

# Quorum-sensing in Gram-negative bacteria

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## Abstract

It has become increasingly and widely recognised that bacteria do not exist as solitary cells, but are colonial organisms that exploit elaborate systems of intercellular communication to facilitate their adaptation to changing environmental conditions. The languages by which bacteria communicate take the form of chemical signals, excreted from the cells, which can elicit profound physiological changes. Many types of signalling molecules, which regulate diverse phenotypes across distant genera, have been described. The most common signalling molecules found in Gram-negative bacteria are *N*-acyl derivatives of homoserine lactone (acyl HSLs). Modulation of the physiological processes controlled by acyl HSLs (and, indeed, many of the non-acyl HSL-mediated systems) occurs in a cell density- and growth phase-dependent manner. Therefore, the term ‘quorum-sensing’ has been coined to describe this ability of bacteria to monitor cell density before expressing a phenotype. In this paper, we review the current state of research concerning acyl HSL-mediated quorum-sensing. We also describe two non-acyl HSL-based systems utilised by the phytopathogens *Ralstonia solanacearum* and *Xanthomonas campestris*. © 2001 Federation of European Microbiological Societies. Published by Elsevier Science B.V. All rights reserved.

**Keywords:** Quorum-sensing; LuxR; LuxI; Bacterial signalling; Pheromone; Acyl homoserine lactone

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## 1. Introduction

Microorganisms are consistently subjected to a myriad of environmental stimuli. Such cues include changes in temperature, osmolarity, pH and nutrient availability. In response, bacteria have developed multiple systems that allow adaptation to these environmental fluctuations. For instance, two-component signal transduction phosphorelay schemes allow microorganisms to sense and respond to multiple environmental factors by the activation or repression of specific target genes [1]. Similarly, the expression of assorted sigma factors in response to various signals enables transcriptional specificity in bacteria [2]. Alterations to DNA topology, protein-mediated or otherwise, can also result in changes to the transcriptional profile of a microorganism [3,4].

A further layer of microbial sensing and response mechanisms has been recently uncovered in the form of cell-to-cell communication via the use of small signalling molecules. Once thought of as a rare phenomenon, restricted to a few obscure examples, it is now increasingly apparent that an extensive range of microorganisms has the ability to perceive and respond to the presence of neighbouring microbial populations. Numerous signalling molecule-mediated sensing and response pathways have now been defined and many fall within the scope of a form of regulation that is commonly known as quorum-sensing. The term quorum-sensing was first used in a review by Fuqua et al. [5]. The process relies on the production of a low-molecular-mass signalling molecule, the extracellular concentration of which is related to the population density of the producing organism. The signalling molecule can be sensed by cells and this allows the whole population to initiate a concerted action once a critical concentration (corresponding to a particular cell density) has been achieved.

Several chemical classes of microbially derived signalling molecule have now been identified. Broadly, these can be split into two main categories: (1) amino acids and short peptides, commonly utilised by Gram-positive bacteria [6–8] and (2) fatty acid derivatives, frequently utilised by Gram-negative bacteria. This review will summarise our current understanding of Gram-negative cell-to-cell signalling systems. Particular emphasis will be placed on the

wide range of quorum-sensing systems that employ *N*-acyl homoserine lactones (acyl HSLs) as the signalling molecule with which to control the expression of diverse physiological functions. This review will begin by revisiting the first phenotype in which regulation via such a quorum-sensing system was identified: the control of bioluminescence in *Vibrio fischeri*.

## 2. The paradigm of acyl HSL-based regulatory systems: the Lux system

When free-living in seawater (i.e. at low cell densities), *V. fischeri* is non-luminescent. However, when grown to high cell densities in the laboratory, a *V. fischeri* culture bioluminesces with a blue-green light. Interestingly, this organism commonly forms symbiotic relationships with some fish and squid species. The most studied example of such a symbiosis is that between *V. fischeri* and *Euprymna scolopes* [9,10]. This small squid lives in the shallow sand flats associated with coral reefs in the Hawaiian archipelago and is a nocturnal feeder. *Eu. scolopes* has a bioluminescent appearance in dark environments, due to the maintenance of high-density *V. fischeri* populations ( $10^{10}$ – $10^{11}$  cells ml<sup>−1</sup>) in a specialised light organ. This bioluminescent phenotype is exploited by the squid in order to perform a behavioural phenomenon called counter-illumination. At night the squid camouflages itself from predators residing below it by controlling the intensity of the light that it projects downward, thus eliminating a visible shadow created by moonlight. In return, *Eu. scolopes* provides the *V. fischeri* population with nutrients [11]. The mutual gain exacted from this particular symbiosis is emphasised by the fact that the presence of luminescent-competent *V. fischeri* cells in the light organ of juvenile squid is crucial for the correct development of this structure [12–15].

### 2.1. The molecular basis of bioluminescence regulation

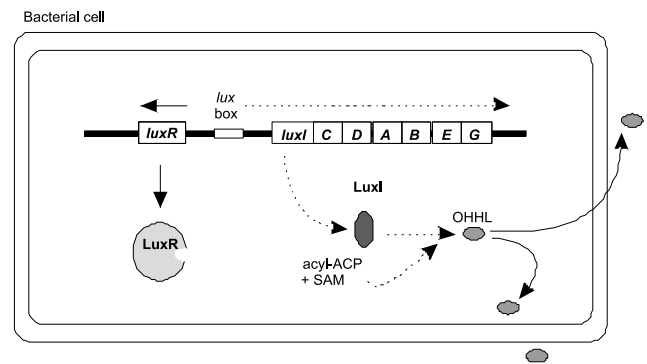
The bioluminescence gene cluster of *V. fischeri* consists of eight *lux* genes (*luxA–E*, *luxG*, *luxI* and *luxR*) which are arranged in two bi-directionally transcribed operons [16,17]. The products of the *luxI* and *luxR* genes function

as regulators of bioluminescence [18]. The *luxA* and *luxB* genes encode the subunits of the heterodimeric luciferase enzyme. This protein catalyses the oxidation of aldehyde and reduced flavin mononucleotide. The products of the reaction are a long-chain fatty acid, water and flavin mononucleotide. The simultaneous liberation of excess free energy, evident as blue-green light, results in the phenotype one associates with bioluminescent microorganisms [19–21]. *luxC–E* encode products that form a multienzyme complex, responsible for the synthesis of the aldehyde substrate utilised by the luciferase [22,23]. *luxG* encodes a probable flavin reductase [24]; the gene is followed by a transcriptional termination site [17].

When grown in liquid culture, populations of *V. fischeri* exhibit an initial lag phase before becoming bioluminescent. Many researchers addressed this observation and accounts, reciting much of this early work, are published elsewhere [25,26]. In short, it was eventually revealed that the delay was due to the need for the attainment of a threshold concentration of a *V. fischeri*-derived signalling molecule before bioluminescence could begin [27–29]. This process was originally defined by Nealson et al. as an autoinduction: the induction of genes due to the conditioning of growth medium by signals produced by a bacterial population [27]. Later work led to the isolation and chemical characterisation of this signal (termed autoinducer), its identity being revealed as *N*-(3-oxohexanoyl)-L-homoserine lactone (OHHL) [30]. More detailed studies have since shown that OHHL is freely diffusible, effective in activating bioluminescence at low concentrations (10 nM), and generated in *V. fischeri* by the *luxI* gene product [18,31].

The molecular basis of the initial stages of bioluminescence induction involves an interaction between OHHL and the transcriptional regulator protein, LuxR (Fig. 1) [16,18]. *V. fischeri* cells express *luxI* at a basal level when in low population densities, so the concentration of OHHL in the medium remains low. However, as the population density increases within the confines of a light organ (or laboratory flask), the concentration of OHHL in the environment also increases. Once a critical concentration of OHHL has been achieved (corresponding to a particular cell density or ‘quorum’ of bacteria), OHHL is thought to bind to LuxR. The protein is subsequently activated, probably by the OHHL-mediated induction of a conformational change in the protein. It is believed that activated LuxR then binds to a 20-bp DNA element of dyad symmetry, known as the *lux* box, which lies around 40 bp upstream of the transcriptional start site of *luxI* [32–34]. From there, the protein induces transcription of *lux-ICDABEG*. This thereby increases the cellular levels of mRNA transcripts encoding both bioluminescence and OHHL synthesis functions, resulting in increased levels of both light output and acyl HSL production. The newly produced diffusible OHHL molecules are able to activate more LuxR protein carried by cells within the *V. fischeri*

### A. Low Cell Density



### B. High Cell Density

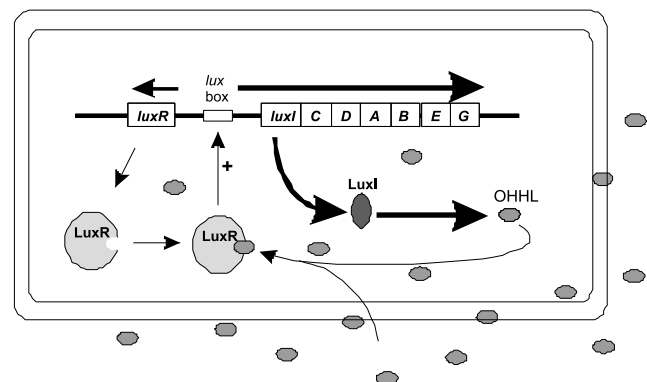


Fig. 1. The regulation of bioluminescence in *V. fischeri*: the quorum-sensing paradigm. A: At low cell density, transcription of the genes for bioluminescence (*luxICDABEG*) is weak and insufficient for light emission due to low levels of OHHL. B: At high cell density, a critical concentration of OHHL is reached. OHHL binds to LuxR and stimulates transcription of *luxICDABEG*, leading to rapid amplification of the OHHL signal and emission of light.

population of the light organ. Thus, the autoinduction ensures that bioluminescence and signalling molecule production continue. Consistent with this, the *V. fischeri* cells contained within the densely populated light organs of *Eu. scolopes* have been predicted to collectively produce an effective OHHL concentration of at least 100 nM [35]. As the autoinduction mechanism is not initiated until a population has achieved a particular cell density (hence quorum-sensing), individual *V. fischeri* cells avoid expending the considerable amount of energy required for the physiological luxury of light emission when not host-associated.

In the presence of low levels of OHHL, LuxR serves to induce transcription of *luxR* [36,37]. However, when OHHL is abundant, activated LuxR represses transcription of *luxR* [36,38]. The mechanism of this autorepression is unknown but it is dependent upon the presence of a *lux* box-type element located within *luxD* [39]. It may function to self-limit autoinduction of bioluminescence.

## 2.2. Modulation of bioluminescence

As reviewed elsewhere [40,41], non-quorum-sensing-related regulatory factors impinge on the expression of both the rightward and leftward *lux* operons of *V. fischeri*. This fact is in harmony with arrangements from other microorganisms where the integration of quorum-sensing within a network of global regulatory systems is commonplace. In *V. fischeri*, activated cAMP receptor protein (CRP) positively regulates the expression of *luxR* [42]. Additionally, the presence of a further *V. fischeri*-derived acyl HSL, distinct from OHHL, is now recognised to have a regulatory effect on light output. This signalling molecule, which has been identified as *N*-octanoyl-L-homoserine lactone (OHL) [43], is believed to bind to LuxR when *V. fischeri* populations are at low cell densities. This competitively inhibits the association between OHHL and LuxR and effectively prevents the premature induction of bioluminescence at early stages of growth [44,45]. A gene, designated *ainS*, encodes the product that directs the synthesis of OHL in *V. fischeri* [43,46,47]. AinS shares no sequence identity with LuxI and is believed to constitute a member of a second group of acyl HSL synthase proteins (Section 3.2.1).

## 2.3. Novel quorum-sensing-regulated processes in *V. fischeri*

New lines of evidence now suggest that bioluminescence is not the only phenotype regulated by the LuxRI quorum-sensing system of *V. fischeri*. Indeed, a two-dimensional polyacrylamide gel electrophoresis (2D PAGE) protein profile of a *V. fischeri* strain defective in *luxI* differs from the profile obtained using the same strain grown in the presence of OHHL. The latter exhibits increased expression of eight Qsr (quorum-sensing regulated) proteins [45]. Amino-terminal sequence analysis of the eight Qsr proteins revealed that at least four of them were distinct from LuxRICDABEG. As might be expected, the promoter regions of the genes encoding two of the Qsr proteins contained *lux* box-type elements. One of the proteins (designated QsrP) is seemingly important for the colonisation of squid light organs [45].

## 3. The molecular interactions behind quorum-sensing

By the mid-1980s, the core components of quorum-sensing regulation in *V. fischeri* had been identified and a molecular mechanism had been proposed. Although Greenberg et al. had also shown that signalling molecules (presumed to be similar to OHHL) were produced by

many other marine bacteria [48], many in the scientific community still viewed the whole process as little more than an interesting means of gene regulation, performed by an esoteric light-emitting marine bacterial species. However, this opinion changed rapidly when, in the early 1990s, independent research groups began to uncover regulatory systems in various other Gram-negative bacterial species that were homologous to the LuxRI system of *V. fischeri*. These systems utilised proteins that shared sequence similarity to LuxRI and signalling molecules that belonged to the same class of chemicals as OHHL. Indeed, there are now over 20 LuxR and LuxI homologues, from multiple microorganisms, in the sequence databases (Figs. 2 and 3). These proteins are involved in the quorum-sensing regulation of assorted physiological functions, as will be illustrated in detail later in this review. However, Sections 3.1 and 3.2 will first describe the proteins responsible for the synthesis of the acyl HSL signal and the mechanism by which this message is converted into a response via LuxR and its respective homologues.

### 3.1. The LuxR family of transcriptional regulatory proteins

Many of the molecular genetic analyses of LuxR have been performed in an *Escherichia coli* background. Such studies on the structure and function of the protein have offered an insight into the mechanisms involved in the activation of quorum-sensing systems by LuxR and its homologues. LuxR is a 250-amino acid polypeptide [49,50] that requires the presence of the GroESL molecular chaperones to enable folding into an active form [51–53]. Indeed, it has been proposed that in the absence of these protein chaperones the instability of LuxR may render it inactive [52]. LuxR does not contain a membrane-spanning domain but it has been shown to localise to the cytoplasmic face of the inner membrane [54]. Amino acid sequence alignments of LuxR with its homologues reveal them to be surprisingly disparate in terms of sequence identity (18–25%). Only five residues are completely conserved in all of the LuxR homologues for which sequence data are currently available (see Fig. 2).

The mapping of molecular functions to LuxR was originally based on a series of early experiments that involved the deletion or alteration of amino acids within the protein. Such studies demonstrated that LuxR is a modular polypeptide. The amino-terminal two-thirds of LuxR are thought to contain a region that acts as the acyl HSL binding domain. This assumption stems from the observation that amino acid changes in this region of LuxR result in a protein that requires higher concentrations of OHHL for induction of bioluminescence compared to the wild-

Fig. 2. A multiple sequence alignment of LuxR homologues. The sequences were aligned using the Clustal alignment algorithm. Absolutely conserved residues are shaded black and indicated under the sequences. Grey shading indicates residues present in at least 60% of the 29 sequences. See text for details of the origin of each LuxR protein.

AhyR	100	120	140
AsaR	100	120	140
CarR(Ecc)	100	120	140
CarR(Ser)	100	120	140
CepR	100	120	140
CinR	100	120	140
EchR	100	120	140
Esar	100	120	140
ExpR(Ecc)	100	120	140
ExpR(Ech)	100	120	140
LacR	100	120	140
LuxR	100	120	140
PhzR(Pau)	100	120	140
PhzR(Pfl)	100	120	140
RaiR	100	120	140
RhlR	100	120	140
SdiA(Eco)	100	120	140
SdiA(Sty)	100	120	140
SolR	100	120	140
Trar(nop)	100	120	140
Trar(Oct)	100	120	140
VanR	100	120	140
YenR	100	120	140
YpsR	100	120	140
YtbR	100	120	140
AhyR	160	180	200
AsaR	160	180	200
CarR(Ecc)	160	180	200
CarR(Ser)	160	180	200
CepR	160	180	200
CinR	160	180	200
EchR	160	180	200
Esar	160	180	200
ExpR(Ecc)	160	180	200
ExpR(Ech)	160	180	200
LacR	160	180	200
LuxR	160	180	200
PhzR(Pau)	160	180	200
PhzR(Pfl)	160	180	200
RaiR	160	180	200
RhlR	160	180	200
SdiA(Eco)	160	180	200
SdiA(Sty)	160	180	200
SolR	160	180	200
Trar(nop)	160	180	200
Trar(Oct)	160	180	200
VanR	160	180	200
YenR	160	180	200
YpsR	160	180	200
YtbR	160	180	200
AhyR	260	280	300
AsaR	260	280	300
CarR(Ecc)	260	280	300
CarR(Ser)	260	280	300
CepR	260	280	300
CinR	260	280	300
EchR	260	280	300
Esar	260	280	300
ExpR(Ecc)	260	280	300
ExpR(Ech)	260	280	300
LacR	260	280	300
LuxR	260	280	300
PhzR(Pau)	260	280	300
PhzR(Pfl)	260	280	300
RaiR	260	280	300
RhlR	260	280	300
SdiA(Eco)	260	280	300
SdiA(Sty)	260	280	300
SolR	260	280	300
Trar(nop)	260	280	300
Trar(Oct)	260	280	300
VanR	260	280	300
YenR	260	280	300
YpsR	260	280	300
YtbR	260	280	300



Fig. 3. A multiple sequence alignment of LuxI homologues. The sequences were aligned using the Clustal alignment algorithm. Absolutely conserved residues are shaded black and indicated under the sequences. Grey shading indicates residues present in at least 60% of the 27 sequences. See text for details of the origin of each LuxI protein.

type protein [55,56]. Residues thought to be important for binding acyl HSL have been identified [55,56]. Another study demonstrated that when the amino-terminal half of LuxR is overexpressed in *E. coli*, the organism is rendered capable of sequestering radioactive OHHL [57]. This is not evident when LuxR truncates, missing portions of their amino-terminal region, are overexpressed in the same organism.

It is perhaps surprising that so few studies have been published that validate the original proposal that a direct interaction between LuxR and acyl HSL molecules actually takes place. However, work using LuxR homologues from organisms other than *V. fischeri* has been far more successful in this respect. Indeed, two reports have documented how TraR (a LuxR homologue from *Agrobacterium tumefaciens*) can be purified as a complex with its cognate acyl HSL [58,59]. Each TraR polypeptide associates with one molecule of ligand. Elsewhere, Welch et al. monitored the intrinsic fluorescence of tryptophan residues within the putative acyl HSL binding region of CarR (a LuxR homologue from *Erwinia carotovora* subspecies *carotovora*) and showed that this was quenched in the presence of acyl HSL molecules [60]. As with TraR, CarR was calculated to bind its cognate acyl HSL with a stoichiometric ratio of one molecule of protein to one molecule of acyl HSL.

One of the proposed consequences of the interaction between many LuxR homologues and their cognate acyl HSLs is promotion of multimerisation of the proteins [61]. This has been demonstrated in *A. tumefaciens*, from which, when grown in the presence of acyl HSL, purified wild-type TraR is found to be in a predominantly dimeric configuration [59,62]. Removal of the acyl HSL causes the TraR dimers to dissociate [59,62]. Indeed, further results from an assay designed to measure the multimerisation properties of proteins illustrated that dimerisation of both TraR and LuxR was dependent upon the presence of acyl HSL molecules [59]. Similarly, in the presence of its cognate ligand, CarR appears to multimerise [60]. Analysis of heterodimers, formed between N- and C-terminal deletion mutants of TraR and His<sub>6</sub>-tagged TraR, indicates that a domain mapping to the middle of the protein (residues 49–156) is critical for its dimerisation [59]. Genetic analysis of deletion mutants also indicates that the dimerisation domain of LuxR maps to a similar region [61]. The importance of dimerisation is underlined by the fact that when defective alleles of LuxR or TraR are co-expressed with their wild-type counterparts, a dominant-negative effect on the level of transcriptional activation of gene expression is observed [61,63].

The carboxy-terminal third of LuxR contains a region

containing a helix-turn-helix motif that is important for the DNA binding activity of the protein [64]. In agreement with this, specific residues that are critical for DNA binding have been recently identified via the generation and analysis of a bank of LuxR polypeptides carrying alanine substitution mutations within the carboxy-terminal third of the protein [65,66]. Interestingly, a truncated LuxR molecule, lacking its amino-terminal region (and therefore designated LuxRΔN), has been shown to activate *lux* genes in an OHHL-independent manner [67]. Although results from work using LuxRΔN should be assessed in the knowledge that this protein may not behave in the same way as its full-sized progenitor protein (as described later), this finding would seem to verify that the DNA binding domain of LuxR is contained within LuxRΔN. It also indicates that the amino-terminal region of LuxR plays a role in retaining LuxR in an inactive conformation in the absence of OHHL. This is probably achieved via structural occlusion of the aforementioned multimerisation and/or DNA binding domains of this protein [61,67]. It is therefore likely that the interaction between OHHL and LuxR serves to free the amino-terminal region from performing such an inhibitory function, effectively activating the protein. To this end, point mutations capable of activating LuxR in the absence of OHHL have been mapped to locations throughout the whole protein [68,69]. This suggests that important amino acid interactions exist within the individual proteins that are responsible for the maintenance of LuxR and its homologues in an inactive form. It may be these very suppressive interactions that acyl HSL binding helps to neutralise in this group of proteins. Studies on TraR and CarR have both offered evidence to show that when these LuxR homologues bind their appropriate ligand, a conformational change in the proteins ensues [59,60].

### 3.1.1. DNA binding

As stated earlier, the regions of DNA to which LuxR binds are known as *lux* boxes. These 20-bp inverted repeat sequences are located around 40 bp upstream of LuxR-regulated genes. Unfortunately, due to the difficulty of obtaining purified full-length LuxR, evidence to demonstrate DNA binding by this protein has not been readily forthcoming. However, recently Egland and Greenberg have utilised artificial promoters with a *lux* box located downstream of the –35 promoter element of a *lacZ* cassette to demonstrate (via the repression of β-galactosidase synthesis) that, when expressed in *E. coli*, LuxR can bind to DNA in a manner that is conditional upon the presence of OHHL [70].

*lux* box-type sequences have been identified upstream of

quorum-sensing-regulated genes in many other organisms (although it is not the case that all quorum-sensing-regulated genes are preceded by *lux* box-type sequences). Data to illustrate DNA binding by at least four LuxR homologues have been presented in the literature. In fact, work that definitively illustrated that a LuxR homologue bound to *lux* box-type sequences and that the presence of signalling molecule was required to do so was first done with TraR. DNA band shift assays and DNase I footprinting experiments showed that, in vitro, ligand-activated TraR dimers bind specifically to a *lux* box-type sequence, known as a *tra* box [58,59,62]. Moreover, Luo and Farrand created *lacZ* reporter constructs that were under the control of artificial promoters with *tra* boxes located downstream of their –35 promoter element and demonstrated that, as was shown more recently with LuxR, TraR could function as a repressor of  $\beta$ -galactosidase activity [63]. Such activity was dependent upon the helix-turn-helix domain at the C-terminus of TraR [63]. Together, these studies show that disruption of the *tra* box sequence abolishes TraR binding activity. Similarly, they also show that DNA binding activity of TraR is dependent upon the prior activation of the protein by its cognate acyl HSL [58,59,62,63].

### 3.1.2. *LuxR* homologues as activators of transcription

The previously mentioned deletion derivative of LuxR (LuxRΔN), in conjunction with RNA polymerase, has the capacity to activate transcription of the *lux* operon in vitro [71]. Such activity is dependent upon the RNA polymerase having a functional  $\alpha$ -subunit C-terminal domain ( $\alpha$ CTD) [72]. Further in vivo studies have indicated that activation of the *lux* operon by both LuxRΔN and LuxR involves an interaction with the RNA polymerase  $\alpha$ CTD and that conservation of the location of the *lux* box is critical for transcriptional activation to ensue [34,72]. A model has therefore been proposed whereby full-length LuxR interacts with the  $\alpha$ CTD of RNA polymerase in order to promote recruitment of the RNA polymerase to the *lux* promoter [72]. The positioning of the *lux* box (centred at –42.5 with respect to the *luxI* transcriptional start site) and the fact that both half-sites of the 20-bp repeat sequence are required for LuxR-dependent activation of *luxI* transcription have also led workers to predict that LuxR may function as an ambidextrous activator [34]. Such activators characteristically make contact with the  $\alpha$ CTD of RNA polymerase upstream of their binding site as well as making contact with other regions of the protein downstream of their binding site [73]. Single amino acid substitution mutants of LuxR that bind DNA but do not activate transcription (positive-control mutants) have been mapped to the carboxy-terminal half of the protein [65]. On the basis of sequence comparisons with other proteins, these particular substitutions are predicted to be of surface-exposed residues. One particular substitution is predicted to be in a tryptophan residue that might interact with the  $\alpha$ CTD of RNA polymerase [65].

In vitro studies have shown that, although LuxRΔN can bind alone to a DNA region upstream of the *lux* box, it can only bind to the *lux* box in the presence of RNA polymerase [71,74]. The protein purifies as a monomer and seems to interact with DNA as a monomer [74]. Egland and Greenberg have also shown recently that, in vivo, LuxRΔN appears to bind DNA in a different manner from that of full-length LuxR [70]. In comparison, TraR purifies as a dimer and binds to the *tra* box sequence in the absence of RNA polymerase [58,59]. Zhu and Winans have also demonstrated that this protein can activate transcription from promoters of two different genes in vitro [58]. Moreover, all positive-control mutants of TraR isolated to date map to the N-terminal half of the protein [63]. This indicates that residues within this region are important for transcriptional activation of TraR. One would predict that LuxR and TraR might use the same mechanism to activate transcription. If this is so, it is difficult to interpret how LuxRΔN can activate transcription whilst being without the corresponding N-terminal residues, shown to be important for TraR activator activity. Thus, the dependence of LuxRΔN upon the presence of RNA polymerase before it can bind to the *lux* box in vitro now seems likely to be a peculiarity of this shortened protein (due to it having a reduced affinity for the *lux* regulatory DNA [65,66]). Similarly, the true mechanism by which members of this family of proteins induce transcription may well differ from the model assembled from data garnered from studies using LuxRΔN.

### 3.1.3. *LuxR* family subgroups

From the evidence described above, a widely accepted picture of the processes involved in the activation of TraR and LuxR emerges. The respective protein firstly interacts with its cognate acyl HSL and this promotes a conformational change that allows the protein to dimerise. Such dimers are then free to bind to relevant DNA promoter sequences and enact their transcriptional regulatory processes in conjunction with RNA polymerase. However, studies performed on other LuxR homologues indicate that this pathway is not conserved in all members of this group of proteins. Indeed, CarR and ExpR (a LuxR homologue from *Erwinia chrysanthemi*) are both capable of binding to promoter sequences of target genes in the absence of acyl HSL [60,75,76]. As such, it is interesting to note that CarR dimerises in the absence of acyl HSL and multimerises in the presence of the molecule [60]. EsaR (from *Pantoea stewartii*) behaves in a similar manner to CarR in that it also appears to form dimers in the absence of its cognate acyl HSL [59]. In the case of both CarR and ExpR, the presence of acyl HSLs alters the DNA binding profiles of the two proteins. Thus, as first proposed by Qin et al., there may be at least two subgroups within the LuxR family of proteins – those that bind DNA in the absence of acyl HSLs and those that require the presence of these ligands to enable initiation of their DNA binding

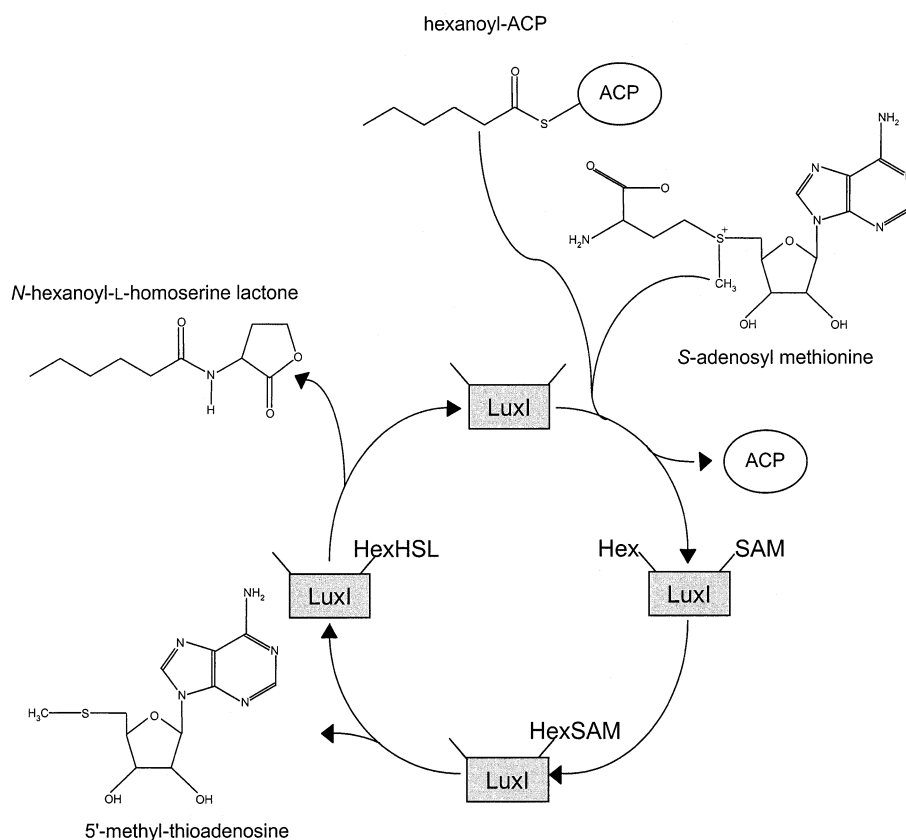


Fig. 4. A putative scheme for HHL synthesis, catalysed by LuxI. SAM binds to the active site on LuxI, and the hexanoyl group is transferred from the appropriately charged ACP. The hexanoyl group forms an amide bond with the amino group of SAM. 5'-Methylthioadenosine is released, and a lactonisation reaction results in the synthesis of HHL [78].

activity [59]. With the finding that many LuxR homologues can seemingly act as transcriptional repressors rather than activators (as will be highlighted during this review), further subgroups may well exist within this family.

### 3.2. The LuxI family of acyl HSL synthase proteins

Although it was recognised from an early stage that LuxI homologues were almost certainly responsible for the synthesis of acyl HSLs, it was not until 1996 that two separate groups offered the first direct evidence for the enzymatic activity of this group of proteins. Moré et al. showed that purified hexahistidyl-TraI (the hexahistidyl conjugate of the LuxI homologue from *A. tumefaciens*) could catalyse the *in vitro* synthesis of its major acyl HSL when supplied with the appropriate substrates [77]. Similarly, Schaefer et al. purified a maltose binding protein–LuxI fusion polypeptide and showed that this was capable of catalysing the synthesis of acyl HSL molecules [78].

The eventual elucidation of the chemical composition of acyl HSL molecules allowed workers to hypothesise that the substrates required for the biological synthesis of such compounds would originate from two distinct classes. It was believed that the acyl chain would probably be de-

rived from fatty acid metabolism whereas the HSL moiety would originate from an amino acid source. Initial work demonstrated that a crude extract of *V. fischeri* cells, when supplemented with *S*-adenosylmethionine (SAM) and a fatty acid derivative, was capable of synthesising OHHL [79]. From this observation, it was suggested that SAM was the amino acid substrate necessary for the synthesis of acyl HSLs. Later work, using amino acid-starved *E. coli* auxotrophs carrying plasmid-borne *luxI*, demonstrated a requirement for methionine or SAM during LuxI-mediated synthesis of OHHL [80]. Inhibition of the methionine adenosyltransferase reaction (which converts methionine to SAM) blocked methionine-dependent OHHL production in this study. Further *in vitro* investigations have demonstrated the necessity for SAM during the synthesis of acyl HSLs by purified LuxI and some of its homologues [77,78,81–83]. Val and Cronan also found that decreasing the intracellular levels of SAM in *E. coli* cells expressing TraI resulted in a significant reduction of acyl HSL synthesis [84]. Thus, it is now widely accepted that SAM serves as the main amino acid substrate during acyl HSL synthesis by LuxI homologues.

Eberhard et al. suggested that 3-oxohexanoyl coenzyme A (CoA) or the acyl carrier protein (ACP) adduct of 3-oxohexanoic acid were the most likely donors of the

fatty acid side chain of LuxI-derived OHHL [79]. Later studies demonstrated that acyl HSL synthesis was unaffected in *E. coli* mutants expressing TraI, but blocked in  $\beta$ -oxidative fatty acid degradation [84]. However, when fatty acid synthesis was inhibited in these cells, acyl HSL synthesis was also blocked. This correlated with work by Hoang and Schweizer who showed that the levels of acyl HSLs produced by a strain of *Pseudomonas aeruginosa* carrying a mutation in the *fabI* gene (which encodes a protein involved in fatty acid synthesis) were severely reduced [85]. Together, these findings offer in vivo evidence that the fatty acid moiety of acyl HSLs is derived from cellular pools of acylated ACP substrates rather than from pools of acylated CoA substrates. Accordingly, in vitro studies have also shown that LuxI and TraI utilise the appropriately charged ACP species, and not CoA derivative, during acyl HSL synthesis [77,78]. It was reported that a CoA derivative could be used as the donor for the side chain of the acyl HSL synthesised by RhlI (a LuxI homologue from *P. aeruginosa*) [81,83]. However, it would seem that the enzyme preferentially utilises the appropriate acylated ACP for this purpose [82,83].

On the basis of the previously described findings, a sequential model for acyl HSL synthesis by LuxI homologues has been described [78,83]. It has been proposed that SAM binds to the active site of the enzyme and that the appropriate acyl group is transferred to this complex from a charged ACP. The acyl group then forms an amide bond with the amino group of SAM. Subsequent lactonisation results in the synthesis of the acyl HSL and

the by-product, 5'-methylthioadenosine (as shown in Fig. 4).

LuxI homologues are around 200 amino acids in size (Fig. 3). Random and site-specific mutagenesis studies of LuxI and RhlI have revealed that the disruption of certain conserved residues within the amino-terminal half of these proteins leads to the loss or severe reduction of synthase activity [86,87]. Conserved amino acids in the carboxy-terminal half of LuxI were thought to be necessary for acyl ACP selection [86]. However, when mutations were made in the corresponding residues in RhlI, a similar loss of activity did not occur [87].

### 3.2.1. Novel non-LuxI-type acyl HSL synthase proteins

As revealed earlier, LuxI homologues are not the only type of protein that are capable of synthesising acyl HSLs. Indeed, AinS and LuxLM (from *Vibrio harveyi*) constitute another family of acyl HSL synthases. Moreover, a protein (HdtS) that is unrelated to members of the LuxI and LuxLM/AinS families has very recently been shown to direct acyl HSL synthesis in *Pseudomonas fluorescens* F113 [88]. AinS resembles LuxI homologues in its biochemical specificity as it has been shown to utilise SAM and octanoyl-ACP for the synthesis of OHL [47]. However, unlike LuxI homologues, AinS can also efficiently use the appropriately charged acyl-CoA derivative instead of octanoyl-ACP as a substrate. The substrate requirements of HdtS have yet to be elucidated, but it does share sequence similarity with members of a family of acyltransferase enzymes.

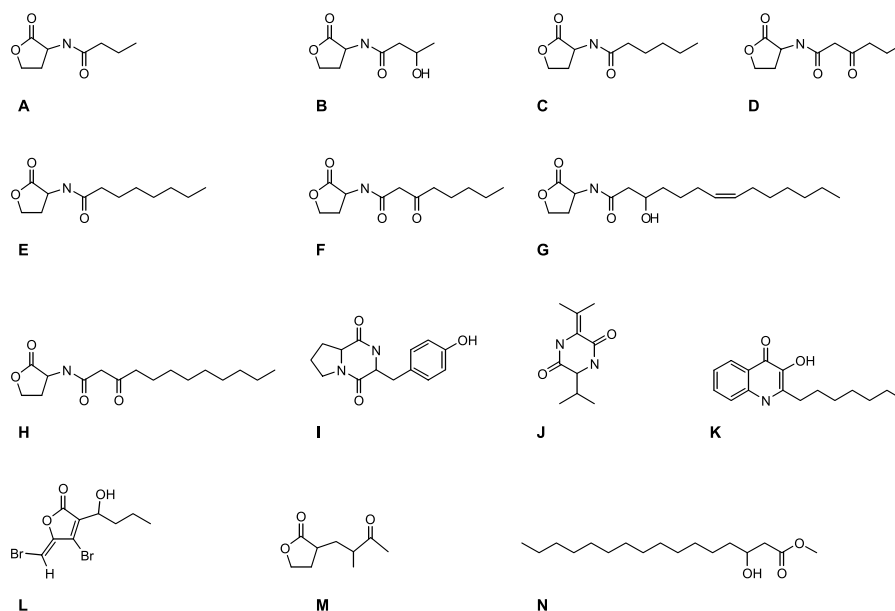


Fig. 5. The quorum-sensing molecules. A–H: Some of the more common microbial acyl HSLs: (A) *N*-butanoyl-L-homoserine lactone (BHL); (B) *N*-(3-hydroxybutanoyl)-L-homoserine lactone (HBHL); (C) *N*-hexanoyl-L-homoserine lactone (HHL); (D) *N*-(3-oxohexanoyl)-L-homoserine lactone (OHHL); (E) *N*-octanoyl-L-homoserine lactone (OHL); (F) *N*-(3-oxooctanoyl)-L-homoserine lactone (OOHL); (G) *N*-(3-hydroxy-7-*cis*-tetradecenoyl)-L-homoserine lactone (HtdeDHL); (H) *N*-(3-oxododecanoyl)-L-homoserine lactone (OdDHL). I, J: Two microbial diketopiperazines: (I) cyclo(L-Pro-L-Tyr); (J) cyclo(ΔAla-L-Val). K: 2-Heptyl-3-hydroxy-4-quinolone (PQS). L: A furanone of *Delisea pulchra*, 4-bromo-5-(bromomethylene)-3-(1'-hydroxybutyl)-2(5*H*)-furanone. M: The butyrolactone putatively produced by *Xanthomonas campestris*. N: 3-Hydroxypalmitic acid methyl ester (3OH PAME).

### 3.3. The acyl HSL molecules

Numerous bioassay and sensor systems have been developed that allow facile detection, characterisation and quantitation of microbial acyl HSLs [89–95]. The structures of those discovered to date vary in the size and composition of their acyl chains (as shown in Fig. 5). The chains range from four to 14 carbon atoms, can contain double bonds, and often contain an oxo or hydroxyl group on the third carbon. The overwhelming majority of microbial acyl HSLs identified so far have an even number of carbons in their acyl chain. Many of the individual acyl HSL species are synthesised by representatives of different bacterial genera. Likewise, many bacterial species can produce more than one type of acyl HSL. This observation is likely to echo the respective substrate specificity of each LuxI homologue. As such, it has been reported that certain LuxI homologues can utilise differently charged ACPs to produce acyl HSLs with correspondingly different chain lengths [78,83]. Whether all of the acyl HSLs synthesised by a single LuxI homologue play roles in the physiology of the producing organism is uncertain. It is not inconceivable that minor acyl HSL products synthesised by a bacterium could act as inhibitors of LuxR homologues expressed by that microorganism. In relation to this, TraR has been shown to be capable of interacting with a range of acyl HSLs but only a few of these can induce the supposed conformational change required to activate the protein [96]. The acyl HSLs that do not induce activation but do bind to the protein therefore behave as antagonists to the cognate signal of this LuxR homologue.

The type of acyl HSL produced by a particular species can be strain-dependent. This may reflect the differing habitats in which individual strains reside. Unfortunately, one cannot predict which acyl HSL(s) will be synthesised by different LuxI homologues by carrying out comparative sequence analysis alone. Similarly, sequence analysis of the LuxR homologues does not offer any clues as to which acyl HSL is preferentially bound by each protein [97].

It was originally believed that acyl HSL molecules were freely diffusible through cellular membranes. However, this view is beginning to change. Indeed, Welch et al. offered evidence that the activation of CarR by a range of acyl HSLs was somewhat dependent upon the ability of these ligands to avoid aggregation in the cellular membrane of *E. carotovora* subsp. *carotovora* [60]. Furthermore, another study identified an active efflux pumping system, necessary for the effective translocation of a long-chain (12-carbon) acyl HSL in *P. aeruginosa* [98]. Thus, it may be that short-chain acyl HSL molecules are capable of diffusing across bacterial membranes whereas long-chain acyl HSLs are actively transported via efflux or influx systems. Such systems, in organisms other than *P. aeruginosa*, await discovery.

## 4. Quorum-sensing systems beyond the LuxRI paradigm

Now that the underlying principles of quorum-sensing have been explained, the remainder of this review will expand on how microorganisms other than *V. fischeri* use such systems to regulate physiological processes. How these quorum-sensing systems aid particular bacterial species to enact functions that impinge on their eukaryotic hosts will be underscored throughout.

### 4.1. Quorum-sensing in *P. aeruginosa*

In recent years, the microorganism on which most quorum-sensing-related studies have been initiated is *P. aeruginosa*, reflecting its importance as a common opportunistic human pathogen. It is often associated with infections of immunocompromised patients, particularly those suffering from AIDS, cystic fibrosis (CF) or severe burn wounds [99–101]. The basis of the pathogenicity of *P. aeruginosa* is its ability to produce and secrete multiple extracellular virulence factors such as proteases, haemolysins, exotoxin A, exoenzyme S and pyocyanin. These exofactors are collectively capable of causing extensive tissue damage in humans and other mammals [102,103].

Regulation of the genes encoding the exoproducts is primarily dependent upon a signalling system that encompasses at least two sets of LuxRI homologues (see Fig. 6). The first of these quorum-sensing systems comprises LasI, which is responsible for the synthesis of *N*-(3-oxododecanoyl)-L-homoserine lactone (OdDHL), and the transcriptional activator, LasR [104–106]. The LasRI system was initially shown to regulate the expression of LasA elastase, LasB elastase, exotoxin A and alkaline protease [105,107–109]. Indeed, a preliminary study indicates that a glutathione *S*-transferase–LasR fusion protein can bind to the promoter of *lasB* in the presence of OdDHL [110]. The expression of LasI is also very sensitive to LasR–OdDHL concentrations. An increase in LasI synthesis leads to a rapid increase in OdDHL production and, therefore, a rapid increase in the formation of LasR–OdDHL activator complex [111]. This autoregulatory loop means that the activation of LasRI-dependent virulence factor production by *P. aeruginosa* is tightly linked to OdDHL concentrations. Interestingly, the expression of *lasR* is also cell density-dependent; levels reach a maximum at stationary phase of growth [112,113].

The second quorum-sensing system of *P. aeruginosa* is controlled by the LuxRI homologues, RhlRI (also referred to as VsmRI). RhlI directs the synthesis of *N*-butanoyl-L-homoserine lactone (BHL) [114,115]. This acyl HSL was shown initially to interact with RhlR in order to activate expression of *rhlAB*, an operon encoding a rhamnosyl-transferase required for the production of rhamnolipid biosurfactants [116]. The presence of these compounds reduces surface tension and thereby allows *P. aeruginosa* cells to swarm over semi-solid surfaces [117]. Subsequent

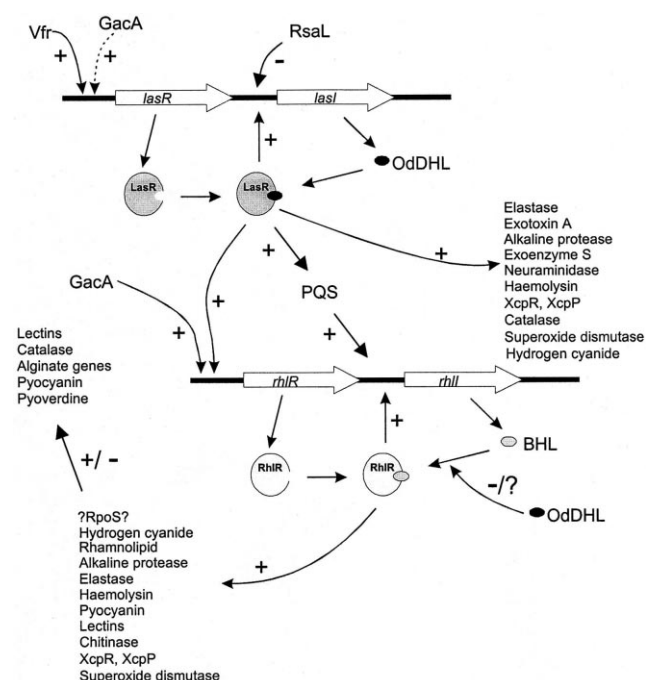


Fig. 6. The use of hierarchical quorum-sensing to control virulence in *P. aeruginosa*. The Las and Rhl quorum-sensing systems of *P. aeruginosa* integrate with other global regulators to control the production of multiple virulence factors. RpoS positively and negatively regulates a further group of physiological processes, some of which are shown. However, it is currently uncertain whether the expression of RpoS is or is not under RhlI/R regulation [124,125]. Similarly, it is also unclear whether OdDHL can inhibit BHL induction of RhlR in a *P. aeruginosa* background [113,122].

studies have revealed that a functional RhlRI system is also required to fully induce expression of other factors, including alkaline protease, pyocyanin, hydrogen cyanide, lectins and elastase [118–123]. Significantly, transcription of *rhlI* is enhanced in the presence of RhlR–BHL, thereby creating a further autoregulatory loop within the LasRI/RhlRI regulons [115]. Interestingly, Latifi et al. have reported that activated RhlR may be capable of activating transcription of *rpoS* (encoding  $\sigma^s$ , the stationary phase sigma factor) [124]. However, quorum-sensing regulation of  $\sigma^s$  in *P. aeruginosa* has recently been questioned [125]. Indeed, the latter study indicates that transcription of *rpoS* is not controlled by RhlR–BHL and, in fact, the sigma factor actually serves to negatively regulate *rhlI* transcription. Obviously, further studies are required to clarify the position of RpoS in the quorum-sensing regulatory network of *P. aeruginosa*.

As both of the described quorum-sensing regulons had been shown to be involved in the regulation of alkaline protease and elastase production, studies were initiated to determine how the LasRI and RhlRI systems interacted with each other. The evidence demonstrated the subordinate nature of the RhlRI system in the hierarchy of regulatory command that exists between these two quorum-sensing regulons. Two independent studies showed that the RhlRI system is functionally dependent upon the

LasRI system, as transcriptional activation of *rhlR* was dependent upon LasR–OdDHL [113,124]. Thus, the activation of the Las system causes the subsequent activation of the Rhl system and together the two LuxR homologues regulate the transcription of genes within their respective regulons (as shown in Fig. 6). Additionally, a level of post-translational control of RhlR activation may operate whereby formation of the RhlR–BHL complex is initially inhibited due to the competitive binding of OdDHL to this transcriptional regulator [113]. It has been hypothesised that this blockage may be required to delay early induction of *rhlI* and, therefore, other genes regulated by the RhlRI quorum-sensing system. However, it should be noted that, although studies have indicated that OdDHL can compete with BHL for binding to RhlR and that *rhlA* expression is reduced in the presence of OdDHL, the work has been performed using a heterologous genetic host (*E. coli*) [113]. Recently, Winzer et al. demonstrated that RhlR–BHL-dependent induction of *lecA* (a lectin structural gene) was similarly decreased in the presence of OdDHL when expressed in *E. coli*. However, they also showed that the presence of the latter acyl HSL had no effect on the RhlR–BHL-induced expression of a *lecA::luxCDABE* gene fusion in a *P. aeruginosa* genetic background [122]. This therefore indicates that OdDHL may not actually block the expression of genes regulated by RhlR–BHL in *P. aeruginosa*. Obviously, more work is necessary to shed light on these conflicting observations.

One study has demonstrated that functional Las and Rhl systems are required for the full expression of two genes (*xcpP* and *xcpR*) that encode components of the Xcp secretory system of *P. aeruginosa* [126]. The Xcp secretory system mediates the passage of exoproteins (such as exotoxin A, elastase and phospholipase) across the outer membrane of the organism [127]. Therefore, by using a quorum-sensing system to regulate the expression of the secretory apparatus through which many of the exoenzymes are translocated, *P. aeruginosa* cells have evolved a further level of control over virulence factor production. This strict control of virulence factor expression may be important in preventing the microorganism from alerting its host to its presence when infecting populations are small. As such, individual *P. aeruginosa* cells are essentially delayed from producing virulence factors until they are in a population (quorum) that is large enough to overwhelm the host.

The importance of functional *las* and *rhl* systems to the pathogenesis of *P. aeruginosa* has been demonstrated by examining quorum-sensing mutant behaviour in numerous model systems. A recent study has shown that a strain of *P. aeruginosa* carrying mutated alleles of *rhlI* and *lasI* displays attenuated virulence and dispersion, in a burned mouse model, compared to the wild-type parental strain [128]. A *lasR* mutant is similarly attenuated in the same model system [128,129]. A functional quorum-sensing system is also necessary for *P. aeruginosa* cells to present

wild-type levels of respiratory infection in neonatal mouse models [130,131]. Intriguingly, Tan et al. identified a *lasR* mutant of *P. aeruginosa* on the basis of its reduced ability to kill cells of the nematode, *Caenorhabditis elegans* [132]. The same study demonstrated that this mutant was less effective at infecting leaves in an *Arabidopsis* leaf infiltration assay. More recently, Jander et al. have shown that the same *lasR*<sup>−</sup> isolate displays reduced virulence capabilities in larvae of the greater wax moth, *Galleria mellonella* [133]. When this information is taken in conjunction with the results from the previously described rodent infection studies, one can conclude that an active Las quorum-sensing system is crucial for full pathogenicity of *P. aeruginosa* cells in at least four different hosts.

Evidence to show quorum-sensing systems are active during *P. aeruginosa* infections is more expansive than the data accumulated from infection studies that use *las* and *rhl* mutants. For instance, analysis of extracted RNA from sputum samples has shown that expression of at least three quorum-sensing regulated genes is co-ordinately controlled with the expression of *lasR* in *P. aeruginosa* populations located in the lungs of CF patients [134]. Similarly, the expression of a RhlR-regulated gene (*migA*) is highly expressed by *P. aeruginosa* cells inhabiting lungs of people afflicted with CF [135]. By using an *E. coli* strain carrying an acyl HSL-inducible *gfp* (green fluorescent protein) reporter construct, Wu et al. have also demonstrated that these signalling molecules are produced by *P. aeruginosa* cells residing in the lungs of infected mice [136].

It is now becoming increasingly apparent that the LasRI and RhlRI systems are not just involved in the regulation of exoenzyme secretion. Production of a siderophore, pyoverdine, is modulated by the LasR–LasI regulatory circuit when cells are grown under iron-limiting conditions [137]. Similarly, Hassett et al. showed that the expression patterns of genes and proteins involved in the degradation of hydrogen peroxide and oxygen radicals were moderated by the Las and Rhl systems [138]. However, it is a study by Whiteley et al. that offers the most compelling evidence to illustrate just how important the quorum-sensing systems of *P. aeruginosa* are in the physiology of the organism [139]. In this illuminating report, over 250 isolates from a library of transposon-induced mutants of *P. aeruginosa* were found to contain insertions within acyl HSL-responsive loci. These were identified by determining the activity of the promoterless *lacZ* cassette, contained within the transposon, in the presence or absence of OdDHL alone or both OdDHL and BHL. Analysis of translated sequence data derived from chromosomal DNA flanking the transposon insertions of 39 of the aforementioned isolates revealed that these had been created via disruption of genes encoding proteins putatively involved in multiple and diverse phenotypes. Conversely, some of the interrupted open reading frames encoded putative proteins that did not share sequence similarity with any other polypeptides in the databases. As expected, genes encoding

proteins that were already known to be regulated by the *P. aeruginosa* quorum-sensing systems were also identified (namely, *rhlI*, *rhlAB* and genes encoding proteins involved in the production of hydrogen cyanide and pyoverdine) [139].

Many of the promoters of the quorum-sensing-regulated genes identified so far in *P. aeruginosa* contain *lux* box-type motifs. How activated LasR and RhlR differentiate between such promoter sequences is currently unclear. Based upon their findings, Whiteley et al. estimate that up to 4% of all genes in *P. aeruginosa* could be regulated, to varying extents, by the quorum-sensing regulons of the organism [139]. It is difficult to ascertain the relative contributions of the Las and Rhl systems towards this figure, primarily because of the degree of regulatory cross-talk between them. This complexity is heightened further by the fact that activated RhlR may regulate the expression of RpoS (although, as stated earlier, this awaits clarification [124,125]). RpoS is likely to participate in the transcriptional regulation of a subset of genes, the expression of which may also be modulated by quorum-sensing. To this end, it has been recently demonstrated that a functional RpoS protein is important for the expression of many activities that are recognised as being regulated by the Las and Rhl regulons (namely lectin, catalase, exotoxin A, pyocyanin and pyoverdine activity) [122,140]. Sequences similar to the consensus binding site for RpoS are also located upstream of the open reading frames encoding XcpP and XcpR [126].

#### 4.1.1. Hierarchical control of quorum-sensing in *P. aeruginosa*

Recently, further levels have been added to the regulatory cascade controlling virulence of *P. aeruginosa*. Firstly, expression of *lasR* has been shown to be dependent on Vfr, a homologue of CRP [112]. It was demonstrated that Vfr binds to a CRP binding consensus sequence located upstream of *lasR*. Secondly, the global activator GacA, a highly conserved response regulator of the two-component family, has also been shown to activate, directly or indirectly, the expression of *rhlR*, and hence modulate the production of BHL and BHL-controlled phenotypes. Although GacA also affects the level of *lasR* expression, for reasons that are currently unclear, the levels of OdDHL do not strictly parallel those of BHL [141]. Thirdly, the product of a gene (*rsaL*) located downstream of *lasR* represses *lasI* transcription at low cell densities [142]. RsaL is able to compete with LasR–OdDHL for binding sites at the *lasI* promoter. It is hypothesised that once the population density is large enough, LasR–OdDHL is able to displace RsaL from these binding sites (Fig. 6). Finally, disruption of *ppk*, a gene involved in the synthesis of inorganic polyphosphate, has a dramatic effect on the synthesis of BHL and OdDHL and (probably as a consequence) the expression of virulence factors by *P. aeruginosa* [143].

#### 4.2. Quorum-sensing in other pseudomonads

Many other species of *Pseudomonas* produce acyl HSLs and possess quorum-sensing systems. Of these, perhaps the best characterised is the sensing apparatus utilised by *P. aureofaciens*. This fluorescent pseudomonad resides in soil and is commonly found within rhizospheres. The organism produces three phenazine antibiotics – namely phenazine 1-carboxylic acid, 2-hydroxy-phenazine 1-carboxylic acid and 2-hydroxy-phenazine (which will collectively be termed phenazine for the purpose of this review). Synthesis of phenazine by *P. aureofaciens* is a beneficial trait in terms of conferring a competitive advantage upon producing strains [144]. The organism can also be used as a potential biocontrol agent [145]. This is because phenazine is a potent inhibitor of fungal pathogens such as *Gaeumannomyces graminis* var. *tritici*, the causative agent of wheat ‘take-all’ disease [146].

Regulation of phenazine production by *P. aureofaciens* is dependent upon a quorum-sensing system involving the LuxRI homologues, PhzRI. The two genes encoding these regulators neighbour each other on the chromosome (they are separated by just 31 bp) and are transcribed convergently [147]. PhzI is responsible for the synthesis of *N*-hexanoyl-L-homoserine lactone (HHL), the cognate acyl HSL sensed by PhzR [147–149]. In common with *P. aeruginosa*, expression of this acyl HSL synthase protein is up-regulated by GacA and GacS [150].

Analogous to other quorum-sensing systems, activation of PhzR is thought to allow this protein to bind to a promoter region upstream of the operon encoding phenazine synthesis function (*phzXYFABCD*) [151–153] and stimulate transcription. Interestingly, Pierson and Pierson proposed that fungal infection of roots could act as the stimulus for phenazine production due to the concomitant release of root exudates during the attack [154]. These chemicals could indeed act as a source of nutrients and thereby allow the subsequent proliferation of *P. aureofaciens* cells to form a quorate population. To this end, Chin-A-Woeng et al. used scanning electron microscopy to show that, after inoculation, fluorescent pseudomonads formed high-density microcolonies on certain locations of tomato roots [155]. From these observations, it has been hypothesised that these microbial accumulations may allow populations of pseudomonads such as *P. aureofaciens* to reach a quorum and therefore express phenazine [155,156]. Coincidentally, certain isolates of *P. fluorescens* also produce phenazine and, perhaps unsurprisingly, *luxRI* homologues (also termed *phzRI*) have been identified [157]. Indeed, studies have shown that *P. fluorescens* does synthesise a large number of acyl HSLs in a strain-dependent manner [88,90,158]. Addition of exogenous signalling molecule has been shown to induce antibiotic production in one such strain [159].

By using several different acyl HSL sensor systems, two separate investigations have demonstrated that many phy-

topathogenic pseudomonads produce these signalling molecules [158,160]. Indeed, it is now recognised that certain strains of *P. corrugata*, *P. savastanoi* and five different pathovars of *P. syringae* (pvs. *syringae*, *tomato*, *angulata*, *coronofaciens* and *tobaci*) all synthesise various acyl HSLs. Likewise, strains of *P. syringae* pvs. *glycinia*, *tabaci* and *phaseolicola* all encode LuxI homologues within their chromosomes [160,161]. Of these microorganisms, the quorum-sensing system of *P. syringae* pv. *syringae* (*Pss*) is the most comprehensively studied. A *Pss*-derived *luxI* homologue has been identified, sequenced and designated *ahII<sub>Pss</sub>* [160]. Inactivation of *ahII<sub>Pss</sub>* results in the loss of acyl HSL production, a possible alteration of colony morphology and a reported loss of viability on plant surfaces [160,162]. The mutation had no effect on the ability of *Pss* to swarm, nor did it cause any changes to the expression pattern of other major virulence factors produced by the organism [160,162]. Unsurprisingly, GacA and GacS also play roles in the regulation of acyl HSL synthesis in *Pss* [160,163].

*Burkholderia cepacia* (formerly classified as *P. cepacia*) is, like *P. aeruginosa*, commonly associated with lung infections of CF patients [164]. One strain of this microorganism (isolated from the respiratory tract of a CF patient) expresses two quorum-sensing-related proteins, CepI (which directs the synthesis of OHL) and CepR. These LuxRI homologues are involved in the regulation of protease and siderophore expression [165]. Interestingly, compounds produced by *P. aeruginosa* (presumably acyl HSLs) serve to up-regulate expression of exoproducts from another *B. cepacia* strain when the latter organism is cultured with cell-free supernatant originating from the *Pseudomonas* species [166]. Therefore, in instances of co-infection, it is not unreasonable to suppose that a degree of acyl HSL-mediated cross-communication transpires between *B. cepacia* and *P. aeruginosa* populations. In fact, analysis of acyl HSL production by isolates of *P. aeruginosa* taken from a patient with a chronic lung infection has revealed that isolates sampled during times of co-infection by *B. cepacia* had a reduced capacity to produce acyl HSLs compared to isolates taken when populations of the co-resident were absent [167]. The mechanism and reason for this lowering of signalling molecule synthesis remain unknown but some form of acyl HSL-mediated cross-communication input cannot be ruled out.

##### 4.2.1. The role of quorum-sensing in the formation of pseudomonad biofilms

Early studies had demonstrated the presence of acyl HSL molecules in biofilms found in both natural and clinical environments [168–170]. Therefore, it was no surprise when cell density-dependent signalling was shown to play a part in the formation and maintenance of these structures [171,172]. Strains of *P. aeruginosa* defective in OdDHL production form abnormal monospecies biofilms that, in contrast to wild-type biofilms, are sensitive to low

concentrations of biocides [172]. The addition of exogenous OddHL to the medium restores the ability of these mutants to form normal biofilms. It would seem that OddHL regulates the expression of developmental processes, important for the differentiation of *P. aeruginosa* cells during the maturation of biofilms [172]. Quorum-sensing may also play an important role in the initial stages of biofilm development by the organism. This is because of the possible involvement of the RhlRI system with the secretion and assembly of pilin, the structural subunit of type IV pili [173]. Expression of these structures has been shown to be an important prerequisite for successful biofilm formation by *P. aeruginosa* cells [174]. It is believed that *P. aeruginosa* cells reside in biofilms within the lungs of CF victims [175]. Therefore, the core components of the quorum-sensing system of *P. aeruginosa* could be targeted by workers seeking novel therapeutic agents for this affliction.

#### 4.2.2. Novel quorum-sensing molecules

Recently, molecules belonging to two new families of quorum-sensing-related signalling molecules have been identified and isolated from spent culture supernatants of *P. aeruginosa*. The first of these groups comprises molecules known as diketopiperazines (DKPs; see Fig. 5). A range of DKPs are synthesised by multiple microorganisms, including *P. aeruginosa*, *P. fluorescens*, *P. alcaligenes*, *Proteus mirabilis*, *Citrobacter freundii* and *Enterobacter agglomerans* [176]. Some of these cyclic dipeptides have the ability to activate supposedly acyl HSL-specific sensor systems. In light of this, it is perhaps not surprising that certain DKPs can also inhibit or activate complete quorum-sensing systems in at least two different microorganisms [176]. It is likely that DKPs interact with LuxR homologues to exert these observed positive and negative effects. However, it should be noted that the concentration of exogenous DKP required to activate or inhibit such quorum-sensing systems is far in excess of the concentration of acyl HSL required for similar activation or inhibition [176]. Thus, it is physiologically unlikely that DKPs actually do interact with acyl HSL-based quorum-sensing systems in organisms that produce both sets of compounds. Nevertheless, it is possible that the expression of non-quorum-sensing-related genes may be co-ordinately controlled via DKP-specific regulatory pathways. Therefore, the demonstration of the widespread synthesis of these molecules may represent the initial step towards the discovery of yet another small molecule-mediated bacterial sensing system. Alternatively, Holden et al. propose that DKPs may actually function as mediators of molecular interactions between microorganisms and their eukaryotic hosts [176].

Pesci et al. identified a further signalling molecule in the spent supernatant of *P. aeruginosa* [177]. The synthesis of this molecule, identified as 2-heptyl-3-hydroxy-4-quinolone (PQS; see Fig. 5), is dependent on LasR. Exogenously

added PQS has the ability to induce expression of *lasB* and *rhlI* (and, to a lesser extent, *lasR* and *rhlR*) and is therefore a further link between the Las and Rhl systems [177,178]. *P. aeruginosa* populations synthesise PQS after exponential phase of growth [178]. It has therefore been hypothesised that *P. aeruginosa* could use PQS to up-regulate *rhlI* expression and, as a consequence, the genes regulated by the *rhl* system during stationary phase of growth, as a means of promoting the fresh supply of nutrients [178].

#### 4.3. Quorum-sensing in *E. carotovora*

Species belonging to the genus *Erwinia* are primarily recognised as plant pathogens belonging to one of three major groups: amylovora, carotovora and herbicola. They are of economic importance due to the diseases they cause to various commercial crops such as potato, carrot, celery, rhubarb, onion, cucumber and pineapple. Although the microorganisms can usually be found as part of the epiphytic flora, transmission is exacerbated via insect vectors. Some *Erwinia* species have also been identified as insect pathogens and as opportunistic pathogens of animals [179].

The soft-rot *Erwinia* spp. include *E. chrysanthemi*, *E. carotovora* subspecies *carotovora* (*Ecc*), *atroseptica* (*Eca*) and *betavascolorum* (*Ecb*). These microorganisms characteristically produce an abundance of exoenzymes, including pectin methylesterases, pectate lyases (Pels), pectin lyases, polygalacturonases, cellulases (Cels) and proteases (Prts), which collectively precipitate maceration of plant tissue [180,181]. The expression of exoenzymes is pivotal to the pathogenic success of the organisms; disruption of genes encoding individual exoenzymes often leads to a reduction of virulence in planta [182,183]. The exoenzymes are transported to the external environment via two discrete pathways, a single-step (type I) secretory pathway for export of Prts and a two-step (type II) pathway for secretion of Pels and Cels. In *Ecc*, translocation of the periplasmic form of the enzymes to outside the cell via the latter pathway requires the products encoded by at least 13 *out* genes [184].

##### 4.3.1. Regulation of antibiotic production

Certain strains of *Ecc* (originating from different sources) have been found to produce small amounts of a simple  $\beta$ -lactam antibiotic, 1-carbapen-2-em-3-carboxylic acid (carbapenem), during the transition between late exponential and stationary phases of growth [185]. There is some evidence that antibiotic production by *Erwinia* spp. is important for the elimination of sensitive *Erwinia* competitors from sites of infection in the plant host [186]. This fact, coupled with the commercial potential of this natural  $\beta$ -lactam and the genetic tractability of *Ecc*, has meant that many research projects have focussed on the production of carbapenem in this organism. One such genetic

complementation study of an *Ecc* carbapenem mutant led to the isolation of a cosmid that restored antibiotic production in the strain [187]. Analysis of the cosmid led to the identification of an operon consisting of eight genes (*carA–H*). The function of the genes in this *car* cluster was subsequently resolved via marker exchange mutagenesis. This showed that *carA–E* encode products involved in carbapenem synthesis while *carF* and *carG* encode a novel  $\beta$ -lactam resistance mechanism [188,189]. The function of CarH is unknown; mutants defective in *carH* make antibiotic and are intrinsically resistant.

The *car* cluster is located approximately 150 bp downstream of a *luxR* homologue termed *carR*. The product of this gene acts as a positive transcriptional regulator of the *car* genes; disruption of *carR* results in a null-carbapenem phenotype [187]. Previous cross-feeding experiments revealed that *Ecc* also produces an acyl HSL (identified as OHHL) that is absolutely required for carbapenem synthesis [190,191]. An acyl HSL-responsive *lux* reporter system was used to isolate the gene responsible for OHHL production in *Ecc* [92]. Analysis of the identified gene revealed that it encoded a LuxI homologue, which was called CarI (ExpI/HsII/OhII) [109,192,193]. Unlike the *V. fischeri* scenario, *carI* is not linked to the *car* gene cluster. Later, a quorum-sensing system was demonstrated to regulate the production of an unidentified antibiotic in *Ecb*. The LuxRI homologues expressed in this organism have been designated EcbRI [194].

It was proposed that activation of CarR by OHHL resulted in promotion of transcription of the *car* operon. The transcriptional start site of *carA* has been mapped to within the 150-bp *carA–carR* intergenic region. No *lux* box-like promoter element has yet been identified near this site but OHHL-dependent transcriptional activation of *carA* has been demonstrated once autoinducer concentrations reach a threshold level of  $0.1 \mu\text{g ml}^{-1}$  [195]. This finding correlates well with that of Bainton et al. who showed that OHHL concentrations need to reach  $0.1 \mu\text{g ml}^{-1}$  before *Ecc* populations initiate production of carbapenem [190]. Such induction does not occur when *Ecc* cells are grown at  $37^\circ\text{C}$  (rather than  $30^\circ\text{C}$ ) as OHHL levels do not reach the required concentration. This may be due to some form of post-transcriptional control of CarI levels [195]. Interestingly, addition of OHHL to *Ecc* cultures at the start of growth results in the precocious induction of *carA* transcription [195]. This implies that OHHL is the limiting factor for carbapenem synthesis at early stages of growth. It also serves to emphasise the apparent simplicity of carbapenem regulation.

CarR exists naturally as a dimer and is capable of binding to the *carA–carR* intergenic region in the absence of OHHL [60]. In the presence of the signalling molecule, each dimer associates with two molecules of OHHL thereby promoting the formation of multimers which can also associate with the aforementioned stretch of DNA [60]. How (or even if) this high-molecular-mass CarR–OHHL

complex acts to promote transcription of the *car* genes is unclear. However, in concert with multimerisation of CarR being necessary for induction of transcription, it has been shown that overexpression of CarR in a *carI*<sup>−</sup> strain of *Ecc* promotes carbapenem production [187].

When CarR is associated with OHHL, it is less susceptible to proteolysis compared with when not associated with the ligand [60]. Expression of *carR* is also CarR- and OHHL-dependent (coincidentally, the DNA sequence directly upstream of *carR* is strikingly similar to that within the *carA–carR* intergenic region) [195]. It is probable that these phenomena aid *Ecc* cells to maintain low levels of CarR at early stages of growth and enable rapid up-regulation of the protein when it is required.

A study by Holden et al. found that 16% of strains of *Ecc* from within a culture collection were cryptic producers of carbapenem [196]. That is to say, when *carR* was expressed *in trans* in these strains, they were rendered capable of producing the antibiotic. This implies that these strains have acquired a mutation in their *carR* gene that disrupts the positive regulation of carbapenem synthesis. Such mutations might be caused by selective pressure that operates on strains of *Ecc* inhabiting niches where production of carbapenem is of no benefit to the organism. The recent discovery of a CarR-independent promoter, within the *car* cluster, which is dedicated to the expression of the carbapenem resistance functions encoded by *carFG* [195], explains how such strains could also survive in the presence of carbapenem-producing strains that shared the same locale. A study in this laboratory has therefore been initiated whereby natural isolates of *Ecc* from potatoes are being tested for their ability to produce carbapenem. Results from this survey may offer clues as to the extent and true role of this antibiotic in the environment.

#### 4.3.2. Regulation of exoenzyme production

The cell density-dependent production of exoenzymes in *Ecc* is also reliant upon the synthesis of OHHL by CarI. Indeed, disruption of *carI* leads to a diminution of exoenzyme synthesis and a consequential reduction of virulence in planta [109,192]. However, inactivation of *carR* has no effect on exoenzyme production and thus CarR cannot be responsible for the OHHL-mediated regulation of enzymes [187]. A decrease in exoenzyme levels was observed when *carR* was expressed on a multicopy plasmid in *Ecc* [187]. This led to the hypothesis that multiple CarR proteins have the ability to sequester OHHL away from an additional LuxR homologue, responsible for the induction of exoenzyme synthesis. Indeed, a second *luxR* homologue, *expR* (*eccR/irexR*), has been identified in *Ecc*. The gene lies adjacent to *carI* and is transcribed convergently with it [197–199]. Andersson et al. have presented data indicating that an *expR* mutant in their particular strain of *Ecc* exhibited slightly increased levels of Pel activity and OHHL production during the early exponential phase of growth [199]. The effects of the *expR* mutation were all modest

but suggested that ExpR might be acting as a repressor in this strain of *Ecc*. The authors postulated that ExpR could function by sequestering OHHL. The role of ExpR in our laboratory strains of *Ecc* is unclear as disruption of *expR* has no perceptible effect on any phenotype, exoenzyme-related or otherwise [198]. Interestingly, further studies in this laboratory have led to the identification of a number of genes, unconnected to exoenzyme or carbapenem synthesis, that are under quorum-sensing control in *Ecc* [200]. ExpR is equally uninvolved in their regulation. Thus, the search continues for the elusive exoenzyme-dedicated LuxR homologue of *Ecc* (if, indeed, one exists).

The overall effect of coupling the expression of exoenzyme synthesis with cell density is that, as with *P. aeruginosa*, a host is not exposed (in any significant way) to these virulence factors until populations of *Ecc* have reached a critical level. Many plants mount a defence response when they sense breakdown products of their own cell walls [201,202]. Hence, it has again been proposed that quorum-sensing control of exoenzyme synthesis may serve to camouflage *Ecc* populations by preventing the elicitation of a host response until a sufficiently powerful army of cells has been assembled. Perhaps, the concomitant production of a broad-spectrum antibiotic with the exoenzymes might help to defend the local nutrient-replete niche from competitors.

#### 4.3.3. Additional inputs to exoenzyme production

Further control of exoenzyme production has been found to take place via the input of many regulators, some of which interact with components of the quorum-sensing system. Perhaps the most important of these regulators constitute the Rsm system. Analysis of mini-transposon mutants displaying up-regulated Pel, Cel and Prt phenotypes resulted in the initial identification of *rsmA* [203]. The protein encoded by this gene bears 95% sequence similarity to CsrA, a regulator of carbon storage in *E. coli* [204]. The 61 amino acid CsrA protein has been shown to work by binding to mRNA transcripts of the genes it regulates and promoting their degradation [205,206]. It is uncertain whether RsmA binds mRNA transcripts. However, disruption of *rsmA* leads to increased cellular levels of exoenzyme mRNA [193]. Similarly, overexpression of RsmA in wild-type *Ecc* cells leads to reduced levels of exoenzyme and *carI* transcripts [203,207].

CsrA co-purifies with a small RNA species (*csrB*) in a ratio of 18 molecules to one [206,208]. *csrB* is believed to antagonise CsrA activity by binding to molecules of the protein and removing them from the cellular pool of regulators [209]. For this purpose, *csrB* contains 18 imperfect repeats within its sequence. An equivalent antagonistic RNA species, called *rsmB* (*aepH*), has been identified in *Ecc* [210,211]. In *Ecc*, *rsmB* may antagonise RsmA in an analogous manner to the *csrB*/CsrA scenario in *E. coli*. However, Liu and colleagues have demonstrated that

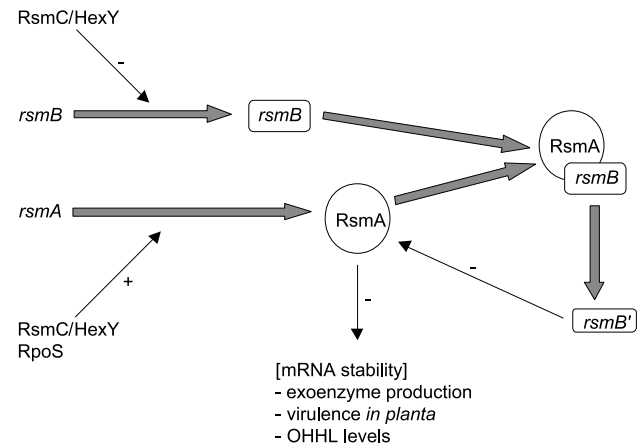


Fig. 7. A model of the RsmA/*rsmB*/RsmC regulatory network in *E. carotovora* subsp. *carotovora*. RsmA destabilises the mRNA transcripts of *CarI* and the exoenzymes. An interaction with *rsmB* potentially removes RsmA from the cellular pool of regulators and promotes the processing of *rsmB* into two species of RNA. The smaller of these RNA molecules (*rsmB'*) down-regulates expression of RsmA in an unknown fashion. RsmC, HexY and RpoS all affect the cellular levels of RsmA and *rsmB*.

*rsmB*, unlike *csrB*, appears to be processed into two RNA species of 479 bp and 259 bp [211]. It is the shorter of these two molecules that has anti-RsmA activity. It has therefore been postulated that RsmA binds to *rsmB* and thereby promotes processing of the RNA molecule. The 259 bp fragment (*rsmB'*) is then able to exert negative regulatory powers over RsmA activity by an unidentified pathway (Fig. 7) [211].

Although various regulators have been shown to interact with the Rsm system, for many the exact mechanism by which they do so remains uncertain. As reviewed elsewhere [212], the product of *hexY* (*rsmC*) somehow activates expression of RsmA but represses expression of *rsmB* [213,214]. Additionally, RpoS and HexA are similarly thought to act by affecting RsmA and *rsmB* expression respectively [215–217].

#### 4.4. Quorum-sensing in other *Erwinia* spp.

Many other species of *Erwinia* have been found to produce acyl HSLs. In fact, different screening programmes have shown that strains of *Eca*, *E. herbicola* and *E. chrysanthemi* synthesise various different acyl HSLs [158,191]. The latter organism produces at least three acyl HSLs: OHHL, HHL, and *N*-decanoyl-L-homoserine lactone (DHL) [75]. The *expI* gene product is responsible for the synthesis of OHHL and HHL but DHL synthesis seems to require another, yet to be identified, locus. Like *Ecc*, a *luxR* homologue (*expR*) is located adjacent to *expI* and is transcribed convergently with it. Although disruption of *expI* or *expR* has very little effect on the gross production of Pels, DNA bandshift and DNase I footprinting experiments have shown that ExpR can interact with the regulatory regions of different *pel* genes in the presence and absence of OHHL. Interestingly, the protein seems to have

disparate affinities for these respective genes. ExpR has also been shown to associate with DNA originating from the promoter regions of *expI*, *expR* and a gene encoding an important regulatory protein, PecS. Indeed, in the absence of acyl HSL, ExpR appears to bind to its own promoter and autorepresses transcription while, in the presence of the signalling molecule, ExpR dissociates from the binding site [76]. As with other quorum-sensing regulons, integration of regulation by CRP within the system is evident. In a *crp* mutant, expression of *expI* is increased but expression of *expR* is decreased [76]. A putative CRP box has been identified upstream of both *expI* and *expR* and CRP has been demonstrated to interact with these regions of DNA.

*Pantoea stewartii* subsp. *stewartii*, formerly classified as *E. stewartii* subsp. *stewartii*, is the causative agent of Stewart's wilt in sweetcorn and leaf blight in maize. The quorum-sensing system of this microorganism consists of the LuxI homologue, EsaI, which directs production of OHHL, and the LuxR homologue, EsaR [218,219]. As is the case with other *Erwinia*-based quorum-sensing regulons, the two genes encoding these regulatory proteins are located next to each other and are convergently transcribed. OHHL is required for the cell density-dependent production of an exopolysaccharide (EPS) virulence factor called stewartan. When expressed in large enough quantities, this compound can block plant xylem vessels and subsequently cause wilting. *esaI* mutants do not produce OHHL or stewartan and show reduced virulence in planta [219]. However, *esaR* mutants display a supermucoid phenotype that is not rescued by the addition of exogenous OHHL [219]. Thus, it has been suggested that the role of EsaR is to repress stewartan production when *P. stewartii* subsp. *stewartii* populations are small [219]. This repression is then relieved at higher cell densities by the interaction of the protein with OHHL (analogous to the ExpR-mediated autorepression observed in *E. chrysanthemi*). To this end, EsaR has been shown to act as a repressor of EPS synthesis *in trans* and this negative activity is alleviated by the addition of exogenous OHHL [219]. Although unproven, one would presume that EsaR interacts with the promoters of genes required for EPS synthesis to manifest its repressive influence. Therefore, it is likely that, in the absence of OHHL, the protein exists in a dimeric configuration that is capable of binding DNA [59]. Interestingly, OHHL does not cause EsaR dimers to dissociate and so how the ligand eases the negative activity of EsaR is yet to be shown [59].

#### 4.5. Quorum-sensing in *Serratia* spp.

##### 4.5.1. *Serratia liquefaciens*

*S. liquefaciens* is an opportunistic pathogen of animals that can be found in many different habitats such as soil, water and animal intestines [220]. Like other species of *Serratia*, *S. liquefaciens* produces and secretes an array

of virulence factors, including nucleases, lipases, phospholipases and chitinases [221–223]. The organism is usually motile, but one strain (MG1) also has the ability to swarm over solid surfaces such as agar. Swarming behaviour requires differentiation into specialised 'swarmer cells' which occurs on the edge of bacterial colonies. Swarmer cells, which are long, multinucleate and hyper-flagellated, propel themselves across surfaces by use of their peritrichously arranged flagella. Such cells are capable of colonising large areas [224].

In 1996, Eberl and co-workers isolated and identified BHL and HHL in cell-free culture supernatants of *S. liquefaciens* MG1. A *luxI* homologue (*swrI*) was shown to encode the enzyme responsible for directing the synthesis of these two acyl HSLs [225]. Later, it was reported that *swrR*, a *luxR* homologue, lay downstream of *swrI* [222]. Disruption of *swrI* led to a five-fold reduction in extracellular proteolytic activity and a substantial reduction in the swarming capability of the strain [225]. However, growth rate, swimming motility and the ability of cells to differentiate into swarmer cells were not affected. Wild-type levels of swarming motility and protease activity could be restored in *swrI*<sup>−</sup> strains by supplementing media with BHL and, to a lesser extent, HHL [225].

In a later study, Lindum et al. demonstrated that the expression of biosurfactant was impaired in a *swrI* mutant of *S. liquefaciens* MG1 [226]. However, wild-type levels of surfactant could be regained by the addition of BHL. In order to identify the gene(s) encoding functions necessary for biosurfactant synthesis, the *swrI* mutant was exposed to further mutagenesis via use of a Tn5-derived transposon carrying a promoterless *luxAB* reporter [226]. The resultant double mutants were screened for bioluminescent activity in the presence and absence of BHL. This led to the isolation of 19 BHL-inducible gene fusion strains. One of these mutants was unable to swarm or produce biosurfactant even in the presence of exogenous BHL. It was therefore concluded that the transposon in this strain had been introduced into a gene (denoted *swrA*) that was essential for biosurfactant production. Analysis of *swrA* revealed that it shared a high degree of sequence similarity with genes encoding non-ribosomal peptide synthetases in other bacteria [226]. The major biosurfactant produced by *S. liquefaciens* MG1 was identified as being a cyclic lipopeptide called serrawettin W2, first isolated from *S. marcescens* [227]; complementation studies revealed that swarming motility of the *swrI*<sup>−</sup> strain of *S. liquefaciens* was restored when cells were inoculated onto agar plates supplemented with serrawettin W2 [226].

The aforementioned findings strongly suggest that the quorum-sensing system in *S. liquefaciens* MG1 is required for the induction of serrawettin W2 production. The role of this biosurfactant is to condition surfaces prior to swarming [226,227] and so it is unsurprising that one of the observed phenotypes in a mutant defective in acyl

HSL synthesis is abolition of swarming motility. Quorum-sensing regulation of such a phenotype presumably allows optimal dissemination of *S. liquefaciens* cells when a population is getting too large to inhabit a single given location.

The other 18 uncharacterised BHL-inducible gene fusion strains isolated in the study by Lindum et al. could include isolates carrying insertions within genes that are involved in the expression of other quorum-sensing-regulated phenotypes [226]. Indeed, 2D PAGE analysis of total protein samples extracted from an *swrI*<sup>−</sup> strain of *S. liquefaciens* MG1 cultured with and without exogenous BHL has revealed that the expression of at least 28 proteins is in some way regulated by the acyl HSL [228]. Thus, the true extent of the quorum-sensing system in this organism still remains to be determined.

#### 4.5.2. Other *Serratia* spp.

Work in this laboratory has concentrated on the quorum-sensing regulon of *Serratia* sp. ATCC39006. This strain of *Serratia* synthesises carbapenem, the production of which was initially thought to be regulated by an acyl HSL-independent LuxR homologue (like its *Ecc* counterpart, also termed CarR) [229]. This interpretation was primarily based on the initial finding that acyl HSL production by *Serratia* sp. ATCC39006 could not be detected via use of a *lux*-based biosensor. However, further analysis, using a different acyl HSL bioassay, capable of detecting a wider range of acyl HSLs, revealed that the microorganism does in fact produce at least two acyl HSLs, identified as BHL and HHL [230]. Both of these molecules are synthesised by the protein product of the *smal* locus [230]. Taking these new findings into account, it was therefore not surprising to discover that expression of carbapenem by *Serratia* sp. ATCC39006 was in fact regulated by a quorum-sensing system involving BHL, HHL and (acyl HSL-dependent) CarR [230]. It is of particular interest that, although the carbapenem synthesis and resistance genes (*carA–H*) of *Serratia* sp. ATCC39006 share a high level of sequence similarity with the *car* genes of *Ecc* [230], the two organisms utilise differing acyl HSLs to activate their respective CarR proteins. Thus, comparative analysis of these two transcriptional activators may explain how LuxR homologues differentially respond to their cognate acyl HSLs. Incidentally, the highly conserved genetic organisation shared between the *car* clusters of both *Serratia* sp. ATCC39006 and *Ecc* suggests that these genes (along with *carR*) have, at some time, experienced horizontal transfer as a complete unit.

A functional quorum-sensing system is also required by *Serratia* sp. ATCC39006 to enable production of the red pigment, 2-methyl-3-pentyl-6-methoxyprodigiosin (prodigiosin) [230]. This secondary metabolite, which is also produced by other species of *Serratia* [231], possesses antimicrobial and immunosuppressive activity [232–234]. Thomson et al. showed that the addition of exogenous

BHL and (to a lesser extent) HHL could induce expression of prodigiosin in a *smal*<sup>−</sup> strain of *Serratia* sp. ATCC39006 [230]. Interestingly, a *luxR* homologue, termed *smar*, overlaps with, and is convergently transcribed with, *smal* [230]. Disruption of this gene has no effect on carbapenem or prodigiosin synthesis. As in *Ecc*, CarR appears to be dedicated to the regulation of carbapenem synthesis in *Serratia* sp. ATCC39006.

A study by Thomson et al. resulted in the identification of a protein involved in the positive regulation of both carbapenem and prodigiosin synthesis in *Serratia* sp. ATCC39006 [235]. This protein, designated Rap (regulation of antibiotic and pigment), is closely related to other Gram-negative bacterial regulators such as MarR, SlyA, RovA and PecS [236–239]. A further homologue, named Hor (homologue of rap), is expressed by *Ecc* [235]. Disruption of the *hor* gene in *Ecc* results in a pleiotropic mutant, defective in the production of both carbapenem and exoenzymes. Thomson et al. showed that Hor was likely to act as a transcriptional activator of the *car* genes [235]. Further analysis in this laboratory has revealed that the protein is capable of binding DNA [240]. Although yet to be pinpointed, a link between the Rap and Hor proteins and the respective quorum-sensing regulons of *Serratia* sp. ATCC39006 and *Ecc* is thought to be likely. As such, it is noteworthy that addition of physiologically artefactual levels of OHHL can override the carbapenem-related effects of a mutation in *hor* [195].

#### 4.6. Quorum-sensing in *Yersinia* spp.

The first *Yersinia* species shown to possess a quorum-sensing system was *Yersinia enterocolitica*. The microorganism synthesises both HHL and OHHL via the product encoded by the *luxI* homologue, *yenI* [241]. A second open reading frame, termed *yenR*, lies downstream of *yenI* and encodes a LuxR homologue. Disruption of *yenI* abolishes acyl HSL production but no other loss of phenotype is observed [241]. However, proteomic studies have indicated that a functional *yenI* is required for the expression of a number of unidentified proteins [241].

It is now known that many of the species belonging to the genus *Yersinia* express quorum-sensing systems. Cell-free supernatants from several *Yersinia* species, including the non-pathogenic species *Y. frederiksenii*, *Y. kristensenii* and *Y. intermedia*, have been shown to contain acyl HSLs [241]. Genes encoding LuxRI homologues have also been obtained from the human pathogens, *Y. pseudotuberculosis* and *Y. pestis* [242,243]. Both microorganisms produce OHHL and HHL whilst *Y. pseudotuberculosis* also synthesises OHL. A preliminary investigation of the quorum-sensing system utilised by *Y. pseudotuberculosis* has revealed that it is complex, involving a hierarchy of at least two LuxRI homologues (YpsRI and YtbRI) and is affected by variations in temperature [242]. Analysis of the phenotype of *Y. pseudotuberculosis* strains carrying muta-

tions in *ypsI* or *ypsR* indicate that this quorum-sensing regulon is involved in temperature-dependent control of motility and cellular aggregation [242]. YpsR negatively regulates both phenotypes. The function of the *ytrRI* regulon has yet to be clarified, although it may be subordinate to the *ypsRI* system [242]. The purpose of the quorum-sensing system in *Y. pestis* is also presently unknown. Indeed, Swift et al. reported that a strain carrying a mutated *ypeR* allele (the respective *luxR* homologue) does not show reduced expression of any tested virulence factors when compared to wild-type strains [243]. However, the same strain did display slightly attenuated pathogenicity in mice.

#### 4.7. Quorum-sensing in *A. tumefaciens*

*A. tumefaciens* is a pathogen that induces crown gall tumours in plants via the transfer of oncogenic DNA to the nucleus of its host. The tumours secrete opines which are used by *A. tumefaciens* as a nutrient source and as a chemical signal to induce conjugal transfer of Ti (tumour-inducing) plasmids to non-Ti plasmid-containing *Agrobacterium* cells within the tumour. Transfer of octopine-type Ti plasmids is induced by octopine [244] while transfer of the nopaline-type Ti plasmids is induced by agrocinopines [245]. The basis of opine-induced induction of the systems required for conjugation and catabolism of the appropriate opine in *A. tumefaciens* has been reviewed elsewhere [246,247].

In octopine-type Ti plasmids, the two systems are coordinately regulated by an activator called OccR [248–250]. This protein acts to positively regulate expression of the octopine catabolism genes and also expression of the LuxR homologue, TraR, required for conjugation [250]. This is because *traR* is located on a Ti plasmid-based, 14-gene, octopine-inducible operon (the *occ* operon) that encodes products required for the transport and catabolism of octopine [251,252]. The octopine signal from the crown gall tumour induces expression of OccR which, in turn, increases expression of *traR* and the other genes on the *occ* operon. However, TraR is unable to activate expression of the genes required for Ti plasmid conjugal transfer (the *tra* and *trb* genes) without a further signal, *N*-(3-oxooctanoyl)-L-homoserine lactone (OOHL). Like other acyl HSLs, OOHL is synthesised by a LuxI homologue, TraI, which is encoded by a gene on the Ti plasmid [77,250]. Regions showing sequence similarity to the *lux* box (known as *tra* boxes) can be found upstream of three operons regulated by TraR (*traAFB*, *traCDG* and *traI-trb*) [250,251,253]. It is noteworthy that, as *traI* is the first gene of the TraR-regulated *trb* operon, the gene encoding the acyl HSL synthase is the first gene of an operon that is regulated by autoinduction (as is the case in some other quorum-sensing systems).

A further LuxR homologue, called TrlR [254] or TraS [255], has also been found to be encoded on octopine-type

Ti plasmids. This protein shares a high degree of sequence similarity with TraR. However, a frameshift in the gene encoding TrlR results in the production of a LuxR homologue lacking part of its carboxy-terminal (DNA binding) domain. TrlR has a dominant-negative effect on the activity of TraR, possibly due to the formation of inactive TraR-TrlR heterodimers [254,255]. Whether this repressive outcome is the purpose of evolving such a hybrid protein as TrlR is unclear. The *trlR* gene is part of a mannopine-inducible operon and so it is possible that TrlR was once a functional LuxR homologue, involved in the induction of conjugal transfer in the presence of mannopine, and that it has subsequently picked up a mutation [254,255]. Although expression of *trlR* is induced in the presence of mannopine, this opine cannot induce expression of *trlR* in cells grown in the presence of succinate and glutamine [254,255]. This suggests that *A. tumefaciens* may be able to modulate its Ti plasmid conjugal transfer activity in a nutrient-dependent manner.

Regulation of conjugal transfer of nopaline-type Ti plasmids also relies on quorum-sensing. In this case, a repressor, AccR, controls the expression of nopaline catabolism genes (*acc*) and the conjugal transfer system [256,257]. Expression of a LuxR homologue (also called TraR) is repressed by the action of AccR [252]. This is because, in this case, TraR is located within a Ti plasmid-based AccR-regulated operon (the *arc* operon) which also includes at least three other genes, none of which are required for plasmid conjugal transfer [252]. The repressive effect of AccR is relieved in the presence of agrocinopines. This thereby allows expression of *traR* and the other genes on the *arc* operon [252]. TraR is then free to activate transcription of the *tra* and *trb* genes required for conjugal transfer, but only in the presence of OOHL. As is the case with *A. tumefaciens* strains carrying octopine-type Ti plasmids, OOHL is synthesised by a LuxI homologue called TraI [258–260].

An antiactivator of TraR, called TraM, has been found to be encoded on both octopine- and nopaline-type Ti plasmids [261,262]. Work using nopaline-type Ti plasmid-encoded TraM has indicated that this 11-kDa protein appears to act by binding to the carboxy-terminal domain of TraR in an OOHL-independent manner. This thereby prevents the LuxR homologue from binding to its target promoter sequences [263,264]. Particular amino acid residues that are important for this interaction have been mapped to both proteins. The repressive activity of TraM is likely to reside in the carboxy-terminus of the protein [263]. The physiological purpose of the protein may be to repress the onset of TraR-mediated activation of conjugal transfer at low cell densities [265]. Similarly, it may act to dampen down this quorum-sensing system and prevent prolonged activation by binding to, and interfering with, active TraR dimers [264]. As such, it is interesting that expression of *traM* is positively regulated by activated TraR [261,262]. It will be interesting to discover

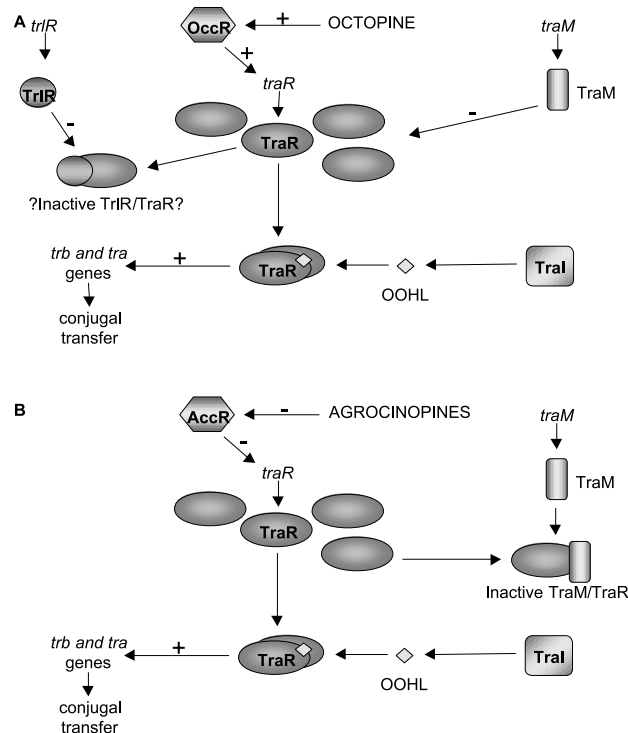


Fig. 8. The regulation of Ti plasmid conjugal transfer in *A. tumefaciens*. A: For octopine-type Ti plasmid conjugal transfer, *OccR*-mediated expression of *TraR* is induced by octopines. At high cell density, *TraR*–*OOHL* is able to activate expression of the *tra* and *trb* genes, resulting in conjugal transfer. Some *TraR* proteins may be sequestered due to the formation of inactive heterodimers with *TrIR* (although a clear physiological role for *TrIR* has not been demonstrated). *TraM* also acts as a negative regulator of *TraR*. However, *TraM* has not yet been demonstrated to interact with *TraR* in the octopine-type system. B: For nopaline-type Ti plasmid conjugal transfer, *AccR*-mediated repression of *TraR* is relieved by agrocinopines. At high cell density, *TraR*–*OOHL* is able to activate expression of the *tra* and *trb* genes, resulting in conjugal transfer. Basal levels of *TraR* are sequestered due to the formation of inactive heterodimers with *TraM*.

whether other quorum-sensing microorganisms carry *traM* homologues within their genomes.

Obviously, the regulation of Ti plasmid conjugal transfer is complex and involves multiple inputs (see Fig. 8). As already stated, *traR* is part of an opine-regulated operon on both nopaline- and octopine-type Ti plasmids. The purpose of regulating Ti plasmid conjugal transfer via opine induction may be to ensure that the ability to catabolise a given opine produced by a tumour is disseminated to all cells within a given population [252]. It is also of note that the quorum-sensing element of Ti plasmid conjugal transfer is controlled by the opine system [265]. Indeed, removing opine control of the system (via mutation of *accR*) results in dramatically lowering the population size at which cells become competent for conjugal transfer of nopaline-type Ti plasmids compared to wild-type [265]. Similarly, mutation of the gene encoding the antiactivator *TraM* separates quorum-sensing (which is lost) from auto-induction (which remains) [265]. That is to say, maintenance of the quorum-sensing component in this particular system requires opine-mediated control of *traR* expression and *TraM*-mediated control of *TraR* activity [265].

A final vexing question is, what are the possible benefits to *A. tumefaciens* cells from regulating Ti plasmid conjugal transfer by use of a quorum-sensing system? Results ob-

tained in a study by Piper and Farrand suggest that the answer may lie with the inefficient nature in which recipient agrobacterial cells inherit the Ti plasmids [265]. As such, maximising the number of donor cells within a population via use of a quorum-sensing system should also increase the chances of successful transfer of Ti plasmids to (transformation-incompetent) recipients [265].

The processes involved in the acyl HSL-mediated activation of *TraR* have been discussed elsewhere within this review (Section 3.1). Briefly, the association of *TraR* monomers with *OOHL* promotes dimerisation of the protein. Dimerisation causes *TraR* to become active thereby allowing it to bind to the promoter regions of the target genes within its regulon and promote transcription [58,59,62,63]. In the absence of *OOHL*, monomers of *TraR* predominantly associate with the cytoplasmic membrane of *A. tumefaciens*. However, in the presence of the ligand, *TraR* dimers can be found in the cytoplasm [59]. Based upon these observations, Farrand and associates have proposed a model whereby monomeric *TraR* is compartmentalised within the membrane from where it can only interact with external *OOHL* molecules. This interaction causes *TraR* monomers to dimerise thereby masking the hydrophobic region of these proteins and allowing them to enter the cytoplasm and interact with specific

DNA promoters [59]. Very recently, Zhu and Winans have proposed an alternative model based on results from an analysis of the proteolytic susceptibility of monomeric TraR compared to ligand-associated dimeric TraR expressed in *E. coli* [62]. They hypothesise that in *A. tumefaciens*, monomeric TraR is subject to rapid proteolytic degradation whereas dimerisation of the protein in the presence of OOHl stabilises TraR against such activity [62]. Whether either model can be extended to other quorum-sensing systems remains to be analysed.

#### 4.8. Quorum-sensing in *Rhizobium* spp.

Species of *Rhizobium* are best known for their ability to sustain symbiotic relationships with leguminous plants via the formation of nitrogen-fixing nodules on roots [266–268]. Many of the gene products required for this symbiotic relationship are often encoded on large, so-called symbiotic (Sym) plasmids carried by *Rhizobium* spp. The quorum-sensing system of one species of this genus, *R. leguminosarum*, is in the early stages of its characterisation. From the evidence so far, it appears to be complex and involves many LuxR and LuxI homologues. The chromosomally encoded CinRI proteins appear to sit at the top of this sensing hierarchy [269]. The product of the *cinI* gene is responsible for the synthesis of *N*-(3-hydroxy-7-*cis*-tetradecenoyl)-L-homoserine lactone (HtdeDHL). This acyl HSL, containing 14 carbons in its acyl side chain, had previously been identified by two separate groups and was originally believed to be a rhizobial bacteriocin (called *small*) [270,271]. This was because HtdeDHL has the apparent ability to inhibit growth in certain strains of *R. leguminosarum* [270]. Rather than being a bacteriocin per se, it may be that HtdeDHL induces inhibitory functions in sensitive strains of the organism.

Expression of *cinI* is dependent upon HtdeDHL-activated CinR [269]. Similarly, the products of *cinI* and *cinR* regulate the expression of at least one other chromosomally encoded acyl HSL synthase and two other acyl HSL synthases encoded on the Sym plasmid, pRL1JI, of *R. leguminosarum*. This observation is based on the fact that mutation of *cinI* and *cinR* has an adverse effect on the different acyl HSL profiles generated by non-pRL1JI- and pRL1JI-carrying strains of the organism [269].

One of the plasmid-encoded *luxI* homologues that is positively regulated by CinRI has been characterised [272]. The protein product of this locus, RhiI, directs the synthesis of HHL, OHL and, possibly, an acyl HSL possessing a seven-carbon acyl side chain [269,272]. The expression of *rhiI* is regulated by RhiR, a LuxR homologue that is also encoded by the Sym plasmid. As CinRI affect the transcription of *rhiR* [269], CinRI-mediated regulation of *rhiI* may be performed indirectly, through RhiR. It is believed that RhiR is activated by the acyl HSLs produced by RhiI and by some of the molecules produced by the non-characterised acyl HSL synthases of *R. legumino-*

*sarum* [272]. As well as regulating *rhiI*, RhiR up-regulates the expression of genes within the *rhiABC* operon [272,273]. The protein products of these genes are strongly expressed in the rhizosphere but are not found in the differentiated bacteroids of root nodules. They bear no significant similarity to any other proteins in databases and their function is unclear [273,274].

At the present time, it is uncertain which cellular processes the quorum-sensing system of *R. leguminosarum* regulates. The RhiRI proteins may be involved in the inhibition of nodulation as disruption of *rhiI* leads to a very slight increase in nodule formation by the strain relative to wild-type [272]. Evidence also suggests that CinRI are involved in the positive regulation of conjugation as the presence of a mutated *cinI* allele in both donor and recipient strains results in a dramatic reduction in transfer of pRL1JI [269]. This regulatory activity is thought to be directed via the induction of one of the non-characterised *luxRI* regulons [269]. It is interesting that sequencing of pNGR234a from another isolate of *Rhizobium* has revealed that this Sym plasmid carries open reading frames sharing high sequence identity with the conjugal transfer genes of *A. tumefaciens* [275]. Genes sharing sequence identity with *traR*, *traI* and *traM* have also been found on pNGR234a [275]. This indicates that regulation of conjugal transfer of Sym plasmids by *Rhizobium* spp. may bear some similarity to conjugal transfer of Ti plasmids by *A. tumefaciens*.

Acyl HSL production is prevalent in other rhizobial species. A survey of bacterial signalling molecule production has revealed that many *Rhizobium* species produce a rich diversity of acyl HSLs, with isolates commonly producing from one to nine detectable signals [158]. For example, *R. meliloti* has been shown to produce an array of compounds with acyl HSL-like activity while *R. fredii* produces just one strongly non-polar compound with such activity [90,158]. *R. etli* CNPA512 also produces multiple acyl HSLs; *luxI* and *luxR* homologues have been identified in this organism and the gene products have been shown to be involved in down-regulating nodulation [276].

#### 4.9. Acyl HSL-based quorum-sensing in other Gram-negative bacteria

Many other Gram-negative bacterial species produce acyl HSLs or possess LuxRI homologues. For example, two common fish pathogens, *Aeromonas hydrophila* and *Aeromonas salmonicida*, express LuxRI homologues termed AhyRI and AsaRI respectively [277]. The major acyl HSLs synthesised by both AhyI and AsaI are BHL and HHL. The signalling systems serve to modulate the production of serine protease in both species [277, 278]. A further fish pathogen, *Vibrio anguillarum*, expresses the LuxRI homologues VanRI [279]. VanI catalyses the synthesis of *N*-(3-oxodecanoyl)-L-homoserine lac-

tone (ODHL). Inactivation of *vanI* did not reduce the virulence of *V. anguillarum* in a fish infection model.

Production of acyl HSL(s) by the insect pathogen *Xenorhabdus nematophilus* has been detected via use of a bioluminescent sensor strain [280]. Although the exact identity of the signalling molecule(s) was not determined in this study, *N*-(3-hydroxybutanoyl)-L-homoserine lactone (HBHL) was shown to restore virulence to an avirulent *X. nematophilus* transposon mutant. In another soil-dwelling inhabitant, *Chromobacterium violaceum*, purple pigment production, chitinolytic activity, antibiotic production and virulence factor production are all regulated, in part, by HHL [89,281,282]. A non-autoinducer-producing mutant of *C. violaceum* is commonly used as a test strain for acyl HSL production in other bacteria by virtue of expressing the pigment in the presence of short-chained acyl HSLs [89,93,283].

*Rhodobacter sphaeroides* is a free-living microorganism that has been shown to utilise an acyl HSL-based quorum-sensing system. The microorganism synthesises *N*-(7-*cis*-tetradecenoyl)-L-homoserine lactone (tdeDHL) via expression of *CerI* [284]. Inactivation of *cerI* results in the formation of large aggregates of cells in liquid cultures. Pre-conditioning of liquid broth by the addition of exogenous tdeDHL prevents *cerI* strains forming these cellular aggregates.

## 5. Quorum-sensing in *V. harveyi*: a model for a new language?

*V. harveyi* has evolved an alternative quorum-sensing mechanism for monitoring cell density and effecting transcription of specialised gene sets. In this microorganism, two independent quorum-sensing systems exist, each of which synthesises, detects and responds to a specific signalling molecule to control bioluminescence. One of these signals is the acyl HSL, HBHL, and its synthesis requires two genes, *luxL* and *luxM*, neither of which shows similarity to *luxI* of *V. fischeri* (Section 3.2.1) [285,286]. The structure of the second signalling molecule (AI-2) is yet to be determined, although the gene encoding the protein possibly responsible for its synthesis (*luxS*) has been identified [287].

Both signals are detected by sensor proteins related to the sensor kinase proteins of two-component regulator systems [285]. HBHL is sensed by LuxN, which is a two-component hybrid sensor kinase, containing both sensor kinase and response regulator domains [285]. AI-2 is also sensed by a two-component hybrid sensor kinase, similar to LuxN, called LuxQ. An additional protein, LuxP, related to the periplasmic ribose binding protein of *E. coli*, is also required for sensing AI-2 [288]. It is proposed that LuxP is the primary receptor for AI-2 and that the LuxP–AI-2 complex interacts with LuxQ. These signalling pathways act in parallel, because null mutations

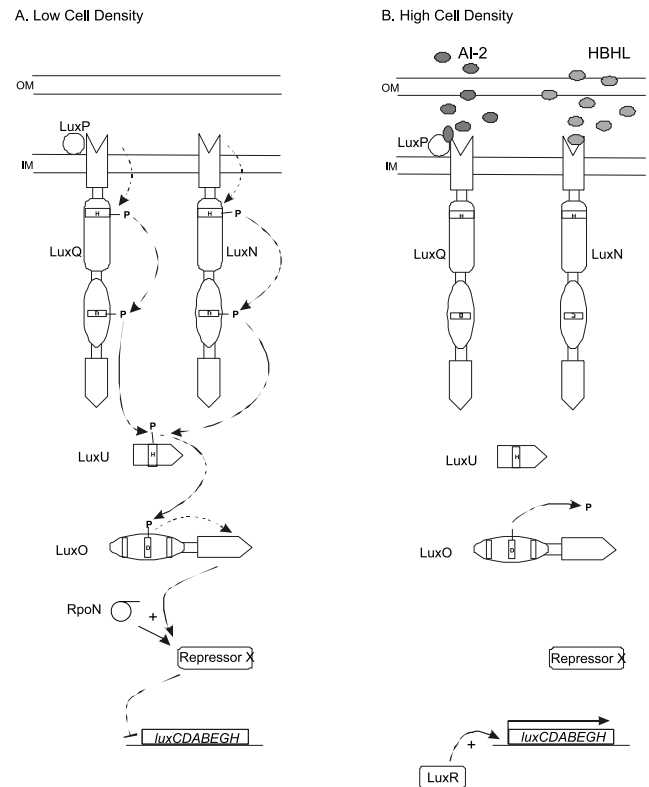


Fig. 9. Quorum-sensing and the regulation of bioluminescence in *V. harveyi*. A: At low cell density, in the absence of HBHL and AI-2, LuxN and LuxQ autophosphorylate. A multistep phosphorelay continues through the shared phosphotransfer protein, LuxU, ultimately phosphorylating the response regulator, LuxO. Phosphorylated LuxO, in conjunction with  $\sigma^{54}$ , is thought to indirectly repress transcription of the genes required for bioluminescence by activating the transcription of an unidentified negative regulator (repressor X). B: At high cell density, corresponding to a critical concentration of signal molecules, LuxN and LuxQ/P sense their cognate signals and switch from kinases to phosphatases. Consequently, dephosphorylation of LuxO results in its inactivation thereby preventing the up-regulation of repressor X activity. Such de-repression allows transcription of *luxCDABEGH* and, hence, emission of light. LuxR is also required for the positive regulation of the *lux* operon [293].

in either *luxN* or *luxQ* retain cell density-dependent expression of bioluminescence [288].

A model for the regulation of quorum-sensing in *V. harveyi* has been proposed (Fig. 9). At low cell density, in the absence of signal molecules HBHL and AI-2, the sensor kinases, LuxN and LuxQ, autophosphorylate at conserved histidine residues. This potentiates a phosphorelay through the shared phosphotransfer protein LuxU [289], ultimately phosphorylating the response regulator LuxO [290–292]. In the phosphorylated state, LuxO, in co-operation with  $\sigma^{54}$ , is thought to activate the transcription of an unidentified gene, the product of which represses the transcription of the *lux* structural genes (*luxCDABEGH*) [293]. Conversely, at high cell densities, in the presence of signal molecules, LuxN and LuxQ lose their kinase activity and switch to being phosphatases [292]. Consequently, dephosphorylation of LuxO results in its

inactivation, meaning that the production of the unknown transcriptional repressor of the *lux* genes is no longer promoted and, hence, transcription of the *lux* structural genes is permitted to ensue [291,293]. A positive transcription factor, called LuxR, is also required for *lux* expression. Although a DNA binding protein, it is not similar to LuxR from *V. fischeri* [294,295].

Indirect evidence suggests that the quorum-sensing systems of *V. harveyi* play roles not only in the regulation of bioluminescence, but also in the regulation of siderophore expression, toxin production and colony morphology [293,296]. Therefore, although these systems regulate global processes, they are mechanistically unlike that of the *V. fischeri* paradigm. This implies that acyl HSLs can also act through a phosphorylation–dephosphorylation cascade, rather than LuxR-like activators. To this end, it is intriguing that *luxO* and *luxU* homologues have been identified in *V. fischeri* and that disruption of *luxO* in this organism positively affects light output [297]. It is therefore possible that *V. fischeri* possesses two systems for the regulation of bioluminescence, the prototypic LuxRI system and a system similar to that utilised by *V. harveyi*.

### 5.1. AI-2-like molecule production by other microorganisms

At first glance, the presence of two parallel signalling systems in *V. harveyi* implies a certain amount of redundancy. However, there is evidence to suggest that the timing and intensity of response to the two quorum-sensing signals of the organism is different [291,292]. Indeed, it has been proposed that while *V. harveyi* recognises HBHL as a signal produced predominantly by other *V. harveyi* cells, the AI-2 input may allow this microorganism to sense and respond to cells of differing species [298]. So far, very few microorganisms have been shown to produce and utilise HBHL as a quorum-sensing molecule and so HBHL-based signalling may well be specific to *V. harveyi* cells. However, if the AI-2 factor does act as an interspecies signalling molecule as surmised, one would predict that other bacterial species should have the capacity to produce AI-2-like molecules. To screen for this possibility, a *V. harveyi* sensor strain (BB170) carrying a mutation in *luxN* (therefore induced to emit light only in the presence of AI-2-like molecules, but not in the presence of acyl HSLs) was used to analyse the cell-free culture supernatants from various bacterial species. Results showed that the culture fluids from strains of *V. cholerae*, *V. parahaemolyticus*, *V. anguillarum*, *V. alginolyticus*, *V. natriegens*, *Y. enterocolitica* and *Photobacterium phosphoreum* all contained a compound with AI-2-like activity [298]. The synthesis of AI-2-like molecules was therefore shown to be more widespread within the bacterial kingdom than had originally been believed.

Recently, Surette and Bassler illustrated that when certain strains of *E. coli* and *Salmonella typhimurium* were grown in Luria–Bertani (LB) medium supplemented with

glucose (or other specific carbohydrates), these two organisms also produced AI-2-like molecules [299]. Genes required for expression of AI-2-like activity by *S. typhimurium* and *E. coli* have now been identified. Both named *luxS*, they share significant sequence identity with each other and with *luxS* of *V. harveyi* [287]. AI-2-like activity is restored to a *S. typhimurium luxS* mutant when the strain is complemented with a plasmid containing the *luxS* gene of either *V. harveyi* or enterohaemorrhagic *E. coli* O157:H7 (EHEC) [287]. Interestingly, one strain of *E. coli* that does not produce AI-2-like activity is DH5 $\alpha$  [287,299]. This highly domesticated strain has been found to contain a frameshift mutation in its *luxS* gene, so that the gene encodes a truncated (and non-functional) LuxS protein [287].

An analysis of databases has revealed that many microorganisms possess *luxS* homologues within their genomes. Such bacterial species include *Haemophilus influenzae*, *Bacillus subtilis*, *Borrelia burgdorferi*, *Neisseria meningitidis*, *Neisseria gonorrhoeae*, *Enterococcus faecalis*, *Yersinia pestis*, *Helicobacter pylori*, *Streptococcus pyogenes*, *Streptococcus pneumoniae*, *Mycobacterium tuberculosis*, *Deinococcus radiodurans* and *Campylobacter jejuni* [287]. It should be noted that, to date, there is not enough evidence for one to label LuxS homologues as the synthases of AI-2 activity. However, AI-2-like activity has been detected in the cytoplasmic fraction of wild-type *H. pylori* cells but not in the cytoplasmic fraction of the equivalent *luxS* mutant [300]. This therefore implies that LuxS is not merely involved in the secretion of the signalling molecule. As stated earlier, the structure of AI-2 (or any molecule with AI-2-like activity) has yet to be elucidated, making it impossible to predict whether all of the molecules possessing AI-2-like activity are identical in structure. Preliminary chemical analysis of AI-2 from *V. harveyi* suggests that it is less than 1 kDa in size, and is polar. It is also resistant to temperatures up to 80°C but is sensitive to high pH [299]. In addition, Forsyth and Cover have shown that the molecule with AI-2-like activity produced by *H. pylori* is unaffected by exposure to proteases [300].

The role (if any) played by AI-2-like compounds in the vast majority of microorganisms producing these signalling molecules or encoding *luxS* homologues remains unknown. However, as reviewed earlier, AI-2 of *V. harveyi* is involved in the induction of bioluminescence in that organism. The AI-2-like molecules produced by enteropathogenic *E. coli* (EPEC) and EHEC may act as signals for quorum-sensing-mediated control of virulence. Evidence for this stems from a study showing that genes encoding components of a type III secretion system in EPEC and EHEC are modestly up-regulated in response to media which have been conditioned by prior growth of other *E. coli* strains (but not DH5 $\alpha$ ) [301]. Consistent with these findings, Sperandio and colleagues also showed that a *luxS* mutant strain of EPEC produces lower amounts of secreted proteins than does its wild-type equivalent [301].

It has been hypothesised that AI-2-like molecules synthesised by the normal flora of the intestine could actually serve to induce this virulence function in pathogenic *E. coli* strains [301].

In another human pathogen, *Shigella flexneri*, expression of a transcriptional regulator VirB (involved in the temperature-dependent up-regulation of an invasion phenotype) has been shown to be slightly responsive to the presence of AI-2-like compounds [302]. However, the expression of some of the genes up-regulated by VirB is unaffected in a *luxS* mutant strain of *S. flexneri*. Moreover, the *luxS* mutation does not impinge on the invasiveness or dissemination capabilities of the strain. Similarly, the overall virulence of the *luxS*<sup>−</sup> strain remains unaffected when tested in a mammalian model system [302]. In *H. pylori*, disruption of the *luxS* gene also has no effect on any discernible phenotype other than preventing production of an AI-2-like compound [300,303]. Comparative 2D PAGE analysis of proteins extracted from wild-type and *luxS* mutant strains of *H. pylori* grown under identical conditions reveals no obvious differences in protein production between the two strains [303].

Analysis of the production profile of the AI-2-like molecule made by a strain of *S. typhimurium* has shown that it is only transiently expressed, being produced in mid-exponential phase of growth and degraded during stationary phase [299,304]. As previously stated, when cells are grown in LB, supplementary glucose (or another carbohydrate) is required to induce the synthesis of the molecule; the amount of factor produced is dependent on the starting concentration of the glucose [299,304]. As well as this link with supplementary carbohydrate metabolism, certain environmental conditions (such as acidic pH and high osmolarity) also induce AI-2-like activity to be expressed by *S. typhimurium* [304]. Initial analysis of the uncharacterised AI-2 degradative machinery of *S. typhimurium* has shown that as well as being induced by depletion of the supplemented carbohydrate, it is also stimulated in conditions of low osmolarity [304]. Since the environmental cues that enhance production of the AI-2-like molecule produced by *S. typhimurium* are likely to be found within a host, while those cues that enhance degradation of the molecule are likely to be encountered as the organism leaves the host, Surette and Bassler have proposed that this AI-2-like molecule is likely to be involved in the control of host-associated phenotypes and even pathogenicity [304].

*E. coli* might also produce other signalling molecules, distinct from the AI-2-like compound just described. One report outlined how *lacZ* fusions had been created randomly in four genes that were activated by two compounds that were present in the conditioned LB medium of a stationary phase *E. coli* culture [305]. Although the nature of these inducing factors has yet to be fully characterised, they are likely to be different from the AI-2-like molecule because, unlike the latter, they are resistant to both alkaline conditions and heat treatment of 100°C for

10 min. They were also found to be present in the conditioned medium of *E. coli* DH5α (which does not produce the AI-2-like compound). Characterisation of the conditioned medium-activated (*cma*) fusions showed them to have been created via insertions within genes encoding functions associated with the metabolism of amino acids [305]. A further study has shown that an extracellular factor within an *E. coli* conditioned medium inhibits the initiation of chromosomal replication in cultures of the same organism [306]. Again, the nature of the active compound remains undefined but it is most prevalent in late exponential and early stationary phases of growth (therefore differing from the production profile of the AI-2-like molecule). The mechanism of inhibition is also unknown. Intriguingly, the factor was still active in cells prevented from carrying out transcription or translation [306].

## 6. Acyl HSL-based cross-communication

### 6.1. Microbial cross-communication

Hopefully, this review will have highlighted how widespread an occurrence acyl HSL-based cell signalling is among Gram-negative bacterial species. As the signalling molecules are so similar (indeed, many organisms utilise the same species of molecule to regulate different respective phenotypes), one would predict that some form of interspecies communication would be likely in environments where different acyl HSL-producing bacterial species inhabit a common locale. The molecular feasibility of such an occurrence has been investigated in numerous studies. Results from these have indicated that various LuxR homologues can interact with non-cognate acyl HSL molecules [48,60,89,96,158,278,307–310]. Depending on the LuxR homologue and acyl HSLs in question, such interactions can result in the activation of the transcriptional regulator. Conversely, when assayed in the presence of the cognate acyl HSL, other species of acyl HSL have been found to essentially block activation of the LuxR homologue, presumably by competing for the ligand binding site on the protein.

Extrapolating from such studies, it seems likely that, in the environment, one bacterial community could produce acyl HSLs that could inhibit the quorum-sensing phenotypes expressed by another community. As such, one might predict that organisms exist which produce acyl HSLs yet do not utilise these molecules to regulate physiological processes of their own. Similarly, LuxI homologues may have evolved to direct the synthesis of multiple acyl HSL molecules as a means of producing such antagonistic weaponry. One reason why bacteria like *Xanthomonas* spp. use non-acyl HSL-based quorum-sensing systems (as described in Section 7.2) could be to gain a competitive advantage over its neighbouring bacteria by avoiding such interference and cross-talk.

It is important to remember that a bacterial species could also respond to the presence of foreign acyl HSLs by utilising the signalling molecules to up- or down-regulate competitively advantageous phenotypes. Examples of such beneficial processes would include the expression of competitor-inhibitory antibiotics. As such, it is relevant that an *in vivo* study, carried out using bacteria in the rhizosphere of wheat, has demonstrated that phenazine biosynthesis can be stimulated in one population of *P. aureofaciens* by acyl HSLs produced by a distinct population of the same organism [148]. Pierson et al. showed that *P. aureofaciens* populations are likely to share their wheat rhizosphere environment with many acyl HSL-producing microorganisms [311]. In another study, Cha et al. demonstrated that TraR of *A. tumefaciens* can also respond to signals (cognate and non-cognate) produced by other microorganisms that occupy its habitat [158]. Thus, it is important to consider the total number of cells within heterogeneous bacterial communities when analysing the dynamics of a single acyl HSL-receptive microorganism in the environment.

Interestingly, LuxR homologues (both termed SdiA) have been identified in *E. coli* and *S. typhimurium*, two organisms that do not produce acyl HSLs [312,313]. It is possible that these proteins are required for the regulation of physiological functions when activated by a host-produced (and as yet unidentified) signalling molecule [314–317]. However, the proteins are not thought to interact with the AI-2-like molecule produced by strains of *E. coli* and *S. typhimurium* [304,316,317].

SdiA of *E. coli* is involved in the regulation of the *ftsQAZ* gene cluster, which encodes proteins important for cell division. It is also involved in the repression of genes encoding an outer membrane protein required for cellular adhesion (intimin) and at least one effector protein (EspD) transported by the previously mentioned (Section 5.1) AI-2-inducible type III secretory system in EHEC O157:H7 [316]. In correlation with these data, SdiA of EHEC O157:H7 has been shown to bind to promoter regions of the genes encoding FtsQAZ, EspD and intimin [316]. The SdiA protein of *S. typhimurium* has been shown to regulate up to 10 different genes, some of which encode virulence-related functions [313]. It has been proposed that the two organisms may use these proteins as a means of sensing and responding to acyl HSL-producing competitors. As such, the addition of exogenous acyl HSLs does mildly stimulate SdiA-mediated transcription of *ftsQAZ* and does repress EspD and intimin production in *E. coli* [315,317].

In contrast to using acyl HSLs for their own advantage, recent evidence suggests that other bacteria may attempt to upset cell-to-cell communication by actively destroying the message. Dong et al. isolated an enzyme (AiiA) from a strain of *B. subtilis* and showed that it was capable of inactivating acyl HSLs [318]. Expression of *aiaA* in *Ecc* caused a reduction in the secretion of extracellular en-

zymes and an attenuated pathogenicity phenotype. Although AiiA does not share significant sequence similarity with any other protein in the database, it does contain a conserved motif commonly found in zinc metalloenzymes.

It is tempting to hypothesise that *B. subtilis* uses AiiA activity to degrade acyl HSLs produced by competing microorganisms. For this reason alone, it will be interesting to discover if enzymes that perform a similar function to AiiA are expressed by other bacterial species. To this end, a soil-based microorganism (*Variovorax paradoxus*) has been isolated recently and shown to have the ability to degrade acyl HSLs [319]. It would also be logical if proteins responsible for the degradation of acyl HSLs were expressed by the very microorganisms that produce such molecules, because the enzyme could be used as a means of damping down quorum-sensing systems. Evidence for the existence of such regulation is, for the most part, circumstantial. However, it is known that OHHL levels decline over time in an *Ecc* culture [196]. The reason for this drop, and the processes involved in its manifestation, are presently unknown.

## 6.2. Microbial–eukaryotic communication

Many of the recognised acyl HSL-producing microorganisms are renowned for their capacity to associate with higher organisms in either pathogenic or symbiotic relationships. Higher organisms may have evolved mechanisms that enable them to detect and respond to acyl HSL messaging systems in order to prevent or limit infection. For example, the macroalga *Delisea pulchra* produces compounds, commonly known as furanones (Fig. 5), which have the ability to specifically interfere with acyl HSL-mediated quorum-sensing systems [296,320–322]. It is thought that these compounds compete with cognate acyl HSL molecules for the binding site on receptor proteins. Although furanones have not yet been shown to physically bind to any LuxR homologues, the compounds are capable of displacing radiolabelled OHHL from the surface of *E. coli* cells that are overexpressing LuxR [323]. Interestingly, a study by Teplitski et al. has revealed that higher plants such as pea, crown vetch and tomato all produce unidentified compounds that are capable of interacting with acyl HSL-dependent quorum-sensing systems [324]. Structural characterisation of these molecules will reveal whether they are related to the furanones secreted by *D. pulchra* or whether they represent a distinct group of acyl HSL mimics.

As well as serving as prokaryotic cell-to-cell signals, some acyl HSLs may act as virulence factors per se. In particular, one of the *P. aeruginosa*-produced molecules, OdDHL, could act as a potential modulatory agent of mammalian immune systems. For example, Telford et al. demonstrated that this acyl HSL could inhibit the proliferation of lymphocytes and tumour necrosis factor pro-

duction by macrophages [325]. A separate study showed that acyl HSLs could hinder nucleotide-stimulated production of an antibacterial factor by CF (but not normal) human tracheal cells [326]. This response is probably due to the inhibition of expression of specific cellular receptors. Elsewhere, Lawrence et al. reported that OdDHL inhibits muscle contraction in porcine blood vessels [327]. They proposed that this could be a method used by *P. aeruginosa* populations to increase blood supply, and therefore nutrient supply, to sites of infection without stimulating a host immune response.

As described in this review, many quorum-sensing systems regulate phenotypes that are undesirable to humans in terms of health and plant crop maintenance. As pathogenic microorganisms with mutations in their quorum-sensing systems display reduced virulence phenotypes, disruption of these signalling pathways would therefore seem to offer a window of opportunity for antimicrobial therapy [328–330]. One target for such therapy would be the inhibition of acyl HSL synthesis by LuxI homologues. Another would be the prevention of acyl HSL-mediated activation of LuxR homologues, perhaps by the use of furanones or other synthetic acyl HSL analogues [331]. Of course, care must be taken to ensure that such compounds do not promote adverse immunological effects in humans, as do some naturally occurring acyl HSLs.

A recent report has highlighted that a transgenic tobacco plant, expressing YenI of *Y. enterocolitica*, is capable of producing acyl HSL molecules [332]. Thus, there seems to be a very real possibility of using a similar approach in other plants to promote quorum-sensing phenotypes that are of benefit to the maintenance of commercial crops (such as phenazine production by *P. aureofaciens*). Conversely, plants could be engineered to synthesise acyl HSLs that are antagonistic to the quorum-sensing systems of pathogens like *Ecc* and *P. stewartii* subsp. *stewartii*. If such technology could be extended to humans, it would have obvious implications on the possible disarmament of pseudomonad communities that flourish in the lungs of patients afflicted with CF.

## 7. Non-acyl HSL-mediated quorum-sensing in Gram-negative bacteria

Two of the better characterised examples of non-acyl HSL quorum-sensing systems are those employed by the plant pathogens *Ralstonia solanacearum* and *Xanthomonas campestris* and this will be the topic of discussion in this section.

### 7.1. Quorum-sensing in *R. solanacearum*

The phytopathogen *R. solanacearum* causes vascular wilt diseases of many plants, primarily by its ability to produce an acidic EPS and plant cell wall-degrading extra-

cellular enzymes [333]. Expression of these virulence determinants occurs in an apparent cell density-dependent manner, with maximal expression at high cell densities [334]. The LysR type regulator, PhcA, is central to the complex regulation of EPS and extracellular enzymes, and hence pathogenicity in *R. solanacearum*. Consequently, a *phcA* mutant has decreased levels of EPS and extracellular enzymes and is almost avirulent [335].

PhcA activity is regulated by a two-component regulatory system, which in turn is responsive to the quorum-sensing signal molecule, 3-hydroxypalmitic acid methyl ester (3OH PAME; see Fig. 5). Unlike other signal molecules, 3OH PAME is active both in solution and in the vapour phase [336,337]. The gene product of *phcB* is essential for the synthesis of this novel molecule. Amino acid sequence analysis of PhcB revealed several motifs typical of small-molecule SAM-dependent methyltransferases [337]. This is indicative that PhcB may catalyse the synthesis of 3OH PAME by conversion of a fatty acid, naturally occurring in the cell, to its methyl ester. Exogenous addition of 3OH PAME to cultures at low cell density does induce precocious production of EPS and enzymes [334,337].

The proteins PhcS and PhcR make up the two-component system responsive to 3OH PAME; PhcS is a predicted histidine kinase sensor and PhcR is similar to response regulators. However, PhcR is quite unusual in that its putative output domain resembles the histidine kinase domain of a sensor protein. The exact biochemical mechanism by which PhcS and PhcR relay sensory information to PhcA is unclear. However, genetic tests revealed that PhcS and PhcR act together to negatively regulate the expression of PhcA-regulated genes in the absence of 3OH PAME [338]. The current hypothesis is that at low cell density, when the concentration of 3OH PAME is low, PhcS phosphorylates PhcR, which acts to repress expression of *phcA*. Because PhcR does not appear to contain a DNA binding domain, it is possible that PhcR acts via other components of the signal cascade, or that it interacts with PhcA to reduce its activity. When 3OH PAME reaches a critical threshold concentration, it is thought to reduce the ability of PhcS to phosphorylate PhcR, resulting in increased expression or activity of PhcA and production of PhcA-regulated virulence factors. Although 3OH PAME synthesis, and the Phc system, was originally thought to be restricted to strains of *R. solanacearum*, it has recently been shown that strains of the chemolithoautotroph *R. eutropha* use a similar mechanism to control expression of motility and siderophore synthesis [339].

*R. solanacearum* also contains a typical acyl HSL-based quorum-sensing system, employing two signals, one of which is probably OHL while the identity of the other is currently under debate [158,338]. Homologues of *luxI* and *luxR* have also been identified and are designated *solI* and *solR*. Although inactivation of *solI* eliminates synthesis of acyl HSLs, production of EPS and extracellular enzymes is

not affected. The ability of *R. solanacearum* to wilt tomato plants is similarly unaffected [338]. Expression of *solR* and *solI* is regulated by the 3OH PAME-dependent system via PhcA, and they show the same cell density-associated expression as other PhcA-dependent virulence genes. Additionally, the acyl HSL quorum-sensing system in *R. solanacearum* requires RpoS [340]. Thus, not only is this quorum-sensing system part of a more complex cell signalling hierarchy, but it is also controlled by another global regulator, implying an important role in the bacterial life cycle. Flavier et al. suggested that because *solR* expression is regulated concomitantly with several virulence genes via PhcA, this system is likely to have a role in planta during pathogenesis [340]. The reader should be aware that an extensive review of the complex regulatory network controlling pathogenesis in *R. solanacearum* is available elsewhere [341]; a simplified model is shown in Fig. 10.

## 7.2. Cell-to-cell signalling in *X. campestris*

*X. campestris* pv. *campestris* (*Xcc*), the black rot pathogen of cruciferous plants [342], produces a range of extracellular enzymes (including proteases, pectinases and endoglucanase) and EPS, which are collectively essential for pathogenesis [343]. As with *R. solanacearum*, the production of these factors is strictly regulated in an apparent cell density-related manner, both during growth in liquid media and during disease. For example, in batch cultures of *Xcc*, high levels of extracellular enzymes are only attained during late exponential and stationary phases of growth [344].

In *Xcc* 8004, production of extracellular enzymes and

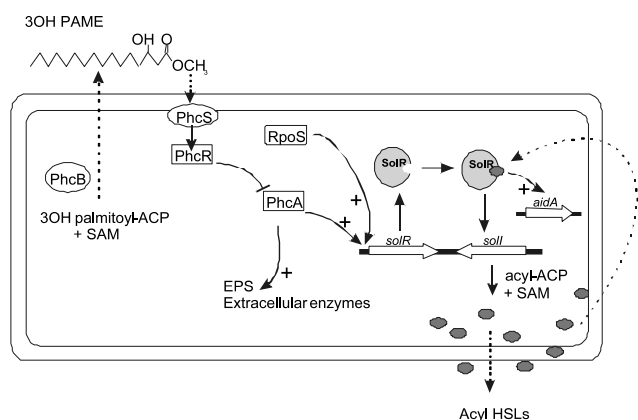


Fig. 10. Hierarchical quorum-sensing in *R. solanacearum*: regulation of pathogenicity and acyl HSL production by a novel signal molecule. At low cell density, when the concentration of 3OH PAME is low, PhcS phosphorylates PhcR. Phosphorylated PhcR negatively regulates the transcriptional regulator, PhcA. As cell density increases and 3OH PAME reaches a critical concentration, it interacts with PhcS, reducing its kinase activity. PhcR becomes dephosphorylated, thus relieving repression of PhcA. This results in increased expression of PhcA-regulated gene products (namely extracellular enzymes and EPS). PhcA also regulates a quorum-sensing system (*solI/solR*) which in turn regulates expression of a gene of unknown function (*aidA*) [338].

EPS has been shown to be subject to regulation by the *rpf* (regulation of pathogenicity factors) cluster, comprising some nine genes (*rpfA–I*) [344–348]. Mutations in many of the genes within the *rpf* cluster lead to qualitatively similar phenotypes, i.e. co-ordinate down-regulation of synthesis of all extracellular enzymes and EPS. This is intriguing as the genes themselves encode products with diverse biochemical roles. Two of the genes, *rpfB* and *rpfF*, are implicated in regulation mediated by a small diffusible molecule called DSF (for diffusible signal factor) [344]. The first intimation of this came from the observation that extracellular enzyme production could be restored to *rpfF* mutants when grown in close proximity to wild-type strains of *Xcc*. This suggested that restoration of the enzyme phenotype was occurring due to cross-feeding via a diffusible substance. Subsequently, it was shown that a low-molecular-mass hydrophobic molecule could be extracted from *Xcc* culture supernatants, which stimulates enzyme production by the *rpfF* mutant 10-fold. Subsequent tests showed that *rpfB* mutants cannot make DSF, nor can they be phenotypically corrected by exogenous addition of DSF.

Despite concerted efforts to solve the structure of DSF, it is as yet undetermined. However, a number of lines of evidence suggest that DSF is not an acyl HSL, although it is probably a fatty acid derivative. Preparations of DSF have no activity in bioassays that detect a range of acyl HSL molecules, nor can purified acyl HSLs substitute for DSF activity. There are no amino acid sequence homologies between the predicted gene products of *rpfF* and *rpfB* and the various LuxI and LuxR families of proteins. RpfB is predicted to be a long-chain fatty acyl CoA ligase, whereas RpfF shows some relatedness to enoyl CoA hydratases. Acid-hydrolysed lipid preparations from *Xcc* cells and C<sub>10</sub> and C<sub>12</sub> purified fatty acids possess DSF-like activity, although chromatographic analysis suggests that DSF itself is more polar than free fatty acids. Taken together these results indicate that DSF may be a fatty acid derivative and Barber et al. have hypothesised that RpfF and RpfB are involved in diverting intermediates of lipid metabolism to DSF production [344].

Transposon fusion studies revealed that transcription of *rpfF* and *rpfB* occurs throughout growth, although the rate appears to increase steadily into the stationary phase. Addition of DSF had no effect on either the kinetics or magnitude of expression of *rpfF* and *rpfB*. Thus, with respect to these steps in its presumed biosynthetic pathway, DSF does not behave in an autoregulatory fashion. Furthermore, early addition of DSF to wild-type bacteria does not stimulate premature expression of the *prtA* gene, which encodes the major protease of the organism. This indicates that DSF cannot be the sole determinant of timing of enzyme synthesis [344].

Recent work by Slater et al. has revealed a functional connection between the DSF system and a two-component regulatory system encoded by the *rpfGHC* operon that is

immediately adjacent to *rpfB* and *rpfF* and is convergently transcribed [348]. RpfC is predicted to encode a hybrid two-component regulator, containing both sensor kinase and response regulator domains. It also contains an additional carboxy-terminal phosphorelay HPt domain [346,349]. RpfH is structurally related to the membrane-spanning sensor domain of RpfC, but does not contain a histidine kinase domain. Under the conditions tested, RpfH appeared largely dispensable for pathogenicity factor synthesis as an in-frame deletion of *rpfH* led to only minor effects on exoenzyme and EPS levels [348]. RpfG encodes a response regulator protein containing a typical receiver domain attached to a specialised version of a HD domain. RpfG belongs to the HD-GYP subgroup of the HD superfamily. The exact role of HD-GYP domain proteins remains unclear although it has been speculated that they are phosphodiesterases involved in di-guanylate signalling [350].

Mutation of either *rpfC* or *rpfG* leads to down-regulation of pathogenicity factor production. This, together with the fact that *rpfG* is in an operon with *rpfC*, strongly suggests that RpfG is the cognate response regulator to RpfC and that this two-component system regulates the synthesis of extracellular enzymes and EPS in response to environmental signals. Mutations within *rpfC* or deletion of the complete *rpfGHC* operon led to overproduction of DSF. Conversely, strains carrying an in-frame deletion of *rpfG* were unable to make DSF. Thus, RpfC appears to be a positive regulator of extracellular enzymes and EPS, but a negative regulator of DSF, whereas RpfG appears to positively regulate both processes. Addition of exogenous DSF to an *rpfG* deletion mutant was unable to restore synthesis of pathogenicity factors, and hence is effectively blind to DSF. This led Slater et al. to propose that the RpfGC two-component system is involved in sensing and controlling the levels of DSF. Transcript levels of *rpfF* and *rpfB*, which encode the presumed DSF biosynthetic enzymes, were found to be two- to three-fold higher in an *rpfC* mutant than in the wild-type. This suggests that regulation of DSF production by the *rpfGHC* operon is, at least in part, at the level of transcription [348].

Although *Xcc* appears to have evolved a unique system for cell-to-cell signalling, it shares similarities with the systems employed by *V. harveyi* and *R. solanacearum*. Most evident is that all three species appear to contain specialised two-component regulators to integrate and/or sense their respective quorum-sensing signals. The current working model for the system of *Xcc* is as follows (Fig. 11). RpfH and RpfC are located in the inner membrane of the bacterial cell envelope and constitute a sensory apparatus. Upon sensing specific environmental signals (possibly including DSF), RpfC undergoes autophosphorylation. RpfH may act as an accessory protein, perhaps to promote the binding of ligand(s). Autophosphorylation of RpfC sets up a phosphorelay to the response regulator RpfG. The phosphorelay is presumed to occur via the

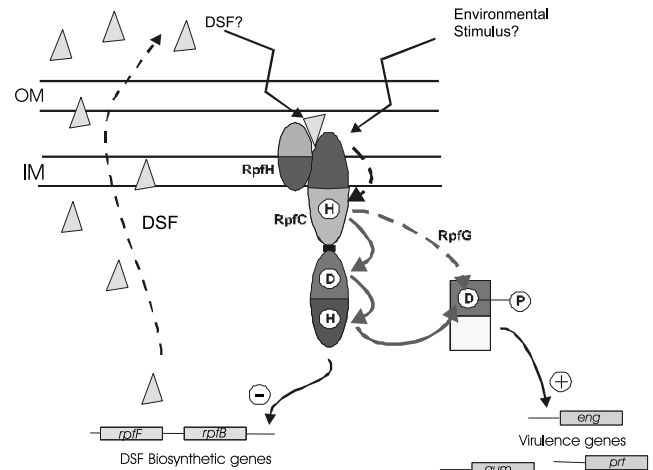


Fig. 11. A model for RpfC, G and H regulation of DSF and pathogenicity factor production in *X. campestris*. The perception of an environmental signal (perhaps DSF itself) stimulates autophosphorylation of RpfC. This sets up a phosphorelay to the response regulator, RpfG. Phosphorylated RpfG is predicted to positively regulate pathogenicity gene expression and to negatively regulate *rpfF* and *rpfB* [348].

His-Asp-His-Asp multi-step pathway, for which one paradigm is the BvgAS system of *Bordetella pertussis* [351,352]. Phosphorylation of RpfG leads to activation of transcription of pathogenicity genes, such as those encoding extracellular enzymes and the biosynthetic enzymes required for EPS synthesis. To do this, RpfG may act to alter the level of an intracellular small-molecular-mass regulator such as cyclic di-guanylate. Negative regulation of the DSF biosynthetic genes, *rpfF* and *rpfB*, occurs via RpfC and is largely independent of RpfG. This would comprise a feedback inhibition loop for DSF biosynthesis. The multi-step phosphorelay may allow for further levels of modulation by other effectors and for fine-tuning of the system. This is significant since it is clear that DSF is not the sole determinant of the timing of synthesis of extracellular enzymes and EPS.

Another group has also reported the involvement of a low-molecular-mass diffusible factor in the regulation of synthesis of pigment (xanthomonadin) and EPS in a different strain of *Xcc* (B24) [353,354]. This factor, named DF, has been tentatively characterised as a butyrolactone (Fig. 5) [355] and, more recently, was shown to contribute to the epiphytic survival of *Xcc*. The locus implicated in the synthesis of DF in *Xcc* strain B24 is *pigB*, mutations in which cause a reduction in levels of EPS and pigment. DF and DSF have been shown to have different  $R_f$  values when analysed by thin-layer chromatography, providing direct physical evidence that these two factors are different. Moreover, the phenotypic data and the results from cross-complementation studies of *pigB*, *rpfF* and *rpfB* mutants implicate both DF and DSF in the regulation of EPS biosynthesis but suggest that they act independently to regulate biosynthesis of pigment and extracellular enzymes respectively [356]. The purpose of using two independent

signals with overlapping roles in *Xcc* may be to allow considerable flexibility when adapting to changing environmental conditions, during epiphytic and pathogenic growth.

Interestingly, a recent survey of plant-associated bacteria revealed that the production of compounds with acyl HSL activity was very rare among *Xanthomonas* species [158]. Further to this, production of DSF appears to be limited to *Xanthomonas* species, and largely to *X. campestris* strains [344]. Similar results were found for the production of DF [353,355]. However, examination of the recently published genomic sequence of the phytopathogen *Xylella fastidiosa* indicates an extremely high degree of sequence relatedness exists between gene products implicated in the synthesis and perception of DSF in *Xcc* and the predicted gene products from *X. fastidiosa* [357]. This provides strong circumstantial evidence for the existence of a DSF regulatory system in *X. fastidiosa*.

## 8. Concluding remarks

In little more than a decade, our knowledge of how bacteria communicate with each other has blossomed. In terms of acyl HSL-mediated communication, we are now at the stage where we can comprehend the intricacies of a language that once seemed alien. Indeed, we now understand how the acyl HSL message is spoken, how it is heard and, to a lesser extent, how bacteria respond to the conversation. Much of the progress in this regard has come about due to a revolution in molecular biology that has greatly extended the range of techniques available for research of microbial genetic systems. We are now at the dawn of a new era, with innovative technologies presenting even more opportunities to rapidly enhance our understanding of quorum-sensing systems. High-throughput methodologies involving proteomics and microarrays lend themselves to analysis and identification of bacterial genes and proteins that fall under the regulatory umbrella of proteins such as LuxRI homologues. Thus, it is likely that many more physiological processes, regulated by bacterial quorum-sensing systems, will be characterised over the next few years. Similarly, the goal of developing successful alternatives to antibiotics via the use of anti-quorum-sensing strategies could be realised.

It is only relatively recently that the complexity and scope of quorum-sensing-specific bacterial regulation has been appreciated by many in the scientific community. Far from being singular entities, it is now apparent that bacteria exist in multifaceted communities and are constantly communicating with each other. However, our current understanding of the extent and significance of bacterial intercellular communication is necessarily naive, superficial and confusing: we see only a prokaryotic tower of Babel at the moment, rather than eavesdropping on illuminating, structured conversations. Although laboratory-

based analysis of quorum-sensing regulation has been enlightening, we now need more information on the importance of such systems in the environment. While the list of bacteria that utilise quorum-sensing systems is large, it is clearly unlikely to be complete. This fact reflects the bias of world-wide research interests towards microorganisms of relevance to human wellbeing, and is truly ironic when one considers the bacterial species in which the prototypic quorum-sensing system was originally discovered. It is therefore certain that our current comprehension of quorum-sensing is severely limited and that the true extent of bacterial cell-to-cell communication in the environment awaits discovery.

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## References

- [1] Stock, A.M., Robinson, V.L. and Goudreau, P.N. (2000) Two-component signal transduction. *Annu. Rev. Biochem.* 69, 183–215.
- [2] Wösten, M.M.S.M. (1998) Eubacterial sigma-factors. *FEMS Microbiol. Rev.* 22, 127–150.
- [3] Atlung, T. and Ingmer, H. (1997) H-NS: a modulator of environmentally regulated gene expression. *Mol. Microbiol.* 24, 7–17.
- [4] Perez-Martin, J. and de Lorenzo, V. (1997) Clues and consequences of DNA bending in transcription. *Annu. Rev. Microbiol.* 51, 593–628.
- [5] Fuqua, W.C., Winans, S.C. and Greenberg, E.P. (1994) Quorum sensing in bacteria: the LuxR-LuxI family of cell density-responsive transcriptional regulators. *J. Bacteriol.* 176, 269–275.
- [6] Kleerebezem, M., Quadri, L.E.N., Kuipers, O.P. and de Vos, W.M. (1997) Quorum sensing by peptide pheromones and two-component signal-transduction systems in Gram-positive bacteria. *Mol. Microbiol.* 24, 895–904.
- [7] Lazazzera, B.A. and Grossman, A.D. (1998) The ins and outs of peptide signaling. *Trends Microbiol.* 7, 288–294.
- [8] Shapiro, J.A. (1998) Thinking about bacterial populations as multicellular organisms. *Annu. Rev. Microbiol.* 52, 81–104.
- [9] Ruby, E.G. (1999) The *Euprymna scolopes-Vibrio fischeri* symbiosis: a biomedical model for the study of bacterial colonization of animal tissue. *J. Mol. Microbiol. Biotechnol.* 1, 13–21.
- [10] Visick, K.L. and McFall-Ngai, M.J. (2000) An exclusive contract: specificity in the *Vibrio fischeri-Euprymna scolopes* partnership. *J. Bacteriol.* 182, 1779–1787.
- [11] Graf, J. and Ruby, E.G. (1998) Host-derived amino acids support the proliferation of symbiotic bacteria. *Proc. Natl. Acad. Sci. USA* 95, 1818–1822.
- [12] Montgomery, M.K. and McFall-Ngai, M. (1994) Bacterial symbionts induce host organ morphogenesis during early postembryonic development of the squid *Euprymna scolopes*. *Development* 120, 1719–1729.

- [13] Lamarca, L.H. and McFall-Ngai, M.J. (1998) Induction of a gradual, reversible morphogenesis of its host's epithelial brush border by *Vibrio fischeri*. *Infect. Immun.* 66, 777–785.
- [14] Lemus, J.D. and McFall-Ngai, M.J. (2000) Alterations in the proteome of the *Euprymna scolopes* light organ in response to symbiotic *Vibrio fischeri*. *Appl. Environ. Microbiol.* 66, 4091–4097.
- [15] Visick, K.L., Foster, J., Doi, J., McFall-Ngai, M. and Ruby, E.G. (2000) *Vibrio fischeri lux* genes play an important role in colonization and development of the host light organ. *J. Bacteriol.* 182, 4578–4586.
- [16] Engebrecht, J., Nealson, K. and Silverman, M. (1983) Bacterial bioluminescence: isolation and genetic analysis of functions from *Vibrio fischeri*. *Cell* 32, 773–781.
- [17] Swartzman, E., Kapoor, S., Graham, A.F. and Meighen, E.A. (1990) A new *Vibrio fischeri lux* gene precedes a bidirectional termination site for the *lux* operon. *J. Bacteriol.* 172, 6797–6802.
- [18] Engebrecht, J. and Silverman, M. (1984) Identification of genes and gene products necessary for bacterial bioluminescence. *Proc. Natl. Acad. Sci. USA* 81, 4154–4158.
- [19] Dunn, G.K., Michaliszyn, G.A., Bogacki, I.G. and Meighen, E.A. (1973) Conversion of aldehyde to acid in the bacterial bioluminescent reaction. *Biochemistry* 12, 4911–4918.
- [20] Hastings, J.W. and Nealson, K.H. (1977) Bacterial bioluminescence. *Annu. Rev. Microbiol.* 31, 549–595.
- [21] Hastings, J.W. (1978) Bacterial bioluminescence: an overview. *Methods Enzymol.* 57, 125–135.
- [22] Boylan, M., Graham, A.F. and Meighen, E.A. (1985) Functional identification of the fatty acid reductase components encoded in the luminescence operon of *Vibrio fischeri*. *J. Bacteriol.* 163, 1186–1190.
- [23] Boylan, M., Miyamoto, C., Wall, L., Graham, A. and Meighen, E. (1989) Lux C, D and E genes of the *Vibrio fischeri* luminescence operon code for the reductase, transferase, and synthetase enzymes involved in aldehyde biosynthesis. *Photochem. Photobiol.* 49, 681–688.
- [24] Zenno, S. and Saigo, K. (1994) Identification of the genes encoding NAD(P)H-flavin oxidoreductases that are similar in sequence to *Escherichia coli* Fre in four species of luminous bacteria: *Photobacterium luminescens*, *Vibrio fischeri*, *Vibrio harveyi*, and *Vibrio orientalis*. *J. Bacteriol.* 176, 3544–3551.
- [25] Nealson, K.H. (1999) Early observations defining quorum-dependent gene expression. In: *Cell-Cell Signaling in Bacteria* (Dunny, G.M. and Winans, S.C., Eds.), pp. 277–289. ASM Press, Washington, DC.
- [26] Hastings, J.W. and Greenberg, E.P. (1999) Quorum sensing: the explanation of a curious phenomenon reveals a common characteristic of bacteria. *J. Bacteriol.* 181, 2667–2668.
- [27] Nealson, K.H., Platt, T. and Hastings, J.W. (1970) Cellular control of the synthesis and activity of the bacterial luminescent system. *J. Bacteriol.* 104, 313–322.
- [28] Eberhard, A. (1972) Inhibition and activation of bacterial luciferase synthesis. *J. Bacteriol.* 109, 1101–1105.
- [29] Nealson, K.H. (1977) Autoinduction of bacterial luciferase. *Arch. Microbiol.* 112, 73–79.
- [30] Eberhard, A., Burlingame, A.L., Eberhard, C., Kenyon, G.L., Nealson, K.H. and Oppenheimer, N.J. (1981) Structural identification of autoinducer of *Photobacterium fischeri* luciferase. *Biochemistry* 20, 2444–2449.
- [31] Kaplan, H.B. and Greenberg, E.P. (1985) Diffusion of autoinducer is involved in regulation of the *Vibrio fischeri* luminescence system. *J. Bacteriol.* 163, 1210–1214.
- [32] Devine, J.H., Countryman, C. and Baldwin, T.O. (1988) Nucleotide sequence of the *luxR* and *luxI* genes and structure of the primary regulatory region of the *lux* regulon of *Vibrio fischeri* ATCC 7744. *Biochemistry* 27, 837–842.
- [33] Devine, J.H., Shadel, G.S. and Baldwin, T.O. (1989) Identification of the operator of the *lux* regulon from the *Vibrio fischeri* strain ATCC7744. *Proc. Natl. Acad. Sci. USA* 86, 5688–5692.
- [34] Eglund, K.A. and Greenberg, E.P. (1999) Quorum sensing in *Vibrio fischeri*: elements of the *luxI* promoter. *Mol. Microbiol.* 31, 1197–1204.
- [35] Boettcher, K.J. and Ruby, E.G. (1995) Detection and quantification of *Vibrio fischeri* autoinducer from symbiotic squid light organs. *J. Bacteriol.* 177, 1053–1058.
- [36] Shadel, G.S. and Baldwin, T.O. (1991) The *Vibrio fischeri* LuxR protein is capable of bidirectional stimulation of transcription and both positive and negative regulation of the *luxR* gene. *J. Bacteriol.* 173, 568–574.
- [37] Shadel, G.S. and Baldwin, T.O. (1992) Positive autoregulation of the *Vibrio fischeri luxR* gene. *J. Biol. Chem.* 267, 7696–7702.
- [38] Dunlap, P.V. and Ray, J.M. (1989) Requirement for autoinducer in transcriptional negative autoregulation of the *Vibrio fischeri luxR* gene in *Escherichia coli*. *J. Bacteriol.* 171, 3549–3552.
- [39] Shadel, G.S. and Baldwin, T.O. (1992) Identification of a distantly located regulatory element in the *luxCD* gene required for negative autoregulation of the *Vibrio fischeri luxR* gene. *J. Biol. Chem.* 267, 7690–7695.
- [40] Ulitzur, S. and Dunlap, P.V. (1995) Regulatory circuitry controlling luminescence autoinduction in *Vibrio fischeri*. *Photochem. Photobiol.* 62, 625–632.
- [41] Dunlap, P.V. (1999) Quorum regulation of luminescence in *Vibrio fischeri*. *J. Mol. Microbiol. Biotechnol.* 1, 5–12.
- [42] Dunlap, P.V. and Greenberg, E.P. (1988) Control of *Vibrio fischeri lux* gene transcription by a cyclic AMP receptor protein-LuxR protein regulatory circuit. *J. Bacteriol.* 170, 4040–4046.
- [43] Kuo, A., Blough, N.V. and Dunlap, P.V. (1994) Multiple *N*-acyl-L-homoserine lactone autoinducers of luminescence in the marine symbiotic bacterium *Vibrio fischeri*. *J. Bacteriol.* 176, 7558–7565.
- [44] Kuo, A., Callahan, S.M. and Dunlap, P.V. (1996) Modulation of luminescence operon expression by *N*-octanoyl-L-homoserine lactone in *ainS* mutants of *Vibrio fischeri*. *J. Bacteriol.* 178, 971–976.
- [45] Callahan, S.M. and Dunlap, P.V. (2000) LuxR- and acyl-homoserine-lactone-controlled non-*lux* genes define a quorum-sensing regulon in *Vibrio fischeri*. *J. Bacteriol.* 182, 2811–2822.
- [46] Gilson, L., Kuo, A. and Dunlap, P.V. (1995) AinS and a new family of autoinducer synthesis proteins. *J. Bacteriol.* 177, 6946–6951.
- [47] Hanzelka, B.L., Parsek, M.R., Val, D.L., Dunlap, P.V., Cronan Jr., J.E. and Greenberg, E.P. (1999) Acylhomoserine lactone synthase activity of the *Vibrio fischeri* AinS protein. *J. Bacteriol.* 181, 5766–5770.
- [48] Greenberg, E.P., Hastings, J.W. and Ulitzur, S. (1979) Induction of luciferase synthesis in *Benickea harveyi* by other marine bacteria. *Arch. Microbiol.* 120, 87–91.
- [49] Kaplan, H.B. and Greenberg, E.P. (1987) Overproduction and purification of the *luxR* gene product: transcriptional activator of the *Vibrio fischeri* luminescence system. *Proc. Natl. Acad. Sci. USA* 84, 6639–6643.
- [50] Devine, J.H., Countryman, C. and Baldwin, T.O. (1988) Nucleotide sequence of the *luxR* and *luxI* genes and structure of the primary regulatory region of the *lux* regulon of *Vibrio fischeri* ATCC 7744. *Biochemistry* 27, 837–842.
- [51] Adar, Y.Y., Simaan, M. and Ulitzur, S. (1992) Formation of the LuxR protein in the *Vibrio fischeri lux* system is controlled by HtpR through the GroESL proteins. *J. Bacteriol.* 174, 7138–7143.
- [52] Adar, Y.Y. and Ulitzur, S. (1993) GroESL proteins facilitate binding of externally added inducer by LuxR protein-containing *E. coli* cells. *J. Biolumin. Chemilumin.* 8, 261–266.
- [53] Dolan, K.M. and Greenberg, E.P. (1992) Evidence that GroEL, not  $\sigma^{32}$ , is involved in transcriptional regulation of the *Vibrio fischeri* luminescence genes in *Escherichia coli*. *J. Bacteriol.* 174, 5132–5135.
- [54] Kolibachuk, D. and Greenberg, E.P. (1993) The *Vibrio fischeri* luminescence gene activator LuxR is a membrane-associated protein. *J. Bacteriol.* 175, 7307–7312.
- [55] Shadel, G.S., Young, R. and Baldwin, T.O. (1990) Use of regulated cell lysis in a lethal genetic selection in *Escherichia coli*: identification

- of the autoinducer-binding region of the LuxR protein from *Vibrio fischeri* ATCC7744. *J. Bacteriol.* 172, 3980–3987.
- [56] Sloock, J., van Riet, D., Kolibachuk, D. and Greenberg, E.P. (1990) Critical regions of the *Vibrio fischeri* LuxR protein defined by mutational analysis. *J. Bacteriol.* 172, 3974–3979.
- [57] Hanzelka, B.L. and Greenberg, E.P. (1995) Evidence that the N-terminal region of the *Vibrio fischeri* LuxR protein constitutes an auto-inducer-binding domain. *J. Bacteriol.* 177, 815–817.
- [58] Zhu, J. and Winans, S.C. (1999) Autoinducer binding by the quorum-sensing regulator TraR increases affinity for target promoters in vitro and decreases TraR turnover rates in whole cells. *Proc. Natl. Acad. Sci. USA* 96, 4832–4837.
- [59] Qin, Y., Luo, Z.Q., Smyth, A.J., Gao, P., Beck von Bodman, S. and Farrand, S.K. (2000) Quorum-sensing signal binding results in dimerization of TraR and its release from membranes into the cytoplasm. *EMBO J.* 19, 5212–5221.
- [60] Welch, M., Todd, D.E., Whitehead, N.A., McGowan, S.J., Bycroft, B.W. and Salmond, G.P.C. (2000) *N*-Acyl homoserine lactone binding to the CarR receptor determines quorum-sensing specificity in *Erwinia*. *EMBO J.* 19, 631–641.
- [61] Choi, S.H. and Greenberg, E.P. (1992) Genetic evidence for multimerization of LuxR, the transcriptional activator of *Vibrio fischeri* luminescence. *Mol. Mar. Biol. Biotechnol.* 1, 408–413.
- [62] Zhu, J. and Winans, S.C. (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, 1507–1512.
- [63] Luo, Z.Q. and Farrand, S.K. (1999) Signal-dependent DNA binding and functional domains of the quorum-sensing activator TraR as identified by repressor activity. *Proc. Natl. Acad. Sci. USA* 96, 9009–9014.
- [64] Choi, S.H. and Greenberg, E.P. (1992) Genetic dissection of DNA binding and luminescence gene activation by the *Vibrio fischeri* LuxR protein. *J. Bacteriol.* 174, 4064–4069.
- [65] Eglund, K.A. and Greenberg, E.P. (2001) Quorum sensing in *Vibrio fischeri*: analysis of the LuxR DNA binding region by alanine-scanning mutagenesis. *J. Bacteriol.* 183, 382–386.
- [66] Trott, A.E. and Stevens, A.M. (2001) Amino acid residues in LuxR critical for its mechanism of transcriptional activation during quorum sensing in *Vibrio fischeri*. *J. Bacteriol.* 183, 387–392.
- [67] Choi, S.H. and Greenberg, E.P. (1991) The C-terminal region of the *Vibrio fischeri* LuxR protein contains an autoinducer-independent *lux* gene activating domain. *Proc. Natl. Acad. Sci. USA* 88, 11115–11119.
- [68] Poellinger, K.A., Lee, J.P., Perales Jr., J.V. and Greenberg, E.P. (1995) Intragenic suppression of a *luxR* mutation: characterization of an autoinducer-independent LuxR. *FEMS Microbiol. Lett.* 129, 97–102.
- [69] Sitnikov, D.M., Shadel, G.S. and Baldwin, T.O. (1996) Autoinducer-independent mutants of the LuxR transcriptional activator exhibit differential effects on the two *lux* promoters of *Vibrio fischeri*. *Mol. Gen. Genet.* 252, 622–625.
- [70] Eglund, K.A. and Greenberg, E.P. (2000) Conversion of the *Vibrio fischeri* transcriptional activator, LuxR, to a repressor. *J. Bacteriol.* 182, 805–811.
- [71] Stevens, A.M. and Greenberg, E.P. (1997) Quorum sensing in *Vibrio fischeri*: essential elements for activation of the luminescence genes. *J. Bacteriol.* 179, 557–562.
- [72] Stevens, A.M., Fujita, N., Ishihama, A. and Greenberg, E.P. (1999) Involvement of the RNA polymerase  $\alpha$ -subunit C-terminal domain in LuxR-dependent activation of the *Vibrio fischeri* luminescence genes. *J. Bacteriol.* 181, 4704–4707.
- [73] Rhodius, V.A. and Busby, S.J.W. (1998) Positive activation of gene expression. *Curr. Opin. Microbiol.* 1, 152–159.
- [74] Stevens, A.M., Dolan, K.M. and Greenberg, E.P. (1994) Synergistic binding of the *Vibrio fischeri* LuxR transcriptional activator domain and RNA polymerase to the *lux* promoter region. *Proc. Natl. Acad. Sci. USA* 91, 12619–12623.
- [75] Nasser, W., Bouillant, M.L., Salmond, G. and Reverchon, S. (1998) Characterization of the *Erwinia chrysanthemi* *expI-expR* locus directing the synthesis of two *N*-acyl-homoserine lactone signal molecules. *Mol. Microbiol.* 29, 1391–1405.
- [76] Reverchon, S., Bouillant, M.L., Salmond, G. and Nasser, W. (1998) Integration of the quorum-sensing system in the regulatory networks controlling virulence factor synthesis in *Erwinia chrysanthemi*. *Mol. Microbiol.* 29, 1407–1418.
- [77] Moré, M.I., Finger, D., Stryker, J.L., Fuqua, C., Eberhard, A. and Winans, S.C. (1996) Enzymatic synthesis of a quorum-sensing auto-inducer through use of defined substrates. *Science* 272, 1655–1658.
- [78] Schaefer, A.L., Val, D.L., Hanzelka, B.L., Cronan Jr., J.E. and Greenberg, E.P. (1996) Generation of cell-to-cell signals in quorum sensing: acyl homoserine lactone synthase activity of a purified *Vibrio fischeri* LuxI protein. *Proc. Natl. Acad. Sci. USA* 93, 9505–9509.
- [79] Eberhard, A., Longin, T., Widrig, C.A. and Stranick, S.J. (1991) Synthesis of the *lux* gene autoinducer in *Vibrio fischeri* is positively autoregulated. *Arch. Microbiol.* 155, 294–297.
- [80] Hanzelka, B.L. and Greenberg, E.P. (1996) Quorum sensing in *Vibrio fischeri*: evidence that *S*-adenosylmethionine is the amino acid substrate for autoinducer synthesis. *J. Bacteriol.* 178, 5291–5294.
- [81] Jiang, Y., Camara, M., Chhabra, S.R., Hardie, K.R., Bycroft, B.W., Lazdunski, A., Salmond, G.P.C., Stewart, G.S.A.B. and Williams, P. (1998) In vitro biosynthesis of the *Pseudomonas aeruginosa* quorum-sensing signal molecule *N*-butanoyl-L-homoserine lactone. *Mol. Microbiol.* 28, 193–203.
- [82] Hoang, T.T., Yufang, M., Stern, R.J., McNeil, M.R. and Schweizer, H.P. (1999) Construction and use of low-copy number T7 expression vectors for purification of problem proteins: purification of *Mycobacterium tuberculosis* RmlD and *Pseudomonas aeruginosa* LasI and RhlI proteins, and functional analysis of purified RhlI. *Gene* 237, 361–371.
- [83] Parsek, M.R., Val, D.L., Hanzelka, B.L., Cronan Jr., J.E. and Greenberg, E.P. (1999) Acyl homoserine-lactone quorum-sensing signal generation. *Proc. Natl. Acad. Sci. USA* 96, 4360–4365.
- [84] Val, D.L. and Cronan Jr., J.E. (1998) In vivo evidence that *S*-adenosylmethionine and fatty acid synthesis intermediates are the substrates for the LuxI family of autoinducer synthases. *J. Bacteriol.* 180, 2644–2651.
- [85] Hoang, T.T. and Schweizer, H.P. (1999) Characterization of *Pseudomonas aeruginosa* enoyl-acyl carrier protein reductase (FabI): a target for the antimicrobial triclosan and its role in acylated homoserine lactone synthesis. *J. Bacteriol.* 181, 5489–5497.
- [86] Hanzelka, B.L., Stevens, A.M., Parsek, M.R., Crone, T.J. and Greenberg, E.P. (1997) Mutational analysis of the *Vibrio fischeri* LuxI polypeptide: critical regions of an autoinducer synthase. *J. Bacteriol.* 179, 4882–4887.
- [87] Parsek, M.R., Schaefer, A.L. and Greenberg, E.P. (1997) Analysis of random and site-directed mutations in *rhlI*, a *Pseudomonas aeruginosa* gene encoding an acylhomoserine lactone synthase. *Mol. Microbiol.* 26, 301–310.
- [88] Laue, B.E., Jiang, Y., Chhabra, S.R., Jacob, S., Stewart, G.S.A.B., Hardman, A., Downie, J.A., O'Gara, F. and Williams, P. (2000) The biocontrol strain *Pseudomonas fluorescens* F113 produces the *Rhizobium* small bacteriocin, *N*-(3-hydroxy-7-*cis*-tetradecenoyl) homoserine lactone, via HdtS, a putative novel *N*-acylhomoserine lactone synthase. *Microbiology* 146, 2469–2480.
- [89] McClean, K.H., Winson, M.K., Fish, L., Taylor, A., Chhabra, S.R., Camara, M., Daykin, M., Lamb, J.H., Swift, S., Bycroft, B.W., Stewart, G.S.A.B. and Williams, P. (1997) Quorum sensing and *Chromobacterium violaceum*: exploitation of violacein production and inhibition for the detection of *N*-acylhomoserine lactones. *Microbiology* 143, 3703–3711.
- [90] Shaw, P.D., Ping, G., Daly, S.L., Cha, C., Cronan Jr., J.E., Rinehart, K.L. and Farrand, S.K. (1997) Detecting and characterizing *N*-acylhomoserine lactone signal molecules by thin-layer chromatography. *Proc. Natl. Acad. Sci. USA* 94, 6036–6041.

- [91] Winson, M.K., Swift, S., Fish, L., Throup, J.P., Jorgensen, F., Chhabra, S.R., Bycroft, B.W., Williams, P. and Stewart, G.S.A.B. (1998) Construction and analysis of *luxCDABE*-based plasmid sensors for investigating *N*-acyl homoserine lactone-mediated quorum sensing. *FEMS Microbiol. Lett.* 163, 185–192.
- [92] Swift, S., Winson, M.K., Chan, P.F., Bainton, N.J., Birdsall, M., Reeves, P.J., Rees, C.E.D., Chhabra, S.R., Hill, P.J., Throup, J.P., Bycroft, B.W., Salmond, G.P.C., Williams, P. and Stewart, G.S.A.B. (1993) A novel strategy for the isolation of *luxI* homologues: evidence for the widespread distribution of a LuxR: LuxI superfamily in enteric bacteria. *Mol. Microbiol.* 10, 511–520.
- [93] Blosser, R.S. and Gray, K.M. (2000) Extraction of violacein from *Chromobacterium violaceum* provides a new quantitative bioassay for *N*-acyl homoserine lactone autoinducers. *J. Microbiol. Methods* 40, 47–55.
- [94] Charlton, T.S., de Nys, R., Netting, A., Kumar, N., Hentzer, M., Givskov, M. and Kjelleberg, S. (2000) A novel and sensitive method for the quantification of *N*-3-oxoacyl homoserine lactones using gas chromatography-mass spectrometry: application to a model bacterial biofilm. *Environ. Microbiol.* 2, 530–541.
- [95] Andersen, J.B., Heydorn, A., Hentzer, M., Eberl, L., Geisenberger, O., Christensen, B.B., Molin, S. and Givskov, M. (2001) *gfp*-based *N*-acyl homoserine-lactone sensor systems for detection of bacterial communication. *Appl. Environ. Microbiol.* 67, 575–585.
- [96] Zhu, J., Beaber, J.W., Moré, M.I., Fuqua, C., Eberhard, A. and Winans, S.C. (1998) Analogs of the autoinducer 3-oxooctanoyl-homoserine lactone strongly inhibit activity of the TraR protein of *Agrobacterium tumefaciens*. *J. Bacteriol.* 180, 5398–5405.
- [97] Eberl, L. (1999) *N*-Acyl homoserine lactone-mediated gene regulation in Gram-negative bacteria. *Syst. Appl. Microbiol.* 22, 493–506.
- [98] Pearson, J.P., van Delden, C. and Igleski, B.H. (1999) Active efflux and diffusion are involved in transport of *Pseudomonas aeruginosa* cell-to-cell signals. *J. Bacteriol.* 181, 1203–1210.
- [99] Pollack, M. (1990) *Pseudomonas aeruginosa*. In: Principles and Practice of Infectious Disease (Mandell, G.L., Douglas, R.G. and Bennett, J.E., Eds.), pp. 1673–1691. Churchill Livingstone, New York.
- [100] Koch, C. and Hoiby, N. (1993) Pathogenesis of cystic fibrosis. *Lancet* 341, 1065–1069.
- [101] Govan, J.R.W. and Deretic, V. (1996) Microbial pathogenesis in cystic fibrosis: mucoid *Pseudomonas aeruginosa* and *Burkholderia cepacia*. *Microbiol. Rev.* 60, 539–574.
- [102] Nicas, T.I. and Igleski, B.H. (1985) The contribution of exoproducts to virulence of *Pseudomonas aeruginosa*. *Can. J. Microbiol.* 31, 387–392.
- [103] van Delden, C. and Igleski, B.H. (1998) Cell-to-cell signaling and *Pseudomonas aeruginosa* infections. *Emerging Infect. Dis.* 4, 551–560.
- [104] Gambello, M.J. and Igleski, B.H. (1991) Cloning and characterization of the *Pseudomonas aeruginosa lasR* gene, a transcriptional activator of elastase expression. *J. Bacteriol.* 173, 3000–3009.
- [105] Passador, L., Cook, J.M., Gambello, M.J., Rust, L. and Igleski, B.H. (1993) Expression of *Pseudomonas aeruginosa* virulence genes requires cell-to-cell communication. *Science* 260, 1127–1130.
- [106] Pearson, J.P., Gray, K.M., Passador, L., Tucker, K.D., Eberhard, A., Igleski, B.H. and Greenberg, E.P. (1994) Structure of the auto-inducer required for expression of *Pseudomonas aeruginosa* virulence genes. *Proc. Natl. Acad. Sci. USA* 91, 197–201.
- [107] Toder, D.S., Gambello, M.J. and Igleski, B.H. (1991) *Pseudomonas aeruginosa* LasA: a second elastase under the transcriptional control of *lasR*. *Mol. Microbiol.* 5, 2003–2010.
- [108] Gambello, M.J., Kaye, S. and Igleski, B.H. (1993) LasR of *Pseudomonas aeruginosa* is a transcriptional activator of the alkaline protease gene (*apr*) and an enhancer of exotoxin A expression. *Infect. Immun.* 61, 1180–1184.
- [109] Jones, S., Yu, B., Bainton, N.J., Birdsall, M., Bycroft, B.W., Chhabra, S.R., Cox, A.J.R., Golby, P., Reeves, P.J., Stephens, S., Winson, M.K., Salmond, G.P.C., Stewart, G.S.A.B. and Williams, P. (1993) The *lux* autoinducer regulates the production of exoenzyme virulence determinants in *Erwinia carotovora* and *Pseudomonas aeruginosa*. *EMBO J.* 12, 2477–2482.
- [110] You, Z., Fukushima, J., Ishiwata, T., Chang, B., Kurata, M., Kuwamoto, S., Williams, P. and Okuda, K. (1996) Purification and characterization of LasR as a DNA-binding protein. *FEMS Microbiol. Lett.* 142, 301–307.
- [111] Seed, P.C., Passador, L. and Igleski, B.H. (1995) Activation of the *Pseudomonas aeruginosa lasI* gene by LasR and the *Pseudomonas* autoinducer PAI: an autoinduction regulatory hierarchy. *J. Bacteriol.* 177, 654–659.
- [112] Albus, A.M., Pesci, E.C., Runyen-Janecky, L.J., West, S.E.H. and Igleski, B.H. (1997) Vfr controls quorum sensing in *Pseudomonas aeruginosa*. *J. Bacteriol.* 179, 3928–3935.
- [113] Pesci, E.C., Pearson, J.P., Seed, P.C. and Igleski, B.H. (1997) Regulation of *las* and *rhl* quorum sensing in *Pseudomonas aeruginosa*. *J. Bacteriol.* 179, 3127–3132.
- [114] Pearson, J.P., Passador, L., Igleski, B.H. and Greenberg, E.P. (1995) A second *N*-acylhomoserine lactone signal produced by *Pseudomonas aeruginosa*. *Proc. Natl. Acad. Sci. USA* 92, 1490–1494.
- [115] Winson, M.K., Camara, M., Latifi, A., Foglino, M., Chhabra, S.R., Daykin, M., Bally, M., Chapon, V., Salmond, G.P.C., Bycroft, B.W., Lazdunski, A., Stewart, G.S.A.B. and Williams, P. (1995) Multiple *N*-acyl-L-homoserine lactone signal molecules regulate production of virulence determinants and secondary metabolites in *Pseudomonas aeruginosa*. *Proc. Natl. Acad. Sci. USA* 92, 9427–9431.
- [116] Ochsner, U.A., Koch, A.K., Fiechter, A. and Reiser, J. (1994) Isolation and characterization of a regulatory gene affecting rhamnolipid biosurfactant synthesis in *Pseudomonas aeruginosa*. *J. Bacteriol.* 176, 2044–2054.
- [117] Köhler, T., Curty, L.K., Barja, F., van Delden, C. and Pechere, J.C. (2000) Swarming of *Pseudomonas aeruginosa* is dependent on cell-to-cell signaling and requires flagella and pili. *J. Bacteriol.* 182, 5990–5996.
- [118] Brint, J.M. and Ohman, D.E. (1995) Synthesis of multiple exoproducts in *Pseudomonas aeruginosa* is under the control of RhlR-RhlI, another set of regulators in strain PAO1 with homology to the autoinducer-responsive LuxR-LuxI family. *J. Bacteriol.* 177, 7155–7163.
- [119] Latifi, A., Winson, M.K., Foglino, M., Bycroft, B.W., Stewart, G.S.A.B., Lazdunski, A. and Williams, P. (1995) Multiple homologues of LuxR and LuxI control expression of virulence determinants and secondary metabolites through quorum sensing in *Pseudomonas aeruginosa* PAO1. *Mol. Microbiol.* 17, 333–343.
- [120] Ochsner, U.A. and Reiser, J. (1995) Autoinducer-mediated regulation of rhamnolipid biosurfactant synthesis in *Pseudomonas aeruginosa*. *Proc. Natl. Acad. Sci. USA* 92, 6424–6428.
- [121] Pearson, J.P., Pesci, E.C. and Igleski, B.H. (1997) Roles of *Pseudomonas aeruginosa las* and *rhl* quorum-sensing systems in control of elastase and rhamnolipid biosynthesis genes. *J. Bacteriol.* 179, 5756–5767.
- [122] Winzer, K., Falconer, C., Garber, N.C., Diggle, S.P., Camara, M. and Williams, P. (2000) The *Pseudomonas aeruginosa* lectins PA-IL and PA-IIL are controlled by quorum sensing and by RpoS. *J. Bacteriol.* 182, 6401–6411.
- [123] Pessi, G. and Haas, D. (2000) Transcriptional control of the hydrogen cyanide biosynthetic genes *hcnABC* by the anaerobic regulator ANR and the quorum-sensing regulators LasR and RhlR in *Pseudomonas aeruginosa*. *J. Bacteriol.* 182, 6940–6949.
- [124] Latifi, A., Foglino, M., Tanaka, K., Williams, P. and Lazdunski, A. (1996) A hierarchical quorum-sensing cascade in *Pseudomonas aeruginosa* links the transcriptional activators LasR and RhlR (VsmR) to expression of the stationary-phase sigma factor RpoS. *Mol. Microbiol.* 21, 1137–1146.
- [125] Whiteley, M., Parsek, M.R. and Greenberg, E.P. (2000) Regulation of quorum sensing by RpoS in *Pseudomonas aeruginosa*. *J. Bacteriol.* 182, 4356–4360.

- [126] Chapon-Hervé, V., Akrim, M., Latifi, A., Williams, P., Lazdunski, A. and Bally, M. (1997) Regulation of the *xcp* secretion pathway by multiple quorum-sensing modulons in *Pseudomonas aeruginosa*. *Mol. Microbiol.* 24, 1169–1178.
- [127] Tommassen, J., Filloux, A., Bally, M., Murgier, M. and Lazdunski, A. (1992) Protein secretion in *Pseudomonas aeruginosa*. *FEMS Microbiol. Rev.* 103, 73–90.
- [128] Rumbaugh, K.P., Griswold, J.A., Iglewski, B.H. and Hamood, A.N. (1999) Contribution of quorum sensing to the virulence of *Pseudomonas aeruginosa* in burn wound infections. *Infect. Immun.* 67, 5854–5862.
- [129] Rumbaugh, K.P., Griswold, J.A. and Hamood, A.N. (1999) Contribution of the regulatory gene *lasR* to the pathogenesis of *Pseudomonas aeruginosa* infection of burned mice. *J. Burn Care Rehabil.* 20, 42–49.
- [130] Tang, H.B., Dimango, E., Bryan, R., Gambello, M.J., Iglewski, B.H., Goldberg, J.B. and Prince, A. (1996) Contribution of specific *Pseudomonas aeruginosa* virulence factors to pathogenesis of pneumonia in a neonatal mouse model of infection. *Infect. Immun.* 64, 37–43.
- [131] Pearson, J.P., Feldman, M., Iglewski, B.H. and Prince, A. (2000) *Pseudomonas aeruginosa* cell-to-cell signaling is required for virulence in a model of acute pulmonary infection. *Infect. Immun.* 68, 4331–4334.
- [132] Tan, M.W., Rahme, L.G., Sternberg, J.A., Tompkins, R.G. and Ausubel, F.M. (1999) *Pseudomonas aeruginosa* killing of *Caenorhabditis elegans* to identify *P. aeruginosa* virulence factors. *Proc. Natl. Acad. Sci. USA* 96, 2408–2413.
- [133] Jander, G., Rahme, L.G. and Ausubel, F.M. (2000) Positive correlation between virulence of *Pseudomonas aeruginosa* mutants in mice and insects. *J. Bacteriol.* 182, 3843–3845.
- [134] Storey, D.G., Ujack, E.E., Rabin, H.R. and Mitchell, I. (1998) *Pseudomonas aeruginosa lasR* transcription correlates with the transcription of *lasA*, *lasB*, and *toxA* in chronic lung infections associated with cystic fibrosis. *Infect. Immun.* 66, 2521–2528.
- [135] Yang, H., Matewish, M., Loubens, I., Storey, D.G., Lam, J.S. and Jin, S. (2000) *migA*, a quorum-responsive gene of *Pseudomonas aeruginosa*, is highly expressed in the cystic fibrosis lung environment and modifies low-molecular-mass lipopolysaccharide. *Microbiology* 146, 2509–2519.
- [136] Wu, H., Song, Z., Hentzer, M., Andersen, J.B., Heydorn, A., Mathée, K., Moser, C., Eberl, L., Molin, S., Hoiby, N. and Givskov, M. (2000) Detection of *N*-acylhomoserine lactones in lung tissues of mice infected with *Pseudomonas aeruginosa*. *Microbiology* 146, 2481–2493.
- [137] Stintzi, A., Evans, K., Meyer, J.M. and Poole, K. (1998) Quorum-sensing and siderophore biosynthesis in *Pseudomonas aeruginosa*: *lasR/lasI* mutants exhibit reduced pyoverdine biosynthesis. *FEMS Microbiol. Lett.* 166, 341–345.
- [138] Hassett, D.J., Ma, J.F., Elkins, J.G., McDermott, T.R., Ochsner, U.A., West, S.E.H., Huang, C.T., Fredericks, J., Burnett, S., Stewart, P.S., McFeters, G., Passador, L. and Iglewski, B.H. (1999) Quorum sensing in *Pseudomonas aeruginosa* controls expression of catalase and superoxide dismutase genes and mediates biofilm susceptibility to hydrogen peroxide. *Mol. Microbiol.* 34, 1082–1093.
- [139] Whiteley, M., Lee, K.M. and Greenberg, E.P. (1999) Identification of genes controlled by quorum sensing in *Pseudomonas aeruginosa*. *Proc. Natl. Acad. Sci. USA* 96, 13904–13909.
- [140] Suh, S.J., Silo-Suh, L., Woods, D.E., Hassett, D.J., West, S.E.H. and Ohman, D.E. (1999) Effect of *rpoS* mutation on the stress response and expression of virulence factors in *Pseudomonas aeruginosa*. *J. Bacteriol.* 181, 3890–3897.
- [141] Reimmann, C., Beyeler, M., Latifi, A., Winteler, H., Foglino, M., Lazdunski, A. and Haas, D. (1997) The global activator GacA of *Pseudomonas aeruginosa* PAO positively controls the production of the autoinducer *N*-butyryl-homoserine lactone and the formation of the virulence factors pyocyanin, cyanide, and lipase. *Mol. Microbiol.* 24, 309–319.
- [142] de Kievit, T., Seed, P.C., Nezezon, J., Passador, L. and Iglewski, B.H. (1999) RsaL, a novel repressor of virulence gene expression in *Pseudomonas aeruginosa*. *J. Bacteriol.* 181, 2175–2184.
- [143] Rashid, M.H., Rumbaugh, K., Passador, L., Davies, D.G., Hamood, A.N., Iglewski, B.H. and Kornberg, A. (2000) Polyphosphate kinase is essential for biofilm development, quorum sensing, and virulence of *Pseudomonas aeruginosa*. *Proc. Natl. Acad. Sci. USA* 97, 9636–9641.
- [144] Mazzola, M., Cook, R.J., Thomashow, L.S., Weller, D.M. and Pierson III, L.S. (1992) Contribution of phenazine antibiotic biosynthesis to the ecological competence of fluorescent pseudomonads in soil habitats. *Appl. Environ. Microbiol.* 58, 2616–2624.
- [145] Weller, D.M. and Cook, R.J. (1983) Suppression of take-all of wheat by seed treatments with fluorescent pseudomonads. *Phytopathology* 73, 463–469.
- [146] Thomashow, L.S. and Weller, D.M. (1988) Role of a phenazine antibiotic from *Pseudomonas fluorescens* in biological control of *Gaeumannomyces graminis* var. *tritici*. *J. Bacteriol.* 170, 3499–3508.
- [147] Wood, D.W. and Pierson III, L.S. (1996) The *phzI* gene of *Pseudomonas aureofaciens* 30-84 is responsible for the production of a diffusible signal required for phenazine antibiotic production. *Gene* 168, 49–53.
- [148] Wood, D.W., Gong, F., Daykin, M.M., Williams, P. and Pierson III, L.S. (1997) *N*-Acyl-homoserine lactone-mediated regulation of phenazine gene expression by *Pseudomonas aureofaciens* 30-84 in the wheat rhizosphere. *J. Bacteriol.* 179, 7663–7670.
- [149] Pierson III, L.S., Keppenne, V.D. and Wood, D.W. (1994) Phenazine antibiotic biosynthesis in *Pseudomonas aureofaciens* 30-84 is regulated by PhzR in response to cell density. *J. Bacteriol.* 176, 3966–3974.
- [150] Chancey, S.T., Wood, D.W. and Pierson III, L.S. (1999) Two component transcriptional regulation of *N*-acyl-homoserine lactone production in *Pseudomonas aureofaciens*. *Appl. Environ. Microbiol.* 65, 2294–2299.
- [151] Pierson III, L.S. and Thomashow, L.S. (1992) Cloning and heterologous expression of the phenazine biosynthetic locus from *Pseudomonas aureofaciens* 30-84. *Mol. Plant-Microbe Interact.* 5, 330–339.
- [152] Pierson III, L.S., Gaffney, T., Lam, S. and Gong, F. (1995) Molecular analysis of genes encoding phenazine biosynthesis in the biological control bacterium *Pseudomonas aureofaciens* 30-84. *FEMS Microbiol. Lett.* 134, 299–307.
- [153] Mavrodi, D.V., Ksenzenko, V.N., Bonsall, R.F., Cook, R.J., Boronin, A.M. and Thomashow, L.S. (1998) A seven-gene locus for synthesis of phenazine-1-carboxylic acid by *Pseudomonas fluorescens* 2-79. *J. Bacteriol.* 180, 2541–2548.
- [154] Pierson III, L.S. and Pierson, E.A. (1996) Phenazine antibiotic production in *Pseudomonas aureofaciens*: role in rhizosphere ecology and pathogen suppression. *FEMS Microbiol. Lett.* 136, 101–108.
- [155] Chin-A-Woeng, T.F.C., de Priester, W., van der Bij, A.J. and Lugtenberg, B.J.J. (1997) Description of the colonization of a gnotobiotic tomato rhizosphere by *Pseudomonas fluorescens* biocontrol strain WC5365, using scanning electron microscopy. *Mol. Plant-Microbe Interact.* 10, 79–86.
- [156] Chin-A-Woeng, T.F.C., Bloemberg, G.V., van der Bij, A.J., van der Drift, K.M.G.M., Schripsema, J., Kroon, B., Scheffer, R.J., Keel, C., Bakker, P.A.H.M., Tichy, H.V., de Bruijn, F.J., Thomas-Oates, J.E. and Lugtenberg, B.J.J. (1998) Biocontrol by phenazine-1-carboxamide-producing *Pseudomonas chlororaphis* PCL1391 of tomato root rot caused by *Fusarium oxysporum* f. sp. *radicis-lycopersici*. *Mol. Plant-Microbe Interact.* 11, 1069–1077.
- [157] Mavrodi, D.V., Ksenzenko, V.N., Chatuev, B.M., Thomashow, L.S. and Boronin, A.M. (1997) Structural and functional organization of *Pseudomonas fluorescens* genes encoding enzymes of phenazine-1-carboxylic acid biosynthesis. *Mol. Biol.* 31, 62–68.
- [158] Cha, C., Gao, P., Chen, Y.C., Shaw, P.D. and Farrand, S.K. (1998)

- Production of acyl-homoserine lactone quorum-sensing signals by Gram-negative plant-associated bacteria. *Mol. Plant-Microbe Interact.* 11, 1119–1129.
- [159] Nakata, K., Yoshimoto, A. and Yamada, Y. (1999) Promotion of antibiotic production by ethanol, high NaCl concentration, or heat shock in *Pseudomonas fluorescens* S272. *Biosci. Biotechnol. Biochem.* 63, 293–297.
- [160] Dumenyo, C.K., Mukherjee, A., Chum, W. and Chatterjee, A.K. (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, 569–582.
- [161] Chen, C.Y. and Shaw, P.D. (1995) GenBank accession number U39802.
- [162] Kinscherf, T.G. and Willis, D.K. (1999) Swarming by *Pseudomonas syringae* B728a requires *gacS* (*lemA*) and *gacA* but not the acyl-homoserine lactone biosynthetic gene *ahlI*. *J. Bacteriol.* 181, 4133–4136.
- [163] Kitten, T., Kinscherf, T.G., McEvoy, J.L. and Willis, D.K. (1998) A newly identified regulator is required for virulence and toxin production in *Pseudomonas syringae*. *Mol. Microbiol.* 28, 917–929.
- [164] Govan, J.R.W., Hughes, J.E. and Vandamme, P. (1996) *Burkholderia cepacia*: medical, taxonomic and ecological issues. *J. Med. Microbiol.* 45, 395–407.
- [165] Lewenza, S., Conway, B., Greenberg, E.P. and Sokol, P.A. (1999) Quorum sensing in *Burkholderia cepacia*: identification of the LuxRI homologs CepRI. *J. Bacteriol.* 181, 748–756.
- [166] McKenney, D., Brown, K.E. and Allison, D.G. (1995) Influence of *Pseudomonas aeruginosa* exoproducts on virulence factor production in *Burkholderia cepacia*: evidence of interspecies communication. *J. Bacteriol.* 177, 6989–6992.
- [167] Geisenberger, O., Givskov, M., Riedel, K., Høiby, N., Tümmler, B. and Eberl, L. (2000) Production of *N*-acyl-L-homoserine lactones by *P. aeruginosa* isolates from chronic lung infections associated with cystic fibrosis. *FEMS Microbiol. Lett.* 184, 273–278.
- [168] Stickler, D.J., Morris, N.S., McLean, R.J.C. and Fuqua, C. (1998) Biofilms on indwelling urethral catheters produce quorum-sensing molecules in situ and in vitro. *Appl. Environ. Microbiol.* 64, 3486–3490.
- [169] McLean, R.J.C., Whiteley, M., Stickler, D.J. and Fuqua, W.C. (1997) Evidence of autoinducer activity in naturally occurring biofilms. *FEMS Microbiol. Lett.* 154, 259–263.
- [170] Bachofen, R. and Schenk, A. (1998) Quorum sensing autoinducers: do they play a role in natural microbial habitats? *Microbiol. Res.* 153, 61–63.
- [171] Allison, D.G., Ruiz, B., Carmen, S., Jaspe, A. and Gilbert, P. (1998) Extracellular products as mediators of the formation and detachment of *Pseudomonas fluorescens* biofilms. *FEMS Microbiol. Lett.* 167, 179–184.
- [172] Davies, D.G., Parsek, M.R., Pearson, J.P., Iglewski, B.H., Costerton, J.W. and Greenberg, E.P. (1998) The involvement of cell-to-cell signals in the development of a bacterial biofilm. *Science* 280, 295–298.
- [173] Glessner, A., Smith, R.S., Iglewski, B.H. and Robinson, J.B. (1999) Roles of *Pseudomonas aeruginosa* *las* and *rhl* quorum-sensing systems in control of twitching motility. *J. Bacteriol.* 181, 1623–1629.
- [174] O'Toole, G.A. and Kolter, R. (1998) Flagellar and twitching motility are necessary for *Pseudomonas aeruginosa* biofilm development. *Mol. Microbiol.* 30, 295–304.
- [175] Singh, P.K., Schaefer, A.L., Parsek, M.R., Moninger, T.O., Welsh, M.J. and Greenberg, E.P. (2000) Quorum-sensing signals indicate that cystic fibrosis lungs are infected with bacterial biofilms. *Nature* 407, 762–764.
- [176] Holden, M.T.G., Chhabra, S.R., de Nys, R., Stead, P., Bainton, N.J., Hill, P.J., Manefield, M., Kumar, N., Labatte, M., England, D., Rice, S., Givskov, M., Salmond, G.P.C., Stewart, G.S.A.B., Bycroft, B.W., Kjelleberg, S. and Williams, P. (1999) Quorum-sensing cross talk: isolation and chemical characterization of cyclic dipeptides from *Pseudomonas aeruginosa* and other Gram-negative bacteria. *Mol. Microbiol.* 33, 1254–1266.
- [177] Pesci, E.C., Milbank, J.B.J., Pearson, J.P., McKnight, S., Kende, A.S., Greenberg, E.P. and Iglewski, B.H. (1999) Quinolone signaling in the cell-to-cell communication system of *Pseudomonas aeruginosa*. *Proc. Natl. Acad. Sci. USA* 96, 11229–11234.
- [178] McKnight, S.L., Iglewski, B.H. and Pesci, E.C. (2000) The *Pseudomonas* quinolone signal regulates *rhl* quorum sensing in *Pseudomonas aeruginosa*. *J. Bacteriol.* 182, 2702–2708.
- [179] Perombelon, M.C.M. and Kelman, A. (1980) Ecology of the soft rot *erwinias*. *Annu. Rev. Phytopathol.* 18, 361–387.
- [180] Py, B., Barras, F., Harris, S., Robson, N. and Salmond, G.P.C. (1998) Extracellular enzymes and their role in *Erwinia* virulence. *Methods Microbiol.* 27, 158–168.
- [181] Thomson, N.R., Thomas, J.D. and Salmond, G.P.C. (1999) Virulence determinants in the bacterial phytopathogen *Erwinia*. *Methods Microbiol.* 29, 347–426.
- [182] Mae, A., Heikinheimo, R. and Palva, E.T. (1995) Structure and regulation of the *Erwinia carotovora* subspecies *carotovora* SCC3193 cellulase gene *celVI* and the role of cellulase in phytopathogenicity. *Mol. Gen. Genet.* 247, 17–26.
- [183] Marits, R., Koiv, V., Laasik, E. and Mae, A. (1999) Isolation of an extracellular protease gene of *Erwinia carotovora* subsp. *carotovora* strain SCC3193 by transposon mutagenesis and the role of protease in phytopathogenicity. *Microbiology* 145, 1959–1966.
- [184] Salmond, G.P.C. (1994) Secretion of extracellular virulence factors by plant-pathogenic bacteria. *Annu. Rev. Phytopathol.* 32, 181–200.
- [185] Parker, W.L., Rathnum, M.L., Wells, J.S., Trejo, W.H., Principe, P.A. and Sykes, R.B. (1982) SQ27850, a simple carbapenem produced by species of *Serratia* and *Erwinia*. *J. Antibiot.* 35, 653–660.
- [186] Axelrood, P.E., Rella, M. and Schroth, M.N. (1988) Role of antibiotic in competition of *Erwinia* strains in potato infection courts. *Appl. Environ. Microbiol.* 54, 1222–1229.
- [187] McGowan, S., Sebaiha, M., Jones, S., Yu, B., Bainton, N., Chan, P.F., Bycroft, B., Stewart, G.S.A.B., Williams, P. and Salmond, G.P.C. (1995) Carbapenem antibiotic production in *Erwinia carotovora* is regulated by CarR, a homologue of the LuxR transcriptional activator. *Microbiology* 141, 541–550.
- [188] McGowan, S.J., Sebaihia, M., Porter, L.E., Stewart, G.S.A.B., Williams, P., Bycroft, B.W. and Salmond, G.P.C. (1996) Analysis of bacterial carbapenem antibiotic production genes reveals a novel  $\beta$ -lactam biosynthesis pathway. *Mol. Microbiol.* 22, 415–426.
- [189] McGowan, S.J., Sebaihia, M., O'Leary, S., Hardie, K.R., Williams, P., Stewart, G.S.A.B., Bycroft, B.W. and Salmond, G.P.C. (1997) Analysis of the carbapenem gene cluster of *Erwinia carotovora*: definition of the antibiotic biosynthetic genes and evidence for a novel  $\beta$ -lactam resistance mechanism. *Mol. Microbiol.* 26, 545–556.
- [190] Bainton, N.J., Stead, P., Chhabra, S.R., Bycroft, B.W., Salmond, G.P.C., Stewart, G.S.A.B. and Williams, P. (1992) *N*-(3-Oxohexanoyl)-L-homoserine lactone regulates carbapenem antibiotic production in *Erwinia carotovora*. *Biochem. J.* 288, 997–1004.
- [191] Bainton, N.J., Bycroft, B.W., Chhabra, S.R., Stead, P., Gledhill, L., Hill, P.J., Rees, C.E.D., Winson, M.K., Salmond, G.P.C., Stewart, G.S.A.B. and Williams, P. (1992) A general role for the *lux* auto-inducer in bacterial cell signalling: control of antibiotic biosynthesis in *Erwinia*. *Gene* 116, 87–91.
- [192] Pirhonen, M., Flego, D., Heikinheimo, R. and Palva, E.T. (1993) A small diffusible signal molecule is responsible for the global control of virulence and exoenzyme production in *Erwinia carotovora*. *EMBO J.* 12, 2467–2476.
- [193] Chatterjee, A., Cui, Y., Liu, Y., Dumenyo, C.K. and Chatterjee, A.K. (1995) Inactivation of *rsmA* leads to overproduction of extracellular pectinases, cellulases, and proteases in *Erwinia carotovora* subsp. *carotovora* in the absence of the starvation/cell density-sensing signal, *N*-(3-oxohexanoyl)-L-homoserine lactone. *Appl. Environ. Microbiol.* 61, 1959–1967.

- [194] Costa, J.M. and Loper, J.E. (1997) Ecbl and EcbR: homologs of LuxI and LuxR affecting antibiotic and exoenzyme production by *Erwinia carotovora* subsp. *betavascularum*. Can. J. Microbiol. 43, 1164–1171.
- [195] McGowan, S.J., Bosgelmez, G., Barnard, A.M.L., Sebahia, M., Todd, D.E., Porter, L.E., Thomson, N.R., Welch, M., Whitehead, N.A., Bycroft, B.W. and Salmond, G.P.C. (2001) Multiple genetic and physiological factors regulate carbapenem antibiotic production in *Erwinia carotovora* by modulating the quorum sensing mechanism. Mol. Microbiol. (in revision).
- [196] Holden, M.T.G., McGowan, S.J., Bycroft, B.W., Stewart, G.S.A.B., Williams, P. and Salmond, G.P.C. (1998) Cryptic carbapenem antibiotic production genes are widespread in *Erwinia carotovora*: facile *trans* activation by the *carR* transcriptional regulator. Microbiology 144, 1495–1508.
- [197] Salmond, G.P.C., Bycroft, B.W., Stewart, G.S.A.B. and Williams, P. (1995) The bacterial 'enigma': cracking the code of cell-cell communication. Mol. Microbiol. 16, 615–624.
- [198] Rivet, M.M. (1998) Investigation into the Regulation of Exoenzyme Production in *Erwinia carotovora* subspecies *carotovora*. PhD Thesis, University of Warwick.
- [199] Andersson, R.A., Eriksson, A.R.B., Heikinheimo, R., Mae, A., Pirhonen, M., Koiv, V., Hyytiäinen, H., Tuikkala, A. and Palva, E.T. (2000) Quorum sensing in the plant pathogen *Erwinia carotovora* subsp. *carotovora* the role of *expR<sub>Ecc</sub>*. Mol. Plant-Microbe Interact. 13, 384–393.
- [200] Whitehead, N.A. (1999) Analysis of the Quorum-sensing System of *Erwinia carotovora* subspecies *carotovora*. PhD Thesis, University of Cambridge.
- [201] Dixon, R.A., Harrison, M.J. and Lamb, C.J. (1994) Early events in the activation of plant defence responses. Annu. Rev. Phytopathol. 32, 479–501.
- [202] Palva, T.K., Holmström, K.O., Heino, P. and Palva, E.T. (1993) Induction of plant defense response by exoenzymes of *Erwinia carotovora* subsp. *carotovora*. Mol. Plant-Microbe Interact. 6, 190–196.
- [203] Cui, Y., Chatterjee, A., Liu, Y., Dumenyo, C.K. and Chatterjee, A.K. (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 in soft-rotting *Erwinia* spp. J. Bacteriol. 177, 5108–5115.
- [204] Romeo, T., Gong, M., Liu, M.Y. and Brun-Zinkernagel, A.M. (1993) Identification and molecular characterization of *csrA*, a pleiotropic gene from *Escherichia coli* that affects glycogen biosynthesis, gluconeogenesis, cell size, and surface properties. J. Bacteriol. 175, 4744–4755.
- [205] Liu, M.Y., Yang, H. and Romeo, T. (1995) Pleiotropic regulation of central carbohydrate metabolism in *Escherichia coli* *csrA*. J. Biol. Chem. 270, 29096–29104.
- [206] Liu, M.Y. and Romeo, T. (1997) The global regulator CsrA of *Escherichia coli* is a specific mRNA-binding protein. J. Bacteriol. 179, 4639–4642.
- [207] Cui, Y., Madi, L., Mukherjee, A., Dumenyo, C.K. and Chatterjee, A.K. (1996) The RsmA<sup>−</sup> mutants of *Erwinia carotovora* subsp. *carotovora* strain Ecc71 overexpress *hrpN<sub>Ecc</sub>* and elicit a hypersensitive reaction-like response in tobacco leaves. Mol. Plant-Microbe Interact. 9, 565–573.
- [208] Liu, M.Y., Gui, G., Wei, B., Preston, J.F., Oakford, L., Yuksel, U., Giedroc, D.P. and Romeo, T. (1997) The RNA molecule CsrB binds to the global regulatory protein CsrA and antagonizes its activity in *Escherichia coli*. J. Biol. Chem. 272, 17502–17510.
- [209] Romeo, T. (1998) Global regulation by the small RNA-binding protein CsrA and the non-coding RNA molecule CsrB. Mol. Microbiol. 29, 1321–1330.
- [210] Murata, H., Chatterjee, A., Liu, Y. and Chatterjee, A.K. (1994) Regulation of the production of extracellular pectinase, cellulase, and protease in the soft rot bacterium *Erwinia carotovora* ssp. *carotovora* evidence that *aepH* of *E. carotovora* subsp. *carotovora* 71 activates gene expression in *E. carotovora* subsp. *carotovora*, *E. carotovora* subsp. *atroseptica*, and *Escherichia coli*. Appl. Environ. Microbiol. 60, 3150–3159.
- [211] Liu, Y., Cui, Y., Mukherjee, A. and Chatterjee, A.K. (1998) Characterization of a novel RNA regulator of *Erwinia carotovora* ssp. *carotovora* that controls production of extracellular enzymes and secondary metabolites. Mol. Microbiol. 29, 219–234.
- [212] Swift, S., Downie, J.A., Whitehead, N.A., Barnard, A.M.L., Salmond, G.P.C. and Williams, P. (2001) Quorum sensing as a population-density-dependent determinant of bacterial physiology. Adv. Microb. Physiol. 45, 199–270.
- [213] Cui, Y., Mukherjee, A., Dumenyo, C.K. and Chatterjee, A.K. (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, 6042–6052.
- [214] Shih, Y.L., Harris, S.J., Borner, G., Rivet, M.M. and Salmond, G.P.C. (1999) The *hexY* genes of *Erwinia carotovora* subsp. *carotovora* and subsp. *atroseptica* encode novel proteins which co-ordinately regulate virulence and motility. Environ. Microbiol. 1, 535–547.
- [215] Harris, S.J., Shih, Y.L., Bentley, S.D. and Salmond, G.P.C. (1998) The *hexA* gene of *Erwinia carotovora* encodes a LysR homologue and regulates motility and the expression of multiple virulence determinants. Mol. Microbiol. 28, 705–717.
- [216] Mukherjee, A., Cui, Y., Ma, W., Liu, Y., Ishihama, A., Eisenstark, A. and Chatterjee, A.K. (1998) RpoS (sigma-S) controls expression of *rsmA*, a global regulator of secondary metabolites, harpin, and extracellular proteins in *Erwinia carotovora*. J. Bacteriol. 180, 3629–3634.
- [217] Mukherjee, A., Cui, Y., Ma, W.L., Liu, Y. and Chatterjee, A.K. (2000) *hexA* of *Erwinia carotovora* ssp. *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, 203–215.
- [218] Beck von Bodman, S. and Farrand, S.K. (1995) Capsular polysaccharide biosynthesis and pathogenicity in *Erwinia stewartii* require induction by an *N*-acyl homoserine lactone autoinducer. J. Bacteriol. 177, 5000–5008.
- [219] Beck von Bodman, S., Majerczak, D.R. and Coplin, D.L. (1998) A negative regulator mediates quorum-sensing control of exopolysaccharide production in *Pantoea stewartii* subsp. *stewartii*. Proc. Natl. Acad. Sci. USA 95, 7687–7692.
- [220] Grimont, P.A.D. and Grimont, F. (1978) The genus *Serratia*. Annu. Rev. Microbiol. 32, 221–248.
- [221] Hines, D.A., Saurugger, P.N., Ihler, G.M. and Benedik, M.J. (1988) Genetic analysis of extracellular proteins of *Serratia marcescens*. J. Bacteriol. 170, 4141–4146.
- [222] Givskov, M., Eberl, L. and Molin, S. (1997) Control of exoenzyme production, motility and cell differentiation in *Serratia liquefaciens*. FEMS Microbiol. Lett. 148, 115–122.
- [223] Givskov, M., Olsen, L. and Molin, S. (1998) Cloning and expression in *Escherichia coli* of the gene for an extracellular phospholipase from *Serratia liquefaciens*. Mol. Microbiol. 20, 127–136.
- [224] Harshey, R.M. (1994) Bees aren't the only ones: swarming in Gram-negative bacteria. Mol. Microbiol. 13, 389–394.
- [225] Eberl, L., Winson, M.K., Sternberg, C., Stewart, G.S.A.B., Christiansen, G., Chhabra, S.R., Bycroft, B., Williams, P., Molin, S. and Givskov, M. (1996) Involvement of *N*-acyl-L-homoserine lactone autoinducers in controlling the multicellular behaviour of *Serratia liquefaciens*. Mol. Microbiol. 20, 127–136.
- [226] Lindum, P.W., Anthoni, U., Christophersen, C., Eberl, L., Molin, S. and Givskov, M. (1998) *N*-Acyl-L-homoserine lactone autoinducers control production of an extracellular lipopeptide biosurfactant re-

- quired for swarming motility of *Serratia liquefaciens* MG1. J. Bacteriol. 180, 6384–6388.
- [227] Matsuyama, T., Kaneda, K., Nakagawa, Y., Isa, K., Hara-Hotta, H. and Yano, I. (1992) A novel extracellular cyclic lipopeptide which promotes flagellum-dependent and -independent spreading growth of *Serratia marcescens*. J. Bacteriol. 174, 1769–1776.
- [228] Givskov, M., Östling, J., Eberl, L., Lindum, P.W., Beck Christensen, A., Christiansen, G., Molin, S. and Kjelleberg, S. (1998) Two separate regulatory systems participate in control of swarming motility of *Serratia liquefaciens* MG1. J. Bacteriol. 180, 742–745.
- [229] Cox, A.R.J., Thomson, N.R., Bycroft, B., Stewart, G.S.A.B., Williams, P. and Salmond, G.P.C. (1998) A pheromone-independent CarR protein controls carbapenem antibiotic synthesis in the opportunistic human pathogen *Serratia marcescens*. Microbiology 144, 201–209.
- [230] Thomson, N.R., Crow, M.A., McGowan, S.J., Cox, A. and Salmond, G.P.C. (2000) Biosynthesis of carbapenem antibiotic and prodigiosin pigment in *Serratia* is under quorum sensing control. Mol. Microbiol. 36, 539–556.
- [231] Grimont, P.A.D. and Grimont, F. (1984) Genus VIII. *Serratia*. In: Bergey's Manual of Systematic Bacteriology (Kreig, N.R. and Holt, J.G., Eds.), Vol. 1, pp. 477–484. Williams and Wilkins, Baltimore, MD.
- [232] Williams, R.P. and Quadri, S.M. (1980) The pigments of *Serratia*. In: The Genus *Serratia* (von Graevenitz, A. and Rubin, S.J., Eds.), pp. 31–75. CRC Press, Boca Raton, FL.
- [233] Tsuji, R.F., Magae, J., Yamashita, M., Nagai, K. and Yamasaki, M. (1992) Immunomodulating properties of prodigiosin 25-C, an antibiotic which preferentially suppresses induction of cytotoxic T-cells. J. Antibiot. 45, 1295–1302.
- [234] Tsuji, R.F., Yamamoto, M., Nakamura, A., Kataoka, T., Magae, J., Nagai, K. and Yamasaki, M. (1990) Selective immunosuppression of prodigiosin 25-C and FK506 in the murine immune-system. J. Antibiot. 43, 1293–1301.
- [235] Thomson, N.R., Cox, A., Bycroft, B.W., Stewart, G.S.A.B., Williams, P. and Salmond, G.P.C. (1997) The Rap and Hor proteins of *Erwinia*, *Serratia* and *Yersinia*: a novel subgroup in a growing superfamily of proteins regulating diverse physiological processes in bacterial pathogens. Mol. Microbiol. 26, 531–544.
- [236] Reverchon, S., Nasser, W. and Robert-Baudouy, J. (1994) PecS – a locus controlling pectinase, cellulase and blue pigment production in *Erwinia chrysanthemi*. Mol. Microbiol. 11, 1127–1139.
- [237] Martin, R.G. and Rosner, J.L. (1995) Binding of purified multiple antibiotic resistance repressor protein (MarR) to *mar* operator sequences. Proc. Natl. Acad. Sci. USA 92, 5456–5460.
- [238] Oscarsson, J., Mizunoe, Y., Uhlin, B.E. and Haydon, D.J. (1996) Induction of haemolytic activity in *Escherichia coli* by the *slyA* gene product. Mol. Microbiol. 20, 191–199.
- [239] Revell, P.A. and Miller, V.L. (2000) A chromosomally encoded regulator is required for expression of the *Yersinia enterocolitica* *inv* gene and for virulence. Mol. Microbiol. 35, 677–685.
- [240] Todd, D.E. (1999) Characterisation of the CarR and Hor Proteins: Regulators of Carbapenem and Exoenzyme Production in *Erwinia carotovora*. PhD Thesis, University of Cambridge.
- [241] Throup, J.P., Camara, M., Briggs, G.S., Winson, M.K., Chhabra, S.R., Bycroft, B.W., Williams, P. and Stewart, G.S.A.B. (1995) Characterisation of the *yenI/yenR* locus from *Yersinia enterocolitica* mediating the synthesis of two *N*-acyl homoserine lactone signal molecules. Mol. Microbiol. 17, 345–356.
- [242] Atkinson, S., Throup, J.P., Stewart, G.S.A.B. and Williams, P. (1999) A hierarchical quorum-sensing system in *Yersinia pseudotuberculosis* is involved in the regulation of motility and clumping. Mol. Microbiol. 33, 1267–1277.
- [243] Swift, S., Isherwood, K.E., Atkinson, S., Oyston, P.C.F. and Stewart, G.S.A.B. (1999) Quorum sensing in *Aeromonas* and *Yersinia*. In: Microbial Signalling and Communication (England, R., Hobbs, G., Bainton, N. and Roberts, D., Eds.), pp. 85–104. Cambridge University Press, Cambridge.
- [244] Klapwijk, P.M., Scheulderman, T. and Schilperoort, R.A. (1978) Coordinated regulation of octopine degradation and conjugative transfer of Ti plasmids in *Agrobacterium tumefaciens*: evidence for a common regulatory gene and separate operons. J. Bacteriol. 136, 775–785.
- [245] Ellis, J.G., Kerr, A., Petit, A. and Tempé, J. (1982) Conjugal transfer of nopaline and agropine Ti-plasmids – the role of agrocino-pines. Mol. Gen. Genet. 186, 269–273.
- [246] Zhu, J., Oger, P.M., Schrammeijer, B., Hooykaas, P.J.J., Farrand, S.K. and Winans, S.C. (2000) The bases of crown gall tumorigenesis. J. Bacteriol. 182, 3885–3895.
- [247] Farrand, S.K. (1998) Conjugation in Rhizobiaceae. In: The Rhizobiaceae, Molecular Biology of Model Plant-Associated Bacteria (Spaink, H.P., Kondorosi, A. and Hooykaas, P.J.J., Eds.), pp. 199–233. Kluwer Academic, Dordrecht.
- [248] Habeeb, L.F., Wang, L. and Winans, S.C. (1991) Transcription of the octopine catabolism operon of the *Agrobacterium* tumor-inducing plasmid pTiA6 is activated by a LysR-type regulatory protein. Mol. Plant-Microbe Interact. 4, 379–385.
- [249] Wang, L., Helmann, J.D. and Winans, S.C. (1992) The *A. tumefaciens* transcriptional activator OccR causes a bend at a target promoter, which is partially relaxed by a plant tumor metabolite. Cell 69, 659–667.
- [250] Fuqua, W.C. and Winans, S.C. (1994) A LuxR-LuxI type regulatory system activates *Agrobacterium* Ti plasmid conjugal transfer in the presence of a plant tumour metabolite. J. Bacteriol. 176, 2796–3806.
- [251] Fuqua, C. and Winans, S.C. (1996) Localization of OccR-activated and TraR-activated promoters that express two ABC-type permeases and the *traR* gene of Ti plasmid pTiR10. Mol. Microbiol. 20, 1199–1210.
- [252] Piper, K.R., Beck von Bodman, S., Hwang, I. and Farrand, S.K. (1999) Hierarchical gene regulatory systems arising from fortuitous gene associations: controlling quorum sensing by the opine regulon in *Agrobacterium*. Mol. Microbiol. 32, 1077–1089.
- [253] Fuqua, C. and Winans, S.C. (1996) Conserved *cis*-acting promoter elements are required for density-dependent transcription of *Agrobacterium tumefaciens* conjugal transfer genes. J. Bacteriol. 178, 435–440.
- [254] Oger, P., Kim, K.S., Sackett, R.L., Piper, K.R. and Farrand, S.K. (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, 277–288.
- [255] Zhu, J. and Winans, C. (1998) Activity of the quorum-sensing regulator TraR of *Agrobacterium tumefaciens* is inhibited by a truncated, dominant defective TraR-like protein. Mol. Microbiol. 27, 289–297.
- [256] Beck von Bodman, S., Hayman, G.T. and Farrand, S.K. (1992) Opine catabolism and conjugal transfer of the nopaline Ti plasmid pTiC58 are coordinately regulated by a single repressor. Proc. Natl. Acad. Sci. USA 89, 643–647.
- [257] Kim, H. and Farrand, S.K. (1997) Characterization of the *acc* operon from the nopaline-type Ti plasmid pTiC58, which encodes utilization of agrocino-pines A and B and susceptibility to agrocin 84. J. Bacteriol. 179, 7559–7572.
- [258] Piper, K.R., Beck von Bodman, S. and Farrand, S.K. (1993) Conjugation factor of *Agrobacterium tumefaciens* regulates Ti plasmid transfer by autoinduction. Nature 362, 448–450.
- [259] Zhang, L., Murphy, P.J., Kerr, A. and Tate, M.E. (1993) *Agrobacterium* conjugation and gene regulation by *N*-acyl-L-homoserine lactones. Nature 362, 446–448.
- [260] Hwang, I., Li, P.L., Zhang, L., Piper, K.R., Cook, D.M., Tate, M.E. and Farrand, S.K. (1994) TraI, a LuxI homologue, is responsible for production of conjugation factor, the Ti plasmid *N*-acylho-

- moserine lactone autoinducer. *Proc. Natl. Acad. Sci. USA* 91, 4639–4643.
- [261] Fuqua, C., Burbea, M. and Winans, S.C. (1995) Activity of the *Agrobacterium* Ti plasmid conjugal transfer regulator TraR is inhibited by the product of the *traM* gene. *J. Bacteriol.* 177, 1367–1373.
- [262] Hwang, I., Cook, D.M. and Farrand, S.K. (1995) A new regulatory element modulates homoserine lactone-mediated autoinduction of Ti plasmid conjugal transfer. *J. Bacteriol.* 177, 449–458.
- [263] Hwang, I., Smyth, A.J., Luo, Z.Q. and Farrand, S.K. (1999) Modulating quorum sensing by antiactivation: TraM interacts with TraR to inhibit activation of Ti plasmid conjugal transfer genes. *Mol. Microbiol.* 34, 282–294.
- [264] Luo, Z.Q., Qin, Y. and Farrand, S.K. (2000) The antiactivator TraM interferes with the autoinducer-dependent binding of TraR to DNA by interfering with the C-terminal region of the quorum-sensing activator. *J. Biol. Chem.* 275, 7713–7722.
- [265] Piper, K.R. and Farrand, S.K. (2000) Quorum sensing but not autoinduction of Ti plasmid conjugal transfer requires control by the opine regulon and the antiactivator TraM. *J. Bacteriol.* 182, 1080–1088.
- [266] van Rhijn, P. and Vanderleyden, J. (1995) The *Rhizobium*-plant symbiosis. *Microbiol. Rev.* 59, 124–142.
- [267] Perret, X., Staechelin, C. and Broughton, W.J. (2000) Molecular basis of symbiotic promiscuity. *Microbiol. Mol. Biol. Rev.* 64, 180–201.
- [268] Oke, V. and Long, S.R. (1999) Bacteroid formation in the *Rhizobium*-legume symbiosis. *Curr. Opin. Microbiol.* 2, 641–646.
- [269] Lithgow, J.K., Wilkinson, A., Hardman, A., Rodelas, B., Wisniewski-Dye, F., Williams, P. and Downie, J.A. (2000) The regulatory locus *cinRI* in *Rhizobium leguminosarum* controls a network of quorum-sensing loci. *Mol. Microbiol.* 37, 81–97.
- [270] Gray, K.M., Pearson, J.P., Downie, J.A., Boboye, B.E.A. and Greenberg, E.P. (1996) Cell-to-cell signaling in the symbiotic nitrogen-fixing bacterium *Rhizobium leguminosarum*: autoinduction of a stationary phase and rhizosphere-expressed genes. *J. Bacteriol.* 178, 372–376.
- [271] Schripsema, J., de Rudder, K.E.E., van Vliet, T.B., Lankhorst, P.P., de Vroom, E., Kijne, J.W. and van Brussel, A.A.N. (1996) Bacteriocin *small* of *Rhizobium leguminosarum* belongs to the class of N-acyl-L-homoserine lactone molecules, known as autoinducers and as quorum sensing co-transcription factors. *J. Bacteriol.* 178, 366–371.
- [272] Rodelas, B., Lithgow, J.K., Wisniewski-Dye, F., Hardman, A., Wilkinson, A., Economou, A., Williams, P. and Downie, J.A. (1999) Analysis of quorum-sensing-dependent control of rhizosphere-expressed (*rhi*) genes in *Rhizobium leguminosarum* bv. *viciae*. *J. Bacteriol.* 181, 3816–3823.
- [273] Cubo, M.T., Economou, A., Murphy, G., Johnston, A.W.B. and Downie, J.A. (1992) Molecular characterization and regulation of the rhizosphere-expressed genes *rhiABCR* that can influence nodulation by *Rhizobium leguminosarum* biovar *viciae*. *J. Bacteriol.* 174, 4026–4035.
- [274] Dibb, N.J., Downie, J.A. and Brewin, N.J. (1984) Identification of a rhizosphere protein encoded by the symbiotic plasmid of *Rhizobium leguminosarum*. *J. Bacteriol.* 158, 621–627.
- [275] Freiberg, C., Fellay, R., Bairoch, A., Broughton, W.J., Rosenthal, A. and Perret, X. (1997) Molecular basis of symbiosis between *Rhizobium* and legumes. *Nature* 387, 394–401.
- [276] Rosemeyer, V., Michiels, J., Verreth, C. and Vanderleyden, J. (1998) *luxI*- and *luxR*-homologous genes of *Rhizobium etli* CNPAF512 contribute to synthesis of autoinducer molecules and nodulation of *Phaseolus vulgaris*. *J. Bacteriol.* 180, 815–821.
- [277] Swift, S., Karlyshev, A.V., Fish, L., Durant, E.L., Winson, M.K., Chhabra, S.R., Williams, P., Macintyre, S. and Stewart, G.S.A.B. (1997) Quorum sensing in *Aeromonas hydrophila* and *Aeromonas salmonicida*: identification of the LuxRI homologs AhyRI and AsaRI and their cognate N-acylhomoserine lactone signal molecules. *J. Bacteriol.* 179, 5271–5281.
- [278] Swift, S., Lynch, M.J., Fish, L., Kirke, D.F., Tomas, J.M., Stewart, G.S.A.B. and Williams, P. (1999) Quorum sensing-dependent regulation and blockade of exoprotease production in *Aeromonas hydrophila*. *Infect. Immun.* 67, 5192–5199.
- [279] Milton, D.L., Hardman, A., Camara, M., Chhabra, S.R., Bycroft, B.W., Stewart, G.S.A.B. and Williams, P. (1997) Quorum sensing in *Vibrio anguillarum*: characterization of the *vanI/vanR* locus and identification of the autoinducer N-(3-oxodecanoyl)-L-homoserine lactone. *J. Bacteriol.* 179, 3004–3012.
- [280] Dunphy, G., Miyamoto, C. and Meighen, E. (1997) A homoserine lactone autoinducer regulates virulence of an insect-pathogenic bacterium, *Xenorhabdus nematophilus* (*Enterobacteriaceae*). *J. Bacteriol.* 179, 5288–5291.
- [281] Throup, J., Winson, M.K., Bainton, N.J., Bycroft, B.W., Williams, P. and Stewart, G.S.A.B. (1995) Signalling in bacteria beyond bioluminescence. In: *Bioluminescence and Chemiluminescence: Fundamentals and Applied Aspects* (Campbell, A., Kricka, L. and Stanley, P., Eds.), pp. 89–92. Wiley, Chichester.
- [282] Chernin, L.S., Winson, M.K., Thompson, J.M., Haran, S., Bycroft, B.W., Chet, I., Williams, P. and Stewart, G.S.A.B. (1998) Chitinolytic activity in *Chromobacterium violaceum*: substrate analysis and regulation by quorum sensing. *J. Bacteriol.* 180, 4435–4441.
- [283] Camara, M., Daykin, M. and Chhabra, S.R. (1998) Detection, purification, and synthesis of N-acylhomoserine lactone quorum sensing signal molecules. *Methods Microbiol.* 27, 319–330.
- [284] Puskas, A., Greenberg, E.P., Kaplan, S. and Schaefer, A.L. (1997) A quorum-sensing system in the free-living photosynthetic bacterium *Rhodospirillum rubrum*. *J. Bacteriol.* 179, 7530–7537.
- [285] Bassler, B.L., Wright, M., Showalter, R.E. and Silverman, M.R. (1993) Intercellular signalling in *Vibrio harveyi*: sequence and function of genes regulating expression of luminescence. *Mol. Microbiol.* 9, 773–786.
- [286] Cao, J.G. and Meighen, E.A. (1989) Purification and structural identification of an autoinducer for the luminescence system of *Vibrio harveyi*. *J. Biol. Chem.* 264, 21670–21676.
- [287] Surette, M.G., Miller, M.B. and Bassler, B.L. (1999) Quorum sensing in *Escherichia coli*, *Salmonella typhimurium*, and *Vibrio harveyi*: a new family of genes responsible for autoinducer production. *Proc. Natl. Acad. Sci. USA* 96, 1639–1644.
- [288] Bassler, B.L., Wright, M. and Silverman, M.R. (1994) Multiple signalling systems controlling expression of luminescence in *Vibrio harveyi*: sequence and function of genes encoding a second sensory pathway. *Mol. Microbiol.* 13, 273–286.
- [289] Freeman, J.A. and Bassler, B.L. (1999) Sequence and function of LuxU: a two-component phosphorelay protein that regulates quorum sensing in *Vibrio harveyi*. *J. Bacteriol.* 181, 899–906.
- [290] Bassler, B.L., Wright, M. and Silverman, M.R. (1994) Sequence and function of LuxO, a negative regulator of luminescence in *Vibrio harveyi*. *Mol. Microbiol.* 12, 403–412.
- [291] Freeman, J.A. and Bassler, B.L. (1999) A genetic analysis of the function of LuxO, a two-component response regulator involved in quorum sensing in *Vibrio harveyi*. *Mol. Microbiol.* 31, 665–677.
- [292] Freeman, J.A., Lilley, B.N. and Bassler, B.L. (2000) A genetic analysis of the functions of LuxN: a two-component hybrid sensor kinase that regulates quorum sensing in *Vibrio harveyi*. *Mol. Microbiol.* 35, 139–149.
- [293] Lilley, B.N. and Bassler, B.L. (2000) Regulation of quorum sensing in *Vibrio harveyi* by LuxO and sigma-54. *Mol. Microbiol.* 36, 940–954.
- [294] Showalter, R.E., Martin, M.O. and Silverman, M.R. (1990) Cloning and nucleotide sequence of *luxR*, a regulatory gene controlling bioluminescence in *Vibrio harveyi*. *J. Bacteriol.* 172, 2946–2954.
- [295] Swartzman, E., Silverman, M. and Meighen, E.A. (1992) The *luxR* gene product is a transcriptional activator of the *lux* promoter. *J. Bacteriol.* 174, 7490–7493.
- [296] Manfield, M., Harris, L., Rice, S.A., de Nys, R. and Kjelleberg, S. (2000) Inhibition of luminescence and virulence in the black tiger

- prawn (*Penaeus monodon*) pathogen *Vibrio harveyi* by intercellular signal antagonists. *Appl. Environ. Microbiol.* 66, 2079–2084.
- [297] Miyamoto, C.M., Lin, Y.H. and Meighen, E.A. (2000) Control of bioluminescence in *Vibrio fischeri* by the LuxO signal response regulator. *Mol. Microbiol.* 36, 594–607.
- [298] Bassler, B.L., Greenberg, E.P. and Stevens, A.M. (1997) Cross-species induction of luminescence in the quorum-sensing bacterium *Vibrio harveyi*. *J. Bacteriol.* 179, 4043–4045.
- [299] Surette, M.G. and Bassler, B.L. (1998) Quorum sensing in *Escherichia coli* and *Salmonella typhimurium*. *Proc. Natl. Acad. Sci. USA* 95, 7046–7050.
- [300] Forsyth, M.H. and Cover, T.L. (2000) Intercellular communication in *Helicobacter pylori*: *luxS* is essential for the production of an extracellular signaling molecule. *Infect. Immun.* 68, 3193–3199.
- [301] Sperandio, V., Mellies, J.L., Nguyen, W., Shin, S. and Kaper, J.B. (1999) Quorum sensing controls expression of the type III secretion gene transcription and protein secretion in enterohemorrhagic and enteropathogenic *Escherichia coli*. *Proc. Natl. Acad. Sci. USA* 96, 15196–15201.
- [302] Day, W.A. and Maurelli, A.T. (2001) *Shigella flexneri* LuxS quorum-sensing system modulates *virB* expression but is not essential for virulence. *Infect. Immun.* 69, 15–23.
- [303] Joyce, E.A., Bassler, B.L. and Wright, A. (2000) Evidence for a signalling system in *Helicobacter pylori*: detection of a *luxS*-encoded autoinducer. *J. Bacteriol.* 182, 3638–3643.
- [304] Surette, M.G. and Bassler, B.L. (1999) Regulation of autoinducer production in *Salmonella typhimurium*. *Mol. Microbiol.* 31, 585–595.
- [305] Baca-DeLancey, R.R., South, M.M., Ding, X. and Rather, P.N. (1999) *Escherichia coli* genes regulated by cell-to-cell signalling. *Proc. Natl. Acad. Sci. USA* 96, 4610–4614.
- [306] Withers, H.L. and Nordström, K. (1998) Quorum-sensing acts at initiation of chromosomal replication in *Escherichia coli*. *Proc. Natl. Acad. Sci. USA* 95, 15694–15699.
- [307] Eberhard, A., Widrig, C.A., McBath, P. and Schineller, J.B. (1986) Analogs of the autoinducer of bioluminescence in *Vibrio fischeri*. *Arch. Microbiol.* 146, 35–40.
- [308] Chhabra, S.R., Stead, P., Bainton, N.J., Salmond, G.P.C., Stewart, G.S.A.B., Williams, P. and Bycroft, B.W. (1993) Autoregulation of carbenem biosynthesis in *Erwinia carotovora* by analogues of *N*-(3-oxohexanoyl)-L-homoserine lactone. *J. Antibiot.* 46, 441–454.
- [309] Passador, L., Tucker, K.D., Guertin, K.R., Journet, M.P., Kende, A.S. and Iglewski, B.H. (1996) Functional analysis of the *Pseudomonas aeruginosa* autoinducer PAI. *J. Bacteriol.* 178, 5995–6000.
- [310] Schaefer, A.L., Hanzelka, B.L., Eberhard, A. and Greenberg, E.P. (1996) Quorum sensing in *Vibrio fischeri*: probing autoinducer-LuxR interactions with autoinducer analogs. *J. Bacteriol.* 178, 2897–2901.
- [311] Pierson, E.A., Wood, D.W., Cannon, J.A., Blachere, F.M. and Pierson III, L.S. (1998) Interpopulation signaling via *N*-acyl-homoserine lactones among bacteria in the wheat rhizosphere. *Mol. Plant-Microbe Interact.* 11, 1078–1084.
- [312] Wang, X.D., de Boer, P.A.J. and Rothfield, L.I. (1991) A factor that positively regulates cell division by activating transcription of the major cluster of essential cell division genes of *Escherichia coli*. *EMBO J.* 10, 3363–3372.
- [313] Ahmer, B.M.M., van Reeuwijk, J., Timmers, C.D., Valentine, P.J. and Heffron, F. (1998) *Salmonella typhimurium* encodes an SdiA homolog, a putative quorum sensor of the LuxR family, that regulates genes on the virulence plasmid. *J. Bacteriol.* 180, 1185–1193.
- [314] Garcia-Lara, J., Shang, L.H. and Rothfield, L.I. (1996) An extracellular factor regulates expression of *sdiA*, a transcriptional activator of cell division genes in *Escherichia coli*. *J. Bacteriol.* 178, 2742–2748.
- [315] Sitnikov, D.M., Schineller, J.B. and Baldwin, T.O. (1996) Control of cell division in *Escherichia coli*: regulation of transcription of *ftsQ*A involves both *rpoS* and SdiA-mediated autoinduction. *Proc. Natl. Acad. Sci. USA* 93, 336–341.
- [316] Kanamaru, K., Kanamaru, K., Tatsuno, I., Tobe, T. and Sasakawa, C. (2000) SdiA, an *Escherichia coli* homologue of quorum-sensing regulators, controls the expression of virulence factors in enterohemorrhagic *Escherichia coli* O157:H7. *Mol. Microbiol.* 38, 805–816.
- [317] Kanamaru, K., Kanamaru, K., Tatsuno, I., Tobe, T. and Sasakawa, C. (2000) Regulation of virulence factors of enterohemorrhagic *Escherichia coli* O157:H7 by self-produced extracellular factors. *Biosci. Biotechnol. Biochem.* 64, 2508–2511.
- [318] Dong, Y.H., Xu, J.L., Li, X.Z. and Zhang, L.H. (2000) AiiA, an enzyme that inactivates the acylhomoserine lactone quorum-sensing signal and attenuates the virulence of *Erwinia carotovora*. *Proc. Natl. Acad. Sci. USA* 97, 3526–3531.
- [319] Leadbetter, J.D. and Greenberg, E.P. (2000) Metabolism of acyl-homoserine lactone quorum-sensing signals by *Variovorax paradoxus*. *J. Bacteriol.* 182, 6921–6926.
- [320] Givskov, M., de Nys, R., Manefield, M., Gram, L., Maximilien, R., Eberl, L., Molin, S., Steinberg, P.D. and Kjelleberg, S. (1996) Eukaryotic interference with homoserine lactone-mediated prokaryotic signalling. *J. Bacteriol.* 178, 6618–6622.
- [321] Kjelleberg, S., Steinberg, P., Givskov, M., Gram, L., Manefield, M. and de Nys, R. (1997) Do marine natural products interfere with prokaryotic AHL regulatory systems? *Aquat. Microb. Ecol.* 13, 85–93.
- [322] Rasmussen, T.B., Manefield, M., Andersen, J.B., Eberl, L., Anthoni, U., Christophersen, C., Steinberg, P., Kjelleberg, S. and Givskov, M. (2000) How *Delisea pulchra* furanones affect quorum sensing and swarming motility in *Serratia liquefaciens* MG1. *Microbiology* 146, 3237–3244.
- [323] Manefield, M., de Nys, R., Kumar, N., Read, R., Givskov, M., Steinberg, P. and Kjelleberg, S. (1999) Evidence that halogenated furanones from *Delisea pulchra* inhibit acylated homoserine lactone (AHL)-mediated gene expression by displacing the AHL signal from its receptor protein. *Microbiology* 145, 283–291.
- [324] Teplitski, M., Robinson, J.B. and Bauer, W.D. (2000) Plants secrete substances that mimic bacterial *N*-acyl homoserine lactone signal activities and affect population density-dependent behaviors in associated bacteria. *Mol. Plant-Microbe Interact.* 13, 637–648.
- [325] Telford, G., Wheeler, D., Williams, P., Tomkins, P.T., Appleby, P., Sewell, H., Stewart, G.S.A.B., Bycroft, B.W. and Pritchard, D.I. (1998) The *Pseudomonas aeruginosa* quorum-sensing signal molecule *N*-(3-oxododecanoyl)-L-homoserine lactone has immunomodulatory activity. *Infect. Immun.* 66, 36–42.
- [326] Saleh, A., Figarella, C., Kammouni, W., Marchand-Pinatel, S., Lazdunski, A., Tubul, A., Brun, P. and Merten, M.D. (1999) *Pseudomonas aeruginosa* quorum-sensing signal molecule *N*-(3-oxododecanoyl)-L-homoserine lactone inhibits expression of P2Y receptors in cystic fibrosis tracheal gland cells. *Infect. Immun.* 67, 5076–5082.
- [327] Lawrence, R.N., Dunn, W.R., Bycroft, B., Camara, M., Chhabra, S.R., Williams, P. and Wilson, V.G. (1999) The *Pseudomonas aeruginosa* quorum-sensing signal molecule, *N*-(3-oxododecanoyl)-L-homoserine lactone, inhibits porcine arterial smooth muscle contraction. *Br. J. Pharmacol.* 128, 845–848.
- [328] Robson, N.D., Cox, A.R.J., McGowan, S.J., Bycroft, B.W. and Salmond, G.P.C. (1997) Bacterial *N*-acyl-homoserine-lactone-dependent signalling and its potential biotechnological applications. *Trends Biotechnol.* 15, 458–464.
- [329] Finch, R.G., Pritchard, D.I., Bycroft, B.W., Williams, P. and Stewart, G.S.A.B. (1998) Quorum sensing: a novel target for anti-infective therapy. *J. Antimicrob. Chemother.* 42, 569–571.
- [330] Hartman, G. and Wise, R. (1998) Quorum sensing: potential means of treating gram-negative infections? *Lancet* 351, 848–849.
- [331] Kline, T., Bowman, J., Iglewski, B.H., de Kievit, T., Kakai, Y. and Passador, L. (1999) Novel synthetic analogs of the *Pseudomonas* autoinducer. *Bioorg. Med. Chem. Lett.* 9, 3447–3452.
- [332] Fray, R.G., Throup, J.P., Daykin, M., Wallace, A., Williams, P., Stewart, G.S.A.B. and Grierson, D. (1999) Plants genetically modi-

- fied to produce *N*-acylhomoserine lactones communicate with bacteria. *Nature Biotechnol.* 17, 1017–1020.
- [333] Schell, M.A. (1996) To be or not to be: how *Pseudomonas solanacearum* decides whether or not to express virulence genes. *Eur. J. Plant Pathol.* 102, 459–469.
- [334] Clough, S.J., Flavier, A.B., Schell, M.A. and Denny, T.P. (1997) Differential expression of virulence genes and motility in *Ralstonia* (*Pseudomonas*) *solanacearum* during exponential growth. *Appl. Environ. Microbiol.* 63, 844–850.
- [335] Brumbley, S.M., Carney, B.F. and Denny, T.P. (1993) Phenotype conversion in *Pseudomonas solanacearum* due to spontaneous inactivation of PhcA, a putative LysR transcriptional regulator. *J. Bacteriol.* 175, 5477–5487.
- [336] Clough, S.J., Lee, K.E., Schell, M.A. and Denny, T.P. (1997) A two-component system in *Ralstonia* (*Pseudomonas*) *solanacearum* modulates production of *phcA*-regulated virulence factors in response to 3-hydroxypalmitic acid methyl ester. *J. Bacteriol.* 179, 3639–3648.
- [337] Flavier, A.B., Clough, S.J., Schell, M.A. and Denny, T.P. (1997) Identification of 3-hydroxypalmitic acid methyl ester as a novel autoregulator controlling virulence in *Ralstonia solanacearum*. *Mol. Microbiol.* 26, 251–259.
- [338] Flavier, A.B., Ganova-Raeva, L.M., Schell, M.A. and Denny, T.P. (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, 7089–7097.
- [339] Garg, R.P., Yindeeyoungyeon, W., Gilis, A., Denny, T.P., van der Lelie, D. and Schell, M.A. (2000) Evidence that *Ralstonia eutropha* (*Alcaligenes eutrophus*) contains a functional homologue of the *Ralstonia solanacearum* Phc cell density sensing system. *Mol. Microbiol.* 38, 359–367.
- [340] Flavier, A.B., Schell, M.A. and Denny, T.P. (1998) A *rpoS* (sigmaS) homologue regulates acylhomoserine lactone-dependent autoinduction in *Ralstonia solanacearum*. *Mol. Microbiol.* 28, 475–486.
- [341] Schell, M.A. (2000) Control of virulence and pathogenicity genes of *Ralstonia solanacearum* by an elaborate sensory network. *Annu. Rev. Phytopathol.* 38, 263–292.
- [342] Onsando, J.M. (1992) Black rot of crucifers. In: *Diseases of Vegetables and Oil Seed Crops* (Chaube, H.S., Kumar, J., Mukhopadhyay, A.N and Singh, U.S., Eds.), pp. 243–252. Prentice Hall, Englewood Cliffs, NJ.
- [343] Dow, J.M. and Daniels, M.J. (1994) Pathogenicity determinants and global regulation of pathogenicity of *Xanthomonas campestris* pv. *campestris*. In: *Bacterial Pathogenesis of Plants and Animals* (Dangl, J.L., Ed.), pp. 29–41. Springer-Verlag, Heidelberg.
- [344] Barber, C.E., Tang, J.L., Feng, J.X., Pan, M.Q., Wilson, T.J.G., Slater, H., Dow, J.M., Williams, P. and Daniels, M.J. (1997) A novel regulatory system required for pathogenicity of *Xanthomonas campestris* is mediated by a small diffusible signal molecule. *Mol. Microbiol.* 24, 555–566.
- [345] Dow, J.M., Feng, J.X., Barber, C.E., Tang, J.L. and Daniels, M.J. (2000) Novel genes involved in the regulation of pathogenicity factor production within the *rpf* gene cluster of *Xanthomonas campestris*. *Microbiology* 146, 885–891.
- [346] Tang, J.L., Liu, Y.N., Barber, C.E., Dow, J.M., Wootton, J.C. and Daniels, M.J. (1991) Genetic and molecular analysis of a cluster of *rpf* genes involved in positive regulation of synthesis of extracellular enzymes and polysaccharide in *Xanthomonas campestris* pathovar *campestris*. *Mol. Gen. Genet.* 226, 409–417.
- [347] Wilson, T.J.G., Bertrand, N., Tang, J.L., Feng, J.X., Pan, M.Q., Barber, C.E., Dow, J.M. and Daniels, M.J. (1998) The *rpfA* gene of *Xanthomonas campestris* pathovar *campestris*, which is involved in the regulation of pathogenicity factor production, encodes an aconitase. *Mol. Microbiol.* 28, 961–970.
- [348] Slater, H., Alvarez-Morales, A., Barber, C.E., Daniels, M.J. and Dow, J.M. (2000) A two-component system involving an HD-GYP domain protein links cell-cell signalling to pathogenicity gene expression in *Xanthomonas campestris*. *Mol. Microbiol.* 38, 986–1003.
- [349] Ishige, K., Nagasawa, S., Tokishita, S. and Mizuno, T. (1994) A novel device of bacterial signal transducers. *EMBO J.* 13, 5195–5202.
- [350] Galperin, M.Y., Natale, D.A., Aravind, L. and Koonin, E.V. (1999) A specialized version of the HD hydrolase domain implicated in signal transduction. *J. Mol. Microbiol. Biotechnol.* 1, 303–305.
- [351] Uhl, A.M. and Miller, J.F. (1996) Central role of the BvgS receiver as a phosphorylated intermediate in a complex two-component phosphorelay. *J. Biol. Chem.* 271, 33176–33180.
- [352] Uhl, A.M. and Miller, J.F. (1996) Integration of multiple domains in a two-component sensor protein: the *Bordetella pertussis* BvgAS phosphorelay. *EMBO J.* 15, 1028–1036.
- [353] Poplawsky, A.R. and Chun, W. (1997) *pigB* determines a diffusible factor needed for extracellular polysaccharide slime and xanthomonadin production in *Xanthomonas campestris* pv. *campestris*. *J. Bacteriol.* 179, 439–444.
- [354] Poplawsky, A.R. and Chun, W. (1998) *Xanthomonas campestris* pv. *campestris* requires a functional *pigB* for epiphytic survival and host infection. *Mol. Plant-Microbe Interact.* 11, 466–475.
- [355] Chun, W., Cui, J. and Poplawsky, A. (1997) Purification, characterization and biological role of a pheromone produced by *Xanthomonas campestris* pv. *campestris*. *Physiol. Mol. Plant Pathol.* 51, 1–14.
- [356] Poplawsky, A.R., Chun, W., Slater, H., Daniels, M.J. and Dow, J.M. (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.
- [357] Dow, J.M. and Daniels, M.J. (2000) *Xylella* genomics and bacterial pathogenicity to plants. *Yeast* 17, 263–271.