xanthomonas campestris* pv. *campestris*. *J. Bacteriol.* 179:439–44
123. Poplawsky AR, Chun W. 2002. *Xanthomonas campestris* pv. *campestris* extracellular polysaccharide is required for natural host infection as well as virulence. *Phytopathology* 92:S66 (Abstr.)
124. Poplawsky AR, Chun W, Slater H, Daniels MJ, Dow JM. 1998. Synthesis of extracellular polysaccharide, extracellular enzymes, and xanthomonadin in *Xanthomonas campestris*: evidence for the involvement of two intercellular regulatory signals. *Mol. Plant-Microbe Interact.* 11:68–70
125. Poplawsky AR, Urban SC, Chun W. 2000. Biological role of xanthomonadin pigments in *Xanthomonas campestris* pv. *campestris*. *Appl. Environ. Microbiol.* 66:5123–27
126. Qin Y, Luo ZQ, Smyth AJ, Gao P, von Bodman S, Farrand SK. 2001. Quorum-sensing signal binding results in dimerization of TraR and its release from membranes into the cytoplasm. *EMBO J.* 19:5212–21
127. Rahme LG, Ausubel FM, Cao H, Dernkard E, Goumnerov BC, et al. 2000. Plants and animals share functionally common bacterial virulence factors. *Proc. Natl. Acad. Sci. USA* 97:8815–21
128. Reimmann C, Ginet N, Michel L, Keel C, Michaux P, et al. 2002. Genetically programmed autoinducer destruction reduces virulence gene expression and swarming motility in *Pseudomonas aeruginosa* PAO1. *Microbiology* 148:923–32
129. Riedel K, Hentzer M, Geisenberger O, Huber B, Steidle A, et al. 2001. *N*-acylhomoserine-lactone-mediated communication between *Pseudomonas aeruginosa* and *Burkholderia cepacia* in mixed biofilms. *Microbiology* 147:3249–62
130. Rodelas B, Lithgow JK, Wisniewski-Dye F, Hardman A, Wilkinson A, et al. 1999. Analysis of quorum-sensing-dependent control of rhizosphere-expressed (*rhi*) genes in *Rhizobium leguminosarum* bv. *viciae*. *J. Bacteriol.* 181:3816–23
131. Schell MA. 2000. Control of virulence and pathogenicity genes of *Ralstonia solanacearum* by an elaborate sensory array. *Annu. Rev. Phytopathol.* 38:263–92
132. Slater H, Alvarez-Morales A, Barber CE, Daniels MJ, Dow JM. 2000. A two-component system involving an HD-GYP domain protein links cell-cell signaling to pathogenicity gene expression in *Xanthomonas campestris*. *Mol. Microbiol.* 38:986–1003
133. Sledjeski DD, Gottesman S. 1996. Osmotic shock induction of capsule synthesis in *Escherichia coli* K-12. *J. Bacteriol.* 178:1204–6
134. Steidle A, Sigl K, Schuhegger R, Ihring A, Schmid M, et al. 2001. Visualization of *N*-acylhomoserine lactone-mediated cell-cell communication between bacteria colonizing the tomato rhizosphere. *Appl. Environ. Microbiol.* 67:5761–70

135. Stevens AM, Greenberg EP. 1997. Quorum sensing in *Vibrio fischeri*: essential elements for activation of the luminescence genes. *J. Bacteriol.* 179:557–62
136. Swiderska A, Berndtson AK, Cha M-R, Li L, Beaudoin GMJ III, et al. 2001. Inhibition of the *Agrobacterium tumefaciens* TraR quorum-sensing regulator: interactions with the TraM anti-activator. *J. Biol. Chem.* 276:49449–58
137. Swift S, Downie JA, Whitehead NA, Barnard AML, Salmond GPC, Williams P. 2001. Quorum sensing as a population-density-dependent determinant of bacterial physiology. *Adv. Microb. Physiol.* 45:199–270
138. Tan MW. 2002. Cross-species infections and their analysis. *Annu. Rev. Microbiol.* 56:539–65
139. Tang JL, Liu YN, Barber CE, Dow JM, Wootton JC, Daniels MJ. 1991. Genetic and molecular analysis of a cluster of *rpf* genes involved in positive regulation of synthesis of extracellular enzymes andpolysaccharide in *Xanthomonas campestris* pv. *campestris. Mol. Gen. Genet.* 226:409–17
140. Tans-Kersten J, Huang H, Allen C. 2001. *Ralstonia solanacearum* needs motility for invasive virulence on tomato. *J. Bacteriol.* 183:3597–605
141. Teplitski M, Robinson JB, Bauer WD. 2000. Plants secrete substances that mimic bacterial *N*-acyl homoserine lactone signal activities and affect population density-dependent behaviors in associated bacteria. *Mol. Plant-Microbe Interact.* 13:637–48
142. Vannini A, Volpari C, Gargioli C, Muraglia E, Cortese R, et al. 2002. The crystal structure of the quorum sensing protein TraR bound to its autoinducer and target DNA. *EMBO J.* 21:4393–401
143. Visick KL, McFall-Ngai MJ. 2000. An exclusive contract: specificity in the *Vibrio fischeri-Euptrymna scolopes* partnership. *J. Bacteriol.* 182:1779–87
144. Vojnov AA, Slater H, Newman MA, Daniels MJ, Dow JM. 2001. Regulation of the synthesis of cyclic glucan in *Xanthomonas campestris* by a diffusible signal molecule. *Arch. Microbiol.* 176:415–20
145. Watson WT, Minogue TD, Val DL, von Bodman S, Churchill ME. 2002. Structural basis and specificity of acyl-homoserine lactone signal production in bacterial quorum sensing. *Mol. Cell* 9:685–94
146. Welch M, Todd DE, Whitehead NA, McGowan SJ, Bycroft BW, Salmond GPC. 2000. *N*-acyl homoserine lactone binding to the CarR receptor determines quorum-sensing specificity in *Erwinia. EMBO J.* 19:631–41
147. Whitehead NA, Barnard AML, Slater H, SNJ, L. Salmond GPC. 2001. Quorum-sensing in Gram-negative bacteria. *FEMS Microbiol. Rev.* 25:365–404
148. Whitehead NA, Byers JT, Commander P, Corbett MJ, Coulthurst SJ, et al. 2002. The regulation of virulence in phytopathogenic *Erwinia* species: quorum sensing, antibiotics and ecological considerations. *Antonie van Leeuwenhoek J. Microbiol. Serol.* 81:223–31
149. Whitehead NA, Welch M, Salmond GPC. 2001. Silencing the majority. *Nat. Biotechnol.* 19:735–36
150. Whiteley M, Lee KM, Greenberg EP. 1999. Identification of genes controlled by quorum sensing in *Pseudomonas aeruginosa. Proc. Natl. Acad. Sci. USA* 96:13904–9
151. Whiteley M, Parsek MR, Greenberg EP. 2000. Regulation of quorum sensing by RpoS in *Pseudomonas aeruginosa. J. Bacteriol.* 182:4356–60
152. Wilkinson A, Danino V, Wisniewski-Dye F, Lithgow JK, Downie JA. 2002. *N*-acyl-homoserine lactone inhibition of rhizobial growth is mediated by two quorum-sensing genes that regulate plasmid transfer. *J. Bacteriol.* 184:4510–19
153. Wilson TJG, Bertrand N, Tang JL, Feng

JX, Pan MQ, et al. 1998. The *rpfA* gene of *Xanthomonas campestris* pv. *campestris*, which is involved in the regulation of pathogenicity factor production, encodes an aconitase. *Mol. Microbiol.* 28:961–50

154. Winans SC. 2002. Bacterial esperanto. *Nat. Struct. Biol.* 9:83–84
155. Winzer K, Falconer C, Garber NC, Diggle SP, Camara M, Williams P. 2000. The *Pseudomonas aeruginosa* lectins PA-IL and PA-IIL are controlled by quorum sensing and by RpoS. *J. Bacteriol.* 182:6401–11
156. Winzer K, Hardie KR, Williams P. 2002. Bacterial cell-to-cell communication: Sorry, can't talk now—gone to lunch! *Curr. Opin. Microbiol.* 5:216–22
157. Wisniewski-Dye F, Jones J, Chhabra SR, Downie JA. 2002. *raiIR* genes are part of a quorum-sensing network controlled by *cinI* and *cinR* in *Rhizobium leguminosarum. J. Bacteriol.* 184:1597–606
158. Withers H, Swift S, Williams P. 2001. Quorum sensing as an integral component of gene regulatory networks in Gram-negative bacteria. *Curr. Opin. Microbiol.* 4:186–93
159. Yamada Y, Nihira T. 1998. Microbial hormones and microbial chemical ecology. In *Comprehensive Natural Products Chemistry. Vol. 8*, ed. DHR Barton, K Nakanishi, pp. 377–413. Oxford, UK: Elsevier
160. Yates EA, Philipp B, Buckley C, Atkinson S, Chhabra SR, et al. 2002. *N*-acyl-homoserine lactones undergo lactonolysis in a pH-, temperature-, and acyl chain length-dependent manner during growth of *Yersinia pseudotuberculosis* and *Pseudomonas aeruginosa*. *Infect. Immun.* 70:5635–46
161. Zhang LH, Murphy PJ, Kerr A, Tate ME. 1993. *Agrobacterium* conjugation and gene regulation by *N*-acyl-L-homoserine lactones. *Nature* 362:446–48
162. Zhang RG, Pappas T, Brace JL, Miller PC, Oulmassov T, et al. 2002. Structure of a bacterial quorum-sensing transcription factor complexed with pheromone and DNA. *Nature* 417:971–74
163. Zhang Z, Pierson LS III. 2001. A second quorum-sensing system regulates cell surface properties but not phenazine antibiotic production in *Pseudomonas aureofaciens. Appl. Environ. Microbiol.* 67:4305–15
164. Zhu J, Oger PM, Schrammeijer B, Hooykaas PJJ, Farrand SK, Winans SC. 2000. The basis of crown gall tumorigenesis. *J. Bacteriol.* 182:3885–95
165. Zhu J, Winans SC. 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* 96:4832–37
166. Zhu J, Winans SC. 2001. The quorum-sensing transcriptional regulator TraR requires its cognate signaling ligand for protein folding, protease resistance, and dimerization. *Proc. Natl. Acad. Sci. USA* 98:1507–12

Annual Review of Phytopathology
Volume 41, 2003

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