Quorum sensing, virulence and secondary metabolite production in plant soft-rotting bacteria

Anne M. L. Barnard, Steven D. Bowden, Tom Burr, Sarah J. Coulthurst,
Rita E. Monson and George P. C. Salmond*

Department of Biochemistry, University of Cambridge, Tennis Court Road, Cambridge CB2 1QW, UK

Quorum sensing describes the ability of bacteria to sense their population density and respond by modulating gene expression. In the plant soft-rotting bacteria, such as *Erwinia*, an arsenal of plant cell wall-degrading enzymes is produced in a cell density-dependent manner, which causes maceration of plant tissue. However, quorum sensing is central not only to controlling the production of such destructive enzymes, but also to the control of a number of other virulence determinants and secondary metabolites. *Erwinia* synthesizes both *N*-acylhomoserine lactone (AHL) and autoinducer-2 types of quorum sensing signal, which both play a role in regulating gene expression in the phytopathogen. We review the models for AHL-based regulation of carbapenem antibiotic production in *Erwinia*. We also discuss the importance of quorum sensing in the production and secretion of virulence determinants by *Erwinia*, and its interplay with other regulatory systems.

Keywords: *Erwinia*; quorum sensing; *N*-acylhomoserine lactone; carbapenem antibiotic; virulence; phytopathogenicity

1. QUORUM SENSING IN PLANT SOFT-ROT BACTERIA

Over the past 15 years or so, it has become clear that bacteria are able to communicate with each other to coordinately regulate gene expression according to population density, in a process which has been termed 'quorum sensing' (reviewed in Whitehead *et al.* 2001). Depending on the bacterial strain, various bacterial processes can come under the control of the quorum sensing regulon, particularly systems involved in the production of secondary metabolites and those involved in virulence and symbiosis (Sebaihia 1999; Whitehead 1999; Corbett *et al.* 2005; Pemberton *et al.* 2005).

Quorum sensing has been well studied in some Gram-negative phytopathogens, including *Erwinia* species. *Erwinia carotovora* ssp. *carotovora* (*Ecc*) and the closely related *E. carotovora* ssp. *atroseptica* (*Eca*) are the causative agents of soft-rot disease in a range of host plants, including carrot and potato. *Eca* in particular is responsible for 'blackleg' infections in potato plants. Virulence depends on the production of an arsenal of plant cell wall-degrading enzymes (PCDWEs; including pectinases and cellulases) and many of these virulence determinants, and others, have been found to be under quorum sensing control. Quorum sensing signalling in *Erwinia* uses *N*-acylhomoserine lactone (AHL)-based systems (table 1; Jones *et al.* 1993; Pirhonen *et al.* 1993) as well as systems that depend on autoinducer-2 (AI-2; Coulthurst *et al.* 2006). It

should be noted that the AHL signalling molecules and quorum sensing systems used can vary between different strains, even within the *erwiniae* (table 1).

Regulation of production of the secondary metabolite, 1-carbapen-2-em-3 carboxylic acid (carbapenem or Car), by *Ecc* strain ATCC 39048 remains one of the best-characterized AHL-mediated quorum sensing-regulated systems and the first section of this review will summarize the current regulatory model. Subsequent sections will focus on AI-2-based quorum sensing signalling and the role of quorum sensing in the virulence of *Erwinia* and related species.

2. QUORUM SENSING REGULATES CARBAPE-NEM PRODUCTION IN *E. CAROTOVORA*

Erwinia carotovora ssp. *carotovora* strain ATCC 39048 first produces detectable levels of the β -lactam antibiotic, Car (figure 1), during the transition between late log and stationary phases of growth in laboratory culture (Bainton *et al.* 1992a,b). The Car biosynthetic and autoreistance functions of this strain are encoded by the *carABCDEFGH* gene cluster (figure 1; McGowan *et al.* 1996, 1997). The enzymes encoded by *carA*, *carB* and *carC* are essential for the production of Car (McGowan *et al.* 1997; reviewed in Coulthurst *et al.* 2005). CarB catalyses the first committed step in Car biosynthesis, the formation of carboxymethylproline, from precursors that are thought to be derived from acetate and glutamate (Sleeman & Schofield 2004). CarD and CarE may be involved in the supply of these precursors, but the *carD* and *carE* genes are not essential; disruption of either genes results in the reduction, but not the complete abolition, of Car production (McGowan *et al.* 1997).

* Author for correspondence (gpacs@mole.bio.cam.ac.uk).

One contribution of 12 to a Theme Issue 'Bacterial conversations: talking, listening and eavesdropping'.

Table 1. Examples of quorum sensing systems in erwiniae and related species. (Features of the quorum sensing systems of selected *Erwinia* strains and related bacterial species are shown. Alternative gene nomenclature is provided where applicable. The quorum sensing signalling molecules are abbreviated as follows: 3-oxo-C6-HSL, *N*-(3-oxohexanoyl)-L-homoserine lactone; 3-oxo-C8-HSL, *N*-(3-oxooctanoyl)-L-homoserine lactone; C6-HSL, *N*-hexanoyl-L-homoserine lactone; C4-HSL, *N*-butanoyl-L-homoserine lactone; C10-HSL, *N*-decanoyl-L-homoserine lactone.)

species/strain	quorum sensing signal	LuxI/R homologue	quorum sensing-regulated phenotypes	reference
<i>Erwinia amylovora</i> OMP-BO 1077/7	3-oxo-C6-HSL(?)	EamI, EamR	virulence (?)	Venturi <i>et al.</i> (2004)
<i>Erwinia carotovora</i> ssp. <i>carotovora</i> ATCC 39048	3-oxo-C6-HSL	CarI, CarR (CarI also known as ExpI) CarI, VirR	carbapenem production exoenzyme and virulence factor production unknown	Jones <i>et al.</i> (1993), McGowan <i>et al.</i> (1995, 2005) and Burr <i>et al.</i> (2006)
SCRI 193	3-oxo-C6-HSL ^a	ExpR (also known as EccR) ExpI, ExpR, VirR (CarR is cryptic)	exoenzyme and virulence factor production	Hinton & Salmond (1987), Jones <i>et al.</i> (1993) Holden <i>et al.</i> (1998) and Burr <i>et al.</i> (2006)
SCC3193	3-oxo-C8-HSL, 3-oxo-C6-HSL, C8-HSL	ExpI, ExpR	exoenzyme and virulence factor production	Pirhonen <i>et al.</i> (1993), Andersson <i>et al.</i> (2000), Toth <i>et al.</i> (2004) and Chatterjee <i>et al.</i> (2005)
EC153 Ecc 71	3-oxo-C8-HSL, 3-oxo-C6-HSL 3-oxo-C6-HSL	AhlI, ExpR ExpI (also known as AhII, HsII), ExpR	exoenzyme production exoenzyme and virulence factor production	Chatterjee <i>et al.</i> (2005) Chatterjee <i>et al.</i> (1995) and Cui <i>et al.</i> (2005)
<i>Erwinia carotovora</i> ssp. <i>atroseptica</i> SCRI 1043	3-oxo-C6-HSL ^a	ExpI, ExpR, VirR	exoenzyme and virulence factor production	Burr <i>et al.</i> (2006)
<i>Erwinia chrysanthemi</i> EC 3937	3-oxo-C6-HSL, C6-HSL, C10-HSL	ExpI, ExpR		Nasser <i>et al.</i> (1998)
<i>Pantoea stewartii</i> DC283	3-oxo-C6-HSL	EsaI, EsaR	exopolysaccharide production and virulence	Von Bodman <i>et al.</i> (1998)
<i>Serratia</i> sp. ATCC 39006	C4-HSL, C6-HSL	SmaI, SmaR, CarR SmaI, SmaR	carbapenem production prodigiosin and virulence factor production	Thomson <i>et al.</i> (2000) and Slater <i>et al.</i> (2003)

^a In addition to the major AHL, 3-oxo-C6-HSL, the presence of 3-oxo-C8-HSL and C6-HSL has been described in these *Erwinia* strains (Toth *et al.* 2004).

Figure 1. The regulation of carbapenem antibiotic production in *Erwinia carotovora* ssp. *carotovora*. The organization of the *car* biosynthetic locus is shown. *carA–E* encode the Car biosynthetic enzymes and *carF–G* encode the Car resistance mechanism. The *carH* product is of unknown function. *carA–H* are expressed from a single, quorum sensing-dependent promoter located upstream of *carA* (P_{carA}). There is also a weak, constitutive promoter located within *carD* (P_{int}), which causes expression of *carEFGH*, and hence primes the Car-resistance mechanism, in the absence of quorum sensing-dependent production of the Car antibiotic. Quorum sensing depends on a diffusible signalling molecule, *N*-(3-oxohexanoyl)-L-homoserine lactone (3-oxo-C6-HSL), produced by the CarI synthase. 3-Oxo-C6-HSL binds to and activates the response regulator CarR, which is encoded immediately upstream of the *carA* gene. CarR/3-oxo-C6-HSL binds to the *carA* promoter and activates transcription of *carA–H*. Car production is also controlled by the *hor* gene product. Various environmental factors such as temperature, pH of the culture medium, carbon source and anaerobiosis also influence expression of *carA–H*. Pointed arrows or (+) symbols indicate a positive effect, while blunted arrows indicate a negative effect. The (?) symbols indicate a possible effect.

CarA is the β -lactam synthetase, acting on the product of the CarB-catalysed reaction, (2*S*-5*S*) carboxymethylproline, to close the β -lactam ring (Miller *et al.* 2002*a*, 2003; Gerratana *et al.* 2003). Finally, CarC catalyses an epimerization and desaturation reaction, forming the active carbapenem antibiotic (Li *et al.* 2000). In addition, ATCC 39048 possesses an intrinsic antibiotic resistance mechanism, encoded by *carF* and *carG*, to counter the action of the antibiotic it produces (McGowan *et al.* 1997). However, the function of the *carH* gene product remains unknown (McGowan *et al.* 1997).

The *car* gene cluster is shown in figure 1. The eight *car* genes are organized as an operon, with a quorum sensing-dependent promoter driving production of a single transcript, initiating 52 bp upstream from the translational start of *carA* (McGowan *et al.* 2005). However, there is also a weak quorum sensing-independent promoter within *carD*, which means that the cells constitutively express CarE–H at low cell densities and are therefore resistant to Car produced

by other bacteria (McGowan *et al.* 2005). This quorum sensing-independent expression of the resistance genes also means that the Car resistance mechanism is in place before the quorum sensing-dependent expression of the antibiotic biosynthesis genes occurs at high cell densities.

Early work showed that Car production in *Ecc* could be induced prematurely (i.e. at lower cell densities) when grown in the presence of filter-sterilized spent culture supernatants from stationary-phase *Ecc* cultures (Bainton *et al.* 1992*a,b*). This suggested the accumulation of a diffusible signal in stationary-phase cultures that could activate Car production. It was subsequently shown that a single gene product was responsible for the production of this signalling molecule. The gene encoded a homologue of the previously identified LuxI protein from *Vibrio fischeri* and was named *carI* (Swift *et al.* 1993). CarI produces the AHL signal, *N*-3-(oxohexanoyl)-L-homoserine lactone (3-oxo-C6-HSL; figure 1 and table 1). Purified synthetic 3-oxo-C6-HSL was shown to induce

Figure 2. Models for quorum sensing-dependent control of Car production in (a,b) *Erwinia carotovora* ssp. *carotovora* strain ATCC 39048 and (c,d) *Serratia* sp. strain ATCC 39006. At low cell densities, *carA-H* are not expressed in both *Erwinia* and *Serratia* (a,c); in *Erwinia*, this is because transcription of *carA-H* is not activated (a). However, in *Serratia*, the SmaR repressor adds an extra layer of control by preventing transcription of *carA-H* and *carR* in the absence of quorum sensing signal (c). At high cell densities in *Erwinia*, the 3-oxo-C6-HSL quorum sensing signal binds and activates CarR, which promotes *carA-H* transcription and hence activates Car production (b). In *Serratia*, Car production is derepressed at high cell densities due to the AHL quorum sensing signal which inactivates the SmaR repressor. The *Serratia* CarR protein is then able to activate *carA-H* transcription in a ligand-independent manner (d).

premature Car production in ATCC 39048 (Bainton *et al.* 1992a,b) and to alleviate the Car[−] phenotype of a *carI* mutant (Jones *et al.* 1993).

AHL-based quorum sensing systems rely on the presence of a transcriptional regulator, a member of the LuxR family of proteins, that detects the AHL signal and responds by bringing about a change in the transcriptional regulation. The LuxR homologue involved in the control of Car production in *Erwinia* is called CarR, and is encoded by the *carR* gene, located immediately upstream of *carA* (figure 1; McGowan *et al.* 1995). The *carR* gene is a separate transcriptional unit from the *carA-H* operon, initiating 108 bp upstream from its translational start site (McGowan *et al.* 2005). The *carI* gene is located elsewhere in the genome and is adjacent to a convergently transcribed gene, *expR*, that encodes a second LuxR homologue in *Erwinia* (Swift *et al.* 1993; Rivet 1998). Finally, there is a gene encoding a third LuxR homologue in the genome, VirR, which is an important regulator of virulence factors in *Erwinia* (see §12; Burr *et al.* 2006). Disruption of *carI* and/or *carR* leads to the abolition of Car production and transcription from the quorum sensing-dependent *carA* promoter (McGowan *et al.* 2005). However, inactivation of either *expR* or *virR* has no obvious influence on Car production, either in the presence or absence of a functional *carI* gene. Therefore, it appears that CarR is the only LuxR homologue in ATCC 39048 that is involved in the regulation of Car production.

It is possible to bypass the dependence of Car production on 3-oxo-C6-HSL in a *carI* mutant by supplying *carR* *in trans* on a plasmid (McGowan *et al.* 1995). Therefore, CarR is capable of activating the *carA* promoter in a ligand-independent fashion when present at high non-physiological concentrations. However, *carR* transcription is tightly controlled via a CarR- and 3-oxo-C6-HSL-dependent autoregulatory mechanism (shown using a *carR::lacZ* reporter with the provision of a functional *carR* *in trans* on a plasmid), and therefore the concentration of CarR within the cell is limited (McGowan *et al.* 2005).

3. THE MODEL FOR QUORUM SENSING CONTROL OF CAR PRODUCTION IN *ERWINIA*

The model for quorum sensing-dependent regulation of antibiotic production in *Erwinia* is shown in figure 2. 3-Oxo-C6-HSL has been shown to interact directly with the CarR protein and increase its DNA-binding affinity for the *carR-A* intergenic region (Welch *et al.* 2000). Upon binding of CarR/3-oxo-C6-HSL to the *carA* promoter, *carA-H* transcription is activated, leading to Car production. Thus, under normal conditions, Car production is not activated by CarR until the concentration of 3-oxo-C6-HSL reaches a high enough level, which occurs during the transition from late log to stationary phase of growth. As 3-oxo-C6-HSL levels decline during the stationary phase of growth, we presume that 3-oxo-C6-HSL begins to dissociate from

CarR, which can no longer bind and activate the *carR* and *carA* promoters, and thus Car production is switched off.

4. THE Hor TRANSCRIPTIONAL REGULATOR

The quorum sensing system in *Ecc* is not the only genetic system responsible for the control of Car production. The *hor* gene product (figure 1) is a member of the SlyA family of transcriptional regulators, homologues of which are present in many bacterial species (Thomson *et al.* 1997). This family of proteins controls a diverse range of physiological functions, such as multidrug resistance, toxin production, secondary metabolite production and virulence, in both plant and animal pathogens. In *Erwinia*, Hor is an activator of Car and PCWDE production (Thomson *et al.* 1997). Analysis of *carA* transcriptional fusion strains has shown that Hor activates transcription from the *carA* promoter, although direct physical binding of Hor to the *carA* promoter has not been demonstrated (McGowan *et al.* 2005). Since Hor does not affect the transcription of either *carR* or *carI*, it appears to regulate Car biosynthesis via a quorum sensing-independent mechanism. However, the Car and exoenzyme phenotype of a *hor* mutant can be overridden (at least partially) by the provision of an excess of exogenously added 3-oxo-C6-HSL, suggesting that there may be some crosstalk between the quorum sensing- and the Hor-dependent pathways controlling Car production (McGowan *et al.* 2005).

5. ENVIRONMENTAL FACTORS INFLUENCING Car PRODUCTION

In addition to the cell density-dependent regulation described above, it is clear that Car production in *Ecc* also responds to a variety of other environmental cues, such as temperature, anaerobiosis, pH and carbon source availability (Byers *et al.* 2002; McGowan *et al.* 2005). Although less well characterized at the molecular genetic level, at least some of these inputs are integrated into the Car regulatory network via the quorum sensing system itself, as summarized in figure 1.

The chemical stability of AHL signalling molecules themselves is greatly influenced by pH (Byers *et al.* 2002; Yates *et al.* 2002). Under alkaline conditions, AHLs are rapidly hydrolysed, and hence 'inactivated', and are unable to function as quorum sensing signals. In a laboratory setting, unbuffered *Erwinia* culture medium becomes alkaline during the stationary phase of growth, owing to metabolism of peptides (L-broth). Under these alkaline conditions, the 3-oxo-C6-HSL signal is degraded and Car production is switched off during stationary phase. However, if the medium is buffered at a neutral pH, the quorum sensing signal is allowed to accumulate and the concentrations of 3-oxo-C6-HSL and Car detected in culture supernatants during stationary phase of growth remain constant (Byers *et al.* 2002). The sensitivity of AHLs to extremes of pH may be important during infection *in planta*, since one of the first plant responses to infection by *Erwinia* is alkalization of the plant infection site (Nachin & Barras 2000). This might aid the plant in resisting *Erwinia*

infection by interfering with its quorum sensing system (Byers *et al.* 2002).

Another environmental factor that can influence Car production in *Ecc* is temperature (McGowan *et al.* 2005). The optimal temperature for both *carA* transcription and Car production in *Ecc* is 34°C. However, higher temperatures are inhibitory to antibiotic biosynthesis and Car production is completely abolished at growth temperatures of 37°C and above. Although *carI* transcription is apparently unaffected by an increase in temperature, the concentration of 3-oxo-C6-HSL detected in culture supernatants is reduced at 37°C, suggesting that a diminution of 3-oxo-C6-HSL may be responsible for the effect of temperature on Car production. The addition of exogenous 3-oxo-C6-HSL can partially restore Car production by *Ecc* grown at 37°C. However, in addition to the effect of temperature on 3-oxo-C6-HSL production, transcription of *hor* (and so production of the Hor transcriptional activator) is repressed at 37°C. Thus, the effect of growth temperature on Car production in *Ecc* is mediated by both Hor and the quorum sensing system (McGowan *et al.* 2005).

Car production by *Erwinia* is also influenced by the nature of the carbon source present in the growth medium (McGowan *et al.* 2005). In minimal medium containing sucrose as the sole carbon source, transcription of *carA* was approximately 50% higher than in minimal medium containing glucose; while growth in medium containing glycerol completely inhibited *carA* transcription. Transcription of *carR* and *hor* were unaffected by the nature of the carbon source, but *carI* transcription was found to be reduced by approximately 50% in minimal glycerol medium compared with minimal sucrose medium. This effect on *carI* transcription was also reflected in 3-oxo-C6-HSL levels detected in the culture supernatants (McGowan *et al.* 2005).

It is also known that oxygen availability affects Car production, which could be an important consideration during plant infection. Antibiotic cannot be detected in laboratory cultures grown under oxygen-limiting conditions. However, since *Ecc* cultured under these conditions is severely affected in growth rate, it is not possible to distinguish between the effect of a diminished growth rate and the effect of anaerobiosis on Car production (McGowan *et al.* 2005).

6. CRYPTIC CARBAPENEM GENE CLUSTERS ARE WIDESPREAD IN *ERWINIA* SPECIES

Upon identifying the *carR-H* locus in ATCC 39048, genetic screens were conducted to determine if the gene cluster was widely distributed among *Erwinia* species (Holden *et al.* 1998). The results of these screens showed that, while production of the AHL quorum sensing signalling molecule appeared to be universal in a diverse range of *erwiniae* (43 *Ecc* and 39 *Eca* isolates were tested), only a few *Erwinia* species produced Car under standard laboratory conditions (only two of the *Ecc* isolates tested; Holden *et al.* 1998). However, Southern blot analyses using probes to the Car biosynthetic genes revealed that the *car* cluster was present in a further seven *Ecc* strains, as well as two of the *Eca* strains tested. The lack of Car production could not

Figure 3. Structures and abbreviations of AHL quorum sensing signalling molecules commonly produced by *erwiniae* and related species.

be attributed to a lack of the AHL quorum sensing signalling molecule and, furthermore, Car production could be activated in these strains by the provision of the *carR* gene from ATCC 39048 *in trans* on a plasmid (Holden *et al.* 1998).

One of the strains containing a cryptic *car* cluster was *Ecc* strain SCRI193. This strain was found to have a complete *carR*–*H* gene locus but did not produce Car, even though it synthesized 3-oxo-C6-HSL. However, the lack of Car production could not be attributed to a defective CarR protein either, as, when supplied on a plasmid, the SCRI193 *carR* gene could activate Car production in this strain (Holden *et al.* 1998). There are a number of hypotheses that could explain this result. It is possible that transcription of *carR* is reduced in SCRI193 and thus, while CarR₁₉₃ is functional, its concentration within the cell is just too low. Alternatively, 3-oxo-C6-HSL and/or DNA binding by CarR₁₉₃ may be weaker than the corresponding CarR protein from strain ATCC 39048. It is also possible that SCRI193 requires additional factors to produce Car that are not necessary for ATCC 39048 when grown under standard laboratory conditions.

Another potential explanation for the presence of cryptic *car* genes is that *Erwinia* species may be in the process of ‘degenerating’ the *car* clusters perhaps because it represents some unknown fitness cost to

the species. If this explanation is correct, then it is interesting that the Car resistance functions are maintained in SCRI193. It might be ideal for some strains to be resistant to Car without the metabolic burden of producing the antibiotic. The real ecological value of antibiotic production by *Erwinia* is not known.

7. Car PRODUCTION IN *SERRATIA* SPECIES ATCC 39006

Serratia sp. ATCC 39006 (39006) is a taxonomically ill-defined strain of *Serratia* that produces pectate lyase and cellulase activity, like the *erwiniae*, and also produces the red pigment, prodigiosin (Slater *et al.* 2003), like other *Serratia* species. Interestingly, this strain also possesses functional homologues of the *Ecc* *car* genes (including *carR*), which are also regulated by quorum sensing in 39006 (Cox *et al.* 1998; Slater *et al.* 2003; Fineran *et al.* 2005). However, the means by which quorum sensing-mediated control of Car production is achieved in 39006 shows striking differences when compared with the model proposed for the control of Car production in *Ecc* (figure 2).

In 39006, the *luxI* homologue is called *smaI* and is found in the genome adjacent to, and transcribed convergently with, a gene called *smaR*, which encodes a second LuxR homologue (Thomson *et al.* 2000;

Slater *et al.* 2003). SmaI is the AHL synthase responsible for the production of the major quorum sensing signal, *N*-butanoyl-L-homoserine lactone (C4-HSL) as well as a small amount of *N*-hexanoyl-L-homoserine lactone (C6-HSL; figure 3). When either *smaI* or *carR* are disrupted in 39006, Car production is switched off (Slater *et al.* 2003). A *smaI* mutation, but not a *carR* mutation, can be complemented by the addition of exogenous C4-HSL. These results confirm that *smaI* is required for Car production because AHL is required to activate Car production. It also confirms that Car₃₉₀₀₆ is essential for Car production even in the presence of AHL.

When the other *luxR* homologue, *smaR*, is disrupted, Car production is unaffected (Slater *et al.* 2003). Surprisingly, however, a *smaR* mutation can phenotypically suppress a *smaI* mutation and switch Car production back on in the presence of a functional Car₃₉₀₀₆ (Slater *et al.* 2003). This result suggests that SmaR is a repressor of *carA-H* transcription in the absence of the AHL quorum sensing signal. This interpretation is supported by bandshift experiments demonstrating that purified SmaR protein can bind to the *carA* promoter region, but binding is reduced in the presence of C4-HSL (Slater *et al.* 2003). However, a *smaR* mutation cannot bypass a *carR* mutation (so that Car remains switched off), confirming that Car₃₉₀₀₆ is essential for Car production in 39006. Finally, in a *smaI*, *smaR* and *carR* triple mutant, Car production is switched off. This confirms once again that Car₃₉₀₀₆ is essential for Car production and, when combined with the data for the *smaIR* double mutant, shows that the Car₃₉₀₀₆ protein is an apparently quorum sensing signal-independent activator of Car production, as proposed by Cox *et al.* (1998).

By analysing the above data, the current model for Car regulation in 39006 is as follows. At low cell density and AHL concentration, SmaR acts as a repressor by binding to the *carA* promoter and preventing the transcription of the *car* cluster, as depicted in figure 2. At high cell density, the AHL concentration increases. The interaction of AHL with SmaR weakens the affinity of SmaR for the *carA* promoter and the SmaR repressor dissociates from the DNA. This allows Car₃₉₀₀₆ to bind to the *carA* promoter independently of AHL and activate *carA-H* transcription. As the AHL concentration declines, SmaR dissociates from AHL and binds to the *carA* promoter, switching off Car production.

It is interesting that the same gene cluster is regulated by AHL-based quorum sensing in two different organisms, ATCC 39048 and 39006, yet by two very different mechanisms. In ATCC 39048, Car production is positively regulated by a ligand-responsive activator, Car_{Ecc}, while in 39006, it is derepressed by the competing actions of the ligand-responsive repressor, SmaR, and the ligand-independent activator, Car₃₉₀₀₆ (figure 2).

8. Car PRODUCTION IN *PHOTORHABDUS LUMINESCENS* STRAIN TT01

A third enterobacterial species known to possess a *carA-H* gene cluster and to produce the carbapenem

antibiotic is *Photobacterium luminescens* strain TT01 (Derzelle *et al.* 2002). TT01 is an insect pathogen and can also exist in a symbiotic relationship with nematode species. In strain TT01, the gene locus is referred to as *cpmA-H* and each gene in the cluster exhibits sequence similarity with the corresponding genes in the ATCC 39048 and *Serratia* 39006 *carA-H* gene clusters. The final three genes in strain TT01, *cpmFGH*, are also duplicated elsewhere in the chromosome and called *cpmIJK*, although it is not known if they are functionally expressed (Derzelle *et al.* 2002). TT01 is not known to produce AHL signal molecules. Therefore, it is perhaps not surprising that there is no LuxR homologue encoded immediately upstream of the *cpmA-H* cluster. However, while it is difficult to measure Car production directly, owing to the many other antibiotics produced by TT01, there is evidence that Car production in this strain is repressed during the stationary phase of growth by a LuxS-type (AI-2-based) quorum sensing system (Derzelle *et al.* 2002). While there is no evidence that LuxS regulates Car production in ATCC 39048, there is evidence that a LuxS mutant of 39006 has reduced Car production suggesting that AI-2 has a positive input into Car production in this strain (Coulthurst *et al.* 2004, 2006). *Photobacterium luminescens* strain TT01 serves as yet another example of how a single gene locus can be regulated very differently by quorum sensing in different genetic backgrounds.

These data suggest that bacterial regulation of secondary metabolites by quorum sensing may be versatile, such that bacterial gene clusters (presumably acquired by horizontal gene transfer) can come under the control of existing regulatory pathways.

9. THE ROLE OF QUORUM SENSING IN *ERWINIA* VIRULENCE

While the role of quorum sensing in the control of Car production has been well characterized, it is by no means the only quorum sensing-controlled phenotype in *Erwinia* (Sebaihia 1999; Whitehead 1999; Corbett *et al.* 2005; Pemberton *et al.* 2005; Burr *et al.* 2006). Many *Erwinia* genes whose products are involved in pathogenesis and virulence in plant hosts also lie within the quorum sensing regulon.

10. QUORUM SENSING AND PROTEIN SECRETION IN *ERWINIA*

The production of secreted proteins is key to virulence in the soft-rot *erwinias*. As reviewed by Toth *et al.* (2003) and Toth & Birch (2005), these secreted proteins include both those involved in 'brute-force' attack on the plant tissue, namely the battery of PCWDEs, and also those involved in more subtle interactions with the plant host, for example, type III effectors (such as DspE/A), Nip and Svx. Production of many of these known secreted virulence factors has been shown to be quorum sensing dependent (Toth *et al.* 2003; Corbett *et al.* 2005; Pemberton *et al.* 2005; Toth & Birch 2005; Burr *et al.* 2006).

Corbett *et al.* (2005) used the proteomic technique of two-dimensional difference gel electrophoresis (2D-DiGE; Unlu *et al.* 1997) to identify quorum

sensing-dependent proteins in the secretome of *Eca* SCRI 1043. This study identified three novel quorum sensing-dependent secreted proteins: Sv_x, a putative cellulase and a putative proteoglycan hydrolase, in addition to previously known quorum sensing-dependent secreted enzymes, e.g. PelC and CelV. Following its identification by proteomic analysis, Sv_x was subsequently shown to be required for full virulence *in planta*, demonstrating the usefulness of this approach for the identification of novel virulence factors. In addition, 2D-DiGE also revealed reduced levels of Pel, Cel and Prt enzymes in the secretome of the *luxS* mutant of *Ecc* ATTn10 (Coulthurst *et al.* 2006). A recent proteomic study of *Ech* strain 3937 showed that secretion of multiple pectinases together with a rhamnogalacturonase and an esterase were induced by the addition of appropriate plant-derived inducing compounds (Kazemi-Pour *et al.* 2004). A novel protein, AvrL, homologous to Sv_x, was also identified, although its role, if any, in *Ech* virulence is unknown (Kazemi-Pour *et al.* 2004).

Although such proteomic analyses are proving valuable in identifying and comparing secreted proteins from wild-type *Erwinia* and defined mutants, such proteomic analyses only provide a 'snapshot' of the secretome. Growth under a variety of different conditions, e.g. in different inducing media, is expected to reveal many more secreted proteins and, of course, some key proteins may only be expressed *in planta*. Furthermore, some proteins cannot be seen on two-dimensional gels, often owing to low abundance and/or solubility (although other approaches, such as mass spectrometry-based, are expected to help address this issue). It is therefore anticipated that there are many more secreted proteins yet to be discovered, possibly with important roles *in planta*. With the increase in genomic sequence information, approaches such as proteomics and transcriptomics are an emerging and attractive way to gain a more global picture of the extent of the quorum sensing regulon in an organism. For example, in *Pseudomonas aeruginosa*, transcriptomic analyses have revealed that quorum sensing regulates, directly or indirectly, the expression of at least 300 genes, including many important for virulence (Schuster *et al.* 2003; Wagner *et al.* 2003). Moreover, a proteomic analysis in this organism has revealed that a significantly greater proportion of the proteome than the transcriptome seems to be affected by quorum sensing, implying that quorum sensing regulation is mediated at a post-transcriptional, as well as a transcriptional, level (Arevalo-Ferro *et al.* 2003).

Given that many important secreted virulence factors are produced by *Erwinia*, many in a quorum sensing-dependent fashion, it is also pertinent to consider how these secreted proteins are moved out of the bacterial cell and, in a few cases, into the plant cell. In both *E. carotovora* and *Ech*, proteases are secreted by the one-step type I secretion system, with the *Ech* Prt system (encompassing the transporter PrtDEF and its substrates, proteases PrtABCG) being one of the model systems for this means of secretion (Delepelaire 2004). The majority of the key secreted virulence factors of *E. carotovora* and *Ech*, including multiple Pels, Peh, Cel and Sv_x, are secreted by a

two-step type II secretion system known as Out (He *et al.* 1991; Reeves *et al.* 1993; Corbett *et al.* 2005). Type II secretion systems are complex assemblages, comprising 12–16 different proteins, which span the periplasm and translocate their substrate proteins from the periplasm to the exterior of the cell, following Sec (or Tat)-dependent export to the periplasm (Filloux 2004). Type II secretion is important for virulence in a variety of Gram-negative bacteria (Sandkvist 2001). Type III secretion systems (complex multiprotein structures that deliver 'effector' proteins directly into host cells) are important for virulence in plant and animal pathogens. In plant pathogens, type III systems are generally known as Hrp ('hypersensitive response and pathogenicity') systems and are involved in the establishment of infection in host plants and eliciting a defence response known as the hypersensitive reaction (HR) in non-host (resistant) plants (Alfano & Collmer 2004; He *et al.* 2004). *Ecc*, *Eca* and *Ech* all have *hrp* gene clusters, but the exact role of these secretion systems in virulence and the identity of all of their substrates remain to be fully clarified (Rantakari *et al.* 2001; Yang *et al.* 2002; Toth *et al.* 2003; Holeva *et al.* 2004). Several type III-dependent proteins have been identified in the soft-rotting erwinias: the harpin, HrpN, has been implicated in virulence and/or HR elicitation in *Ecc*, *Eca* and *Ech*, and the effector, DspE/A, has been implicated in pathogenicity in *Eca* (Mukherjee *et al.* 1997; Yang *et al.* 2002; Holeva *et al.* 2004). Interestingly, expression of HrpN is reported to be under AHL quorum sensing control in *Ecc* and *Eca*, but not in *Ech* (Mukherjee *et al.* 1997; Ham *et al.* 2004; Smadja *et al.* 2004).

There are a number of questions that are currently the subject of further investigation: is the secretion process itself also quorum sensing controlled? Alternatively, are the secretion systems expressed and 'primed' ready to secrete their substrates as soon as their expression is activated by quorum sensing, or are they simply able to secrete both quorum sensing-dependent and -independent substrates whenever required? Quorum sensing-dependent secretion systems have been reported in other bacteria, e.g. the Lip type I secretion system of *S. liquefaciens* and the Xcp type II secretion system of *P. aeruginosa* (Chapon-Herve *et al.* 1997; Riedel *et al.* 2001).

11. MECHANISM OF QUORUM SENSING-MEDIATED CONTROL OF VIRULENCE FACTOR PRODUCTION

As illustrated by the cell density-dependent control of Car production in *Erwinia* and *Serratia*, members of the LuxR family of proteins play a key role in quorum sensing. Indeed, in the majority of the known cases, LuxR-type proteins are crucial for detecting the AHL signal produced by LuxI family members and modulating gene expression accordingly. It has been known for some time that the *E. carotovora* LuxI homologue, CarI (in *Ecc*) or its equivalent, ExpI (in *Eca*), is required for optimal virulence factor production (Jones *et al.* 1993; Pirhonen *et al.* 1993) and thus the involvement of a LuxR protein in regulating virulence factor production was postulated (McGowan & Salmond 1999;

Whitehead *et al.* 2002). However, for a long time, the details of this quorum sensing-dependent regulatory pathway eluded investigators. It is only in the light of recent studies that the nature of this regulation is becoming clear.

As discussed, the regulation of carbapenem antibiotic production by CarR in *Erwinia* is a particularly well-studied example of LuxR-regulated, AHL-dependent gene expression. However, mutations in the *Ecc carR* gene have no effect on virulence factor production in this strain. Additionally, as previously discussed, like many so-called LuxIR systems, the *Ecc carI* gene has an adjacent overlapping *luxR* gene (*expR*) that encodes a second LuxR-type regulator (Swift *et al.* 1993). The same organization is seen in *Eca*, with the *expI* and *expR* genes. The corresponding *luxIR* genes in *Ecc* strain 71 are also called *expI* and *expR*, and recent studies have revealed a potential role for ExpR₇₁ in controlling virulence factor production (Chatterjee *et al.* 2005). In the absence of an AHL signal, ExpR₇₁ stimulates the production of the RsmA protein (regulator of secondary metabolites), which in turn is known to negatively regulate the production of virulence factors (Chatterjee *et al.* 1995; Cui *et al.* 2005). In a recent study by Cui *et al.* (2005), ExpR₇₁ was shown to bind *in vitro* to the *rsmA* promoter in the absence of AHL and this binding was abolished by the addition of the AHL signal, 3-oxo-C6-HSL. This correlates with the observation that RsmA levels in an *expI* mutant are higher than in an *expI*, *expR* double mutant. However, while virulence factor production in an *expI* mutant is increased by the *expR* mutation, it is not restored to the wild-type level seen in *Ecc* 71 cells expressing 3-oxo-C6-HSL (Cui *et al.* 2005). It seems then that some additional factor(s) must be contributing to the quorum sensing-dependent control of these genes in *Ecc* strain 71. A recent investigation by Welch *et al.* (2005), that investigated ligand binding by *Ecc* ATCC 39048 LuxR proteins, supports this notion. They studied binding using the ligands derived from the physiological 3-oxo-C6-HSL quorum sensing signal and the results show that, of the various ligands tested, 3-oxo-C6-HSL is the ligand most tightly bound by *Ecc* CarR and results in the greatest induction of carbapenem production. In contrast to this result, 3-oxo-C6-HSL is not the ligand most tightly bound by ExpR, but it is the ligand that most strongly induces production of PCWDEs. Thus, it is possible that the part of stimulating virulence factor production in response to 3-oxo-C6-HSL is performed (or supported) by another player.

12. THE *ERWINIA* VirR REGULATOR

Another LuxR protein has now been described in both *Eca* and *Ecc*, which is intimately involved in the control of virulence factor production in response to quorum sensing. Like *Ecc*71 ExpR, this additional LuxR protein (named VirR for virulence repressor) acts to negatively regulate a number of virulence products in the absence of its presumed ligand, 3-oxo-C6-HSL (Burr *et al.* 2006). The complement of factors regulated by VirR is extensive and includes PCWDEs such as Pel and Cel, as well as less well-characterized virulence factors such

as the recently described Svx and Nip proteins (Corbett *et al.* 2005; Pemberton *et al.* 2005).

By homology with other LuxR family members, VirR possesses an N-terminal 'autoinducer domain' (used by other LuxR proteins to bind AHL ligands) and a C-terminal DNA-binding domain, characterized by the presence of a helix-turn-helix motif. The fact that VirR acts as a repressor only in the absence of the AHL signal explains why it has not previously been isolated in genetic screens looking for genes involved in the quorum sensing-dependent regulation of PCWDE production. Analysis of the *Eca* 1043 genome sequence identified the gene encoding VirR in the genome of this strain and prompted further investigation of its role in quorum sensing (Bell *et al.* 2004). Critically, a *virR* mutation completely restores virulence factor production in an *Eca expI* mutant to wild-type levels. This increase in expression is reflected in an increase in the level of virulence gene mRNA and, importantly, translates into a corresponding restoration of virulence *in planta* to wild-type levels. Thus, VirR has a central role in the quorum sensing regulatory network controlling *Eca* virulence.

The simplest model for repression of virulence gene expression by VirR is that it binds directly to the promoter regions of those genes to prevent transcription. However, it is currently unknown whether the repression of transcription by VirR is direct, or indirect perhaps, as suggested in the case of ExpR₇₁, via an additional factor. The mechanism by which VirR represses transcription of its target genes requires further study. Indeed, members of the LuxR family have been shown to vary in their response to the AHL signal. Some function only in the presence of the AHL signal (e.g. CarR_{*Ecc*}, *Escherichia coli* SdiA and *Agrobacterium tumefaciens* TraR; Qin *et al.* 2000; Welch *et al.* 2000; Yao *et al.* 2006; Van Houdt *et al.* 2006), while others are functional only in the absence of the signal (e.g. *Pantoea stewartii* EsaR, *Ecc* ExpR₇₁ and *Serratia* Smar₃₉₀₀₆; Minogue *et al.* 2002; Slater *et al.* 2003; Chatterjee *et al.* 2005). Other LuxR family members do not appear to respond to an AHL signal at all (e.g. *Serratia* CarR₃₉₀₀₆; Cox *et al.* 1998).

Like *E. carotovora*, the related soft-rotting phytopathogen, *Erwinia chrysanthemi* (*Ech*), also has the *expI/R* locus but, significantly, *virR* does not appear to be present in the genome of *Ech* (Burr *et al.* 2006; *E. chrysanthemi* sequencing project at <http://www.tigr.org>). This can be explained by the observation that in *Ech*, production of most PCWDEs (Pels), while being growth-phase dependent, is not significantly affected by an *expI* mutation (Nasser *et al.* 1998; Ham *et al.* 2004). Similarly, in agreement with this observation, the virulence of *Ech* strains is not affected by the *expI* mutation. Thus, it would not be necessary for this strain to possess the VirR protein and, presumably, cell density-dependent control of PCWDEs is achieved via an alternative mechanism.

That both VirR and ExpR are known to repress the expression of virulence factors in the absence of AHL suggests that, in *E. carotovora*, the expression of these factors is actively prevented when AHL levels are low, i.e. when the population of cells is low. It should be noted that, in an *E. carotovora expI*, *virR* double

mutant, PCWDEs are still produced in a cell density-dependent manner (Burr *et al.* 2006). This suggests that, while VirR plays a key regulatory role, there are additional players in this complex regulatory network that are yet to be characterized.

13. INTEGRATION OF OTHER SIGNALS TO CONTROL *ERWINIA* VIRULENCE

It is important to note that quorum sensing is not the only means by which *Erwinia* virulence factors can be regulated and therefore the contribution of quorum sensing to gene regulation must be examined in context. A number of other regulators from a variety of *erwiniae* have been characterized, which integrate signals from other stimuli in order to regulate the expression of PCWDEs (reviewed in Brencic & Winans 2005).

14. THE Rsm REGULATORY NETWORK

The Rsm (regulator of secondary metabolites) system is proving to be a major, global gene regulatory system, homologues of which are widely distributed among Gram-negative bacteria. RsmA is a homologue of the global regulator, CsrA, from *E. coli*, which operates in the post-exponential phases of growth to negatively regulate a number of metabolic pathways, such as glycogen biosynthesis and gluconeogenesis. CsrA binds to selected mRNA transcripts in a region that overlaps the ribosome-binding site, blocking ribosome binding and promoting transcript degradation (Cui *et al.* 1995; Liu *et al.* 1995; Baker *et al.* 2002; Dubey *et al.* 2003). Recent SELEX (systematic evolution of ligands by exponential enrichment) studies have confirmed the importance of a conserved GGA motif for preferential CsrA binding *in vitro* (Dubey *et al.* 2005). However, CsrA does not degrade mRNA itself, so other factors (potentially nucleases) must be involved (Liu *et al.* 1995). CsrA has also been shown to act as a positive regulator of other genes by stabilizing these target mRNA transcripts. For example, CsrA activates flagellum biosynthesis and motility in *E. coli* (Wei *et al.* 2001). The regulator RsmA was originally identified in *Ecc* strain 71 (Chatterjee *et al.* 1995; Cui *et al.* 1995), but homologues of *rsmA* have been found in many different *erwiniae*, indicating its potential universal importance in the regulation of gene expression (Cui *et al.* 1995). Mutants of *rsmA* in *Ecc* strain 71 were found to be hyper-virulent and overproduced PCWDEs. In addition, mutants also produced more 3-oxo-C6-HSL than the wild-type strain, suggesting a link between the Rsm system and quorum sensing. In support of this idea, the LuxR homologue, ExpR, from *Ecc* 71 has been shown to activate transcription of *rsmA* (Cui *et al.* 2005).

A second component of the Rsm system is the small untranslated RNA, *rsmB* (also known as *csrB* or *aepH*; Murata *et al.* 1994; Liu *et al.* 1998), whose predicted secondary structure contains multiple GGA repeats exposed in the loop regions of stem-loop structures. It was originally noted that the number of these repeats correlates with the number of RsmA molecules sequestered by *rsmB* (Liu *et al.* 1998) and, later, direct binding of RsmA to these repeats was demonstrated in the *Pseudomonas fluorescens* CHAO system (Valverde

et al. 2004). Thus, *rsmB* enhances expression of genes encoding PCWDEs by sequestering RsmA and therefore antagonizing the action of RsmA on its mRNA targets (Liu *et al.* 1998). A similar method of regulation exists between CsrA and *csrB* in *E. coli*.

The third component of this *Ecc* system, RsmC (Cui *et al.* 1999), also known as HexY (Shih *et al.* 1999), was identified by two independent groups as a gene which, when mutated, caused elevated levels of exoenzyme production. RsmC negatively controls the expression of *rsmB*. It also has a positive effect on the expression of *rsmA*, although this might be mediated by *rsmB* rather than being a direct effect.

15. KdgR

It has long been known that full expression of the genes encoding PCWDEs in *Erwinia* requires the presence of plant cell wall breakdown products in the culture medium. The implication for *erwiniae* is that large quantities of PCWDEs are not made unless there is a suitable (plant) substrate nearby on which the enzymes can act. This requirement is mediated at a molecular level by the KdgR repressor, which acts as a global repressor of genes involved in pectin degradation. The *kdgR* gene can be found in the genome of *Ech* and of multiple *Ecc* strains, as well as in the *Eca* strain for which the genome sequence has recently become available (SCRI 1043; Hugouvieux-Cotte-Pattat *et al.* 1996; Liu *et al.* 1999; Bell *et al.* 2004). KdgR is a DNA-binding transcriptional repressor that binds to defined KdgR boxes located within the promoter regions of its target genes (*Ech* reviewed in Barras *et al.* 1994; *Ecc* in Liu *et al.* 1999). These targets include genes encoding enzymes involved in pectin catabolism, such as *pel1* and *peh1* in *Ecc* (Liu *et al.* 1999). KdgR has also been shown to bind to the promoter region of the gene encoding the small regulatory RNA, *rsmB* (Liu *et al.* 1999). The DNA binding of KdgR is abolished by plant cell wall breakdown products such as KDG (2-keto-3-deoxygluconate), DKI (5-keto-4-deoxyuronate) and DKII (2,5-diketo-3-deoxygluconate). Thus, these products induce expression of PCWDE-encoding genes by a depression mechanism.

16. OTHER REGULATORS

An additional *kdgR* homologue, *rexZ* (regulator of exoenzymes), exists in the genomes of both *Ecc* and *Eca* (Thomson *et al.* 1999; Bell *et al.* 2004). However, unlike other KdgR homologues, RexZ activates the production of PCWDEs (Thomson *et al.* 1999). RexZ clearly has a role distinct from that played by KdgR, but its true function requires clarification. Various other regulators of PCWDE production are also known, although their functions have not been well characterized. AepA (activator of extracellular proteins) has been shown to act as a transcriptional activator of *pel* and affects levels of protease and cellulase production in the cell (Liu *et al.* 1993; reviewed in detail by Barras *et al.* 1994). The Hor regulator, previously described, coordinately activates PCWDE and Car production in *Ecc* ATCC 39048 (Thomson *et al.* 1997). Recently, *hor* was identified as one of the two genes required for infection of *Drosophila* by a strain of *Erwinia* (*Ecc* 15;

Basset *et al.* 2003), suggesting that Hor may have additional roles distinct from those involved in the regulation of Car and PCWDE production. The *Erwinia* stationary-phase sigma factor, RpoS, has been shown to be important in virulence, since *rpoS* mutants are less able to infect tobacco plants and exhibit impaired growth (Andersson *et al.* 1999). Interestingly, *rpoS* mutants show elevated production of PCWDEs, an effect that is mediated, at least in part, by a reduction in the levels of the *rsmA* transcript (Mukherjee *et al.* 1998; Andersson *et al.* 1999). Therefore, although PCWDEs are clearly important virulence determinants, there must be additional factors that are required for successful infection of a plant host. The HexA global regulator (Harris *et al.* 1998) also appears to influence PCWDE production by affecting both RpoS (RpoS levels are elevated in a *hexA* mutant; Mukherjee *et al.* 2000) and *rsmB* transcription (which is reduced in a *hexA* mutant; Mukherjee *et al.* 2000). Finally, the ExpA/ExpS (GacA/GacS) two-component system also influences the Rsm system (Eriksson *et al.* 1998). In a *gacA* mutant, *rsmB* transcription is reduced, while RsmA levels remain unaffected, thus resulting in a reduction in the production of PCWDEs (Cui *et al.* 2001).

Clearly, the regulation of virulence factor production in the *erwiniae* is extremely complex to allow the bacteria to effect rapid and precise changes in the expression profile of PCWDEs and other virulence determinants, in response to changes in environmental conditions. Additionally, this intricate system of interconnecting gene regulatory networks has obvious implications for the design of laboratory experiments to study the role of quorum sensing in *Erwinia*.

17. *luxS*/AUTOINDUCER-2-MEDIATED QUORUM SENSING IN *E. CAROTOVORA*

Another type of quorum sensing system, distinct from AHL-mediated quorum sensing, has also been described in Gram-negative bacteria: the AI-2 signalling system. This system has been reviewed recently by Vendeville *et al.* (2005). The AI-2 quorum sensing system was first discovered in the marine bacterium *Vibrio harveyi*, where it is one of at least three parallel quorum sensing systems that converge to regulate bioluminescence and several other phenotypes including type III secretion (Bassler *et al.* 1994; Henke & Bassler 2004a,b). As reviewed by Federle & Bassler (2003), Henke & Bassler (2004c) and Vendeville *et al.* (2005), at high cell densities, AI-2 is bound by the periplasmic protein LuxP, which interacts with the hybrid sensor kinase, LuxQ. This initiates a dephosphorylation cascade resulting in the dephosphorylation of the phosphotransfer protein, LuxU, and thus inactivation of the response regulator, LuxO. Inactivation of LuxO alleviates small RNA-mediated repression of the master regulator, LuxR_{VH}. The other quorum sensing signals feed into the phosphotransfer relay at LuxU, via dedicated sensor kinases.

Production of AI-2 is dependent on the *luxS* gene (Surette *et al.* 1999). The observation that many species of bacteria, both Gram-positive and Gram-negative, produce AI-2-like activity and/or possess a *luxS* homologue led to the suggestion that AI-2 might

be a universal (non-species specific) and/or interspecies signalling molecule (Schauder & Bassler 2001; Sun *et al.* 2004). Inactivation of *luxS* in a variety of bacterial species has led to a range of phenotypes, from very little impact through to altered production of virulence determinants and decreased virulence (reviewed in Vendeville *et al.* 2005). For example, phenotypes of *luxS* mutants have been reported to include decreased expression of type III secretion and motility genes in *E. coli*, decreased production of secreted virulence factors in *Streptococcus pyogenes* and *Porphyromonas gingivalis*, impaired biofilm formation in *Streptococcus mutans*, and reduced virulence in *Neisseria meningitidis* and *Streptococcus pneumoniae* (Sperandio *et al.* 1999, 2001; Lyon *et al.* 2001; Burgess *et al.* 2002; Winzer *et al.* 2002a; Stroher *et al.* 2003; Wen & Burne 2004). Hence, *luxS* appears to have an important role in virulence-associated phenotypes, and therefore potentially in virulence, in many bacterial species.

However, the elucidation of the biosynthetic pathway for AI-2 revealed that LuxS has a metabolic role in the S-adenosyl methionine (SAM) utilization pathway. The product of SAM-dependent methyltransferase reactions, S-adenosyl homocysteine, is detoxified by the nucleosidase Pfs to give S-ribosyl homocysteine (SRH), and then LuxS acts on SRH, yielding homocysteine, which can be recycled to methionine, and AI-2 (Schauder *et al.* 2001; Winzer *et al.* 2002b). The immediate product of LuxS, 4,5-dihydroxypentanedione (DPD), spontaneously forms multiple, interconvertible, cyclized derivatives that exhibit AI-2 activity. As revealed by the crystal structures of the cognate receptors, the structure of AI-2 detected by *V. harveyi* is a furanosyl borate diester, whereas in *Salmonella typhimurium*, it is a hydrated furanone (Chen *et al.* 2002; Miller *et al.* 2004). Indeed, these findings indicate that 'AI-2' is actually a collective term for multiple chemical species (Vendeville *et al.* 2005).

Since LuxS has a metabolic role, it is currently unclear how many of the phenotypes described for *luxS* mutants are actually the result of a signalling defect due to the absence of AI-2, and how many are simply the result of a metabolic defect due to the loss of methionine recycling. In some cases, either conditioned medium from a LuxS⁺/AI-2⁺ strain or, rarely, *in vitro*-synthesized AI-2 have been used to complement *luxS* mutant phenotypes or show an impact on gene expression, implying an extracellular signalling role for *luxS* (Vendeville *et al.* 2005). For example, conditioned medium from a LuxS⁺/AI-2⁺ strain has been used to complement the toxin production defect of a *Clostridium perfringens luxS* mutant and the reduced prodigiosin production of a *Serratia marcescens luxS* mutant, while *Borrelia burgdorferi* shows altered expression of several proteins in response to synthetic DPD (Ohtani *et al.* 2002; Coulthurst *et al.* 2004; Babb *et al.* 2005).

Quorum sensing signal transduction systems similar to that of *V. harveyi* have been found in other *Vibrio* spp., detecting AI-2 and, in some cases, AHLs and/or a third signal, CAI-1. These quorum sensing systems have been implicated in virulence and biofilm formation in *V. cholerae*, colonization in *V. fischeri* and virulence in *V. vulnificus* (Miller *et al.* 2002b; Kim *et al.* 2003;

Hammer & Bassler 2004; Lupp & Ruby 2004). In *S. typhimurium* and *E. coli*, a system for the ABC-transporter-mediated uptake and subsequent phosphorylation and degradation of AI-2, the Lsr system, has been described (Taga *et al.* 2001, 2003; Xavier & Bassler 2005). However, whether this uptake of AI-2 is for the purpose of signal detection/termination or simply for metabolic scavenging of AI-2 is currently unclear.

The potential role of *luxS*/AI-2 has recently been examined in *E. carotovora*, the first such report in a plant pathogen (Coulthurst *et al.* 2006). Both *Ecc* ATTN10 (a derivative of ATCC 39048) and *Eca* SCRI 1043 produce *luxS*-dependent extracellular AI-2 activity. In each strain, a defined *luxS* mutant was constructed and the phenotypes of the resulting mutant characterized. In *Ecc* ATTN10, a proteomic analysis revealed that a defined set of intracellular proteins were altered in abundance in the *luxS* mutant compared with the wild-type, including several potentially involved in virulence. For example, both PelX (periplasmic exopolysaccharide lyase) and TogB (component of the TogMNAB transporter), involved in the generation and uptake, respectively, of pectic oligomers during pectin catabolism, were found to be reduced in the *luxS* mutant. In contrast, a similar analysis of the *Eca* SCRI 1043 *luxS* mutant revealed a negligible impact on the intracellular proteome. Analysis of the secreted proteome of *Ecc* ATTN10 found that levels of key secreted enzymes, Pel, Cel and Prt, were reduced in the *luxS* mutant. Similarly, the levels of Pel, Cel and Prt activities were reduced in the *luxS* mutant of *Eca* SCRI 1043. As has been observed in the *luxS* mutants of other bacteria, the motility of the *luxS* mutant of *Eca* SCRI 1043 was found to be reduced when compared with the wild-type.

Emphasizing the strain-dependent nature of the impact of *luxS* inactivation, the virulence of the *luxS* mutant of *Ecc* ATTN10 was found to be reduced compared with that of the wild-type *in vivo*, whereas no statistically significant decrease in virulence was observed in the *luxS* mutant of *Eca* SCRI 1043, at least in the models used (Coulthurst *et al.* 2006). Strain dependence in the impact of *luxS* has also been noted in *Serratia* spp. (Coulthurst *et al.* 2004). In addition, no link was detected between this potential AI-2 quorum sensing system and the AHL quorum sensing system in *Erwinia*. Although the molecular mechanism(s) of *luxS* regulation in *Erwinia* remain to be determined, it appears that *luxS* plays a strain-dependent role in virulence in this organism.

18. QUORUM SENSING IN RELATIVES OF THE SOFT-ROT ERWINIAS

(a) *Pantoea stewartii*

Pantoea stewartii ssp. *stewartii* (*P. stewartii*, formerly known as *Erwinia stewartii*) is the causative agent of Stewart's wilt and leaf blight in maize and sweetcorn. Symptoms result from the production by *P. stewartii* of large amounts of exopolysaccharide (EPS), which block the plant xylem vessels (Von Bodman *et al.* 1998, 2003a,b). The synthesis and translocation of EPS is encoded by the *cps* gene cluster and EPS-deficient

mutants are avirulent (Minogue *et al.* 2005). Production of EPS is cell density dependent and under the control of the *EsaI*/*EsaR* AHL quorum sensing system. *EsaI*, a LuxI homologue, produces 3-oxo-C6-HSL, and *EsaR*, a LuxR homologue, provides the cognate 3-oxo-C6-HSL receptor. Mutation of *esaI* eliminates EPS synthesis and virulence (Von Bodman *et al.* 1998). *EsaI* was the first LuxI family protein for which a crystal structure was reported, identifying critical residues for catalysis and substrate specificity (Watson *et al.* 2002).

EsaR was the first LuxR homologue identified as being a negative regulator, rather than an activator, of gene expression (Von Bodman *et al.* 1998). Genetic studies revealed that *EsaR* represses production of EPS in the absence of 3-oxo-C6-HSL (at low cell densities) and that 3-oxo-C6-HSL relieves this repression, allowing expression of EPS. Mutation of *esaR* leads to growth-phase-independent overexpression of EPS, in either a wild-type or an *esaI* background (Von Bodman *et al.* 1998). Interestingly, both the *esaR* and the *esaIR* mutants display impaired virulence *in planta*, implying that quorum sensing-mediated repression of EPS production in the early stages of an infection is crucial to its success, perhaps by allowing initial attachment of the bacterium to the host (Von Bodman *et al.* 1998, 2003a). *EsaR* represses expression of *esaR* in the absence of 3-oxo-C6-HSL by binding to a *lux* box overlapping its own promoter, with binding of 3-oxo-C6-HSL relieving this repression. However, *EsaR* does not regulate the expression of *esaI* (Minogue *et al.* 2002). *EsaR*-mediated repression of *cps* expression has recently been shown to be mediated via direct transcriptional repression of *rcaA*. *RcsA* is an essential transcriptional coactivator (together with the response regulator *RcsB*) of the *cps* genes (Minogue *et al.* 2005). The authors suggest that, since *EsaR* can act as a transcriptional activator in the absence of AHL (if its binding site is appropriately positioned with reference to the promoter sequences), *EsaR* may also positively regulate other genes at low AHL concentrations (Von Bodman *et al.* 2003b; Minogue *et al.* 2005).

(b) *Erwinia amylovora*

Erwinia amylovora (*Ea*) causes the necrotic disease fire blight in apple, pear and related plant species, with key virulence traits being production of EPS and type III secretion of Hrp proteins into host cells. The existence of AHL quorum sensing systems in *Ea* has only recently been reported. Venturi *et al.* (2004) describe the production of a single AHL, most probably 3-oxo-C6-HSL, by several Italian strains of *Ea*; for one strain, production of an AHL was observed *in planta*. Molina *et al.* (2005) describe the detection of AHL activity in culture supernatant of a Swiss strain of *Ea*. In this strain, overexpression of the *AiiA* lactonase eliminated detectable AHL activity and considerably reduced EPS production, oxidative stress tolerance and virulence on apple leaves, indicating that AHL is likely to play an important role in *Ea* virulence (Molina *et al.* 2005). Both reports describe the detection and partial sequencing of pairs of convergent *luxI* and *luxR* homologues, named *eamI*/*eamR*. However, we await analysis of *eamI*/*eamR* mutants in order to investigate

the quorum sensing mechanisms and definition of the quorum sensing regulon in this species.

(c) *Serratia* sp. 39006

As described previously, the atypical *Serratia*, species 39006, produces extracellular pectate lyase and cellulase activity, suggesting that, like the *erwinias*, it may be capable of soft rotting in its natural environment. The quorum sensing-mediated control of Car production in this strain has already been discussed, but production of both Pel and Cel as well as the red pigment, prodigiosin, is also under quorum sensing control in 39006 (Slater *et al.* 2003). The LuxR homologue, SmaR, has been shown to repress the expression of the prodigiosin biosynthetic genes (*pigA–O*) and exoenzyme production (as well as Car as described above) in the absence of AHL (i.e. at low cell density). This repression is relieved in the presence of AHL (i.e. at high cell density).

The quorum sensing system of 39006 is integrated into a complex regulatory network controlling production of Car and prodigiosin in response to a variety of environmental inputs (Slater *et al.* 2003; Fineran *et al.* 2005). Regulatory components acting above, below and independently of the quorum sensing system have been detected. Expression of *smaI* (and also of *pigA–O* independently of *smaI*) is upregulated under conditions of phosphate limitation, via the Pst phosphate transport system (Slater *et al.* 2003). The *smaIR* system controls the expression of several regulators, *rap*, *pigR* and *pigQ* (predicted to encode a SlyA-family transcriptional regulator, an adenylate cyclase and a GacA-family response regulator, respectively), which themselves control expression of *pigA–O* in response to unknown signals (Fineran *et al.* 2005). A novel pleiotropic regulator, *pigP*, activates or represses the expression of multiple prodigiosin regulators, some of which are also regulated by *smaIR* (Fineran *et al.* 2005). Hence, multiple cues, including quorum sensing/cell density, can be integrated to finely tune the production of secondary metabolites in response to environmental signals.

19. WHY QUORUM SENSE?

One of the fundamental questions in the field of quorum sensing is that of why bacteria regulate gene expression according to population density. There are, of course, many possibilities for the role of quorum sensing and this topic has been hotly debated.

In *Erwinia*, there are several potential advantages that might be conferred by placing production of the carbapenem antibiotic under quorum sensing control. Car production in *Ecc* is an energetically demanding process for each cell in the population. As discussed above, it requires the expression of the Car biosynthetic and resistance genes, together with the precursors for Car production (glutamate semi-aldehyde and malonyl-CoA) and a supply of ATP to drive Car biosynthesis. Therefore, it makes sense that the bacteria should control antibiotic synthesis, so that it is only produced when it could provide a beneficial effect.

It has been hypothesized that Car production may be involved in eliminating competing bacteria from the

site of infection *in planta*. If these competing bacteria produce an AHL signal to which *Erwinia* can respond, Car production by *Erwinia* would be switched on and any Car-sensitive bacteria would be eliminated. It is probable that *Ecc* might encounter other AHL-producing organisms occupying a similar environmental niche. 3-Oxo-C6-HSL production has been demonstrated in a variety of *Erwinia* strains, including strains that do not make the carbapenem antibiotic (table 1). In addition, it has been shown (both *in vitro* and *in vivo*) that Car_{*Ecc*} is capable of responding to a variety of AHL signals, not just 3-oxo-C6-HSL, further widening the scope for cross-activation of Car production in *Ecc* by exogenously provided AHLs (Welch *et al.* 2000, 2005). The presence of other bacteria may also help to increase the local 3-oxo-C6-HSL concentration (in the absence of further AHL synthesis), which could serve to activate Car production by *Ecc*.

As discussed, the quorum sensing system in *Erwinia* also prevents the production of secreted proteins, such as PCWDEs, at low cell densities. One suggested rationale for this is that *Erwinia* might be better able to prevent detection by the plant host defence systems if it delays secretion of virulence factors until a suitable population density has been achieved at which it can overcome the host by brute-force (induction of those genes). In support of this idea, Mäe *et al.* (2001) reported that resistance to infection by wild-type *Ecc* was improved by the presence of AHL produced in a tobacco host harbouring the *Ecc expI* gene. In contrast, however, Toth *et al.* reported that potato plants engineered to express an AHL synthase (YenI from *Yersinia enterocolitica*) were in fact more susceptible to disease (as measured by the 'disease score' of Toth *et al.* (1999)) caused by wild-type *Erwinia* strains, even when inoculated with numbers as low as 10² cells (Toth *et al.* 2004). It is important to note that the conclusions of Mäe *et al.* (2001) are drawn from the observation that a reduction was seen in the fraction of plants showing disease. However, no difference in disease progression was observed in cases where an infection had been established. Therefore, although quorum sensing is clearly important in *Erwinia* virulence and infection, its actual role may have more subtleties than previously thought, and thus is an area that requires further clarification.

20. QUORUM SENSING: A SENSOR OF POPULATION DENSITY... OR SOMETHING ELSE?

It has long been thought that quorum sensing systems are a means for bacteria to sense population density, owing to the observation that the activity of quorum sensing systems varies according to cell density of the bacterial culture. However, growth of bacterial cultures in defined, rich medium, in shake flasks, is a highly artefactual growth condition that bacteria do not experience outside the laboratory setting. This begs the question: are bacteria in fact using their quorum sensing systems to sense 'population density', or does quorum sensing play some alternative role?

One suggestion is that 'quorum sensing' may be a means of detecting 'diffusion' rather than cell density *per se* (Redfield 2002). It could be envisaged that the

accumulation of AHL may serve to signal to the cells that they are in a plant host environment and not, for example, in the less-confined environments of the soil. Alternatively, for organisms that are able to form biofilms, the AHL signal may allow the population of bacteria to distinguish between growth in planktonic culture and growth in a biofilm, and allow the population to modulate gene expression accordingly.

A related suggestion is simply that *Erwinia* is responding to a limitation of nutrients, which is the inevitable consequence of population increase in a finite space. In this case, on sensing a population increase, expression of genes that result in the liberation of additional nutrients from a plant host would allow further growth. In this way, *Erwinia* might be able to put off expression of PCWDEs for as long as possible, thus preventing the premature death of its host.

Antibiotics are effective against competing organisms only if they are produced and secreted in large enough amounts, such that the local concentration of antibiotic is greater than the minimum inhibitory concentration required for cell death. If Car were produced under circumstances when 3-oxo-C6-HSL concentrations are low, owing to extensive diffusion of the signalling molecule away from the producer cells, Car would also be subject to similar diffusion and so would not reach the concentrations required for it to be effective as an antibiotic. However, by linking Car production to the detection of high 3-oxo-C6-HSL concentrations, it is ensured that Car is only made when diffusion is limited sufficiently to allow antibiotic concentrations to reach high enough levels to be effective against competitor organisms. Finally, it is always possible that 3-oxo-C6-HSL and Car production may have other, as yet unidentified, advantages to *Erwinia*.

Of course, the reality is likely to involve aspects of each of these models, but the fact remains that, in *Erwinia*, virulence is responsive to quorum sensing, a fact that has made it the subject of intense study as a potential target of therapeutics aimed at preventing or reducing disease. One approach has exploited the existence of enzymes capable of degrading AHLs, the AHL-lactonases, which act on AHLs by hydrolysing the homoserine lactone ring and are discussed in more detail elsewhere in this issue. In one study, host plants (both tobacco and potato) expressing the *Bacillus thuringiensis aiiA* gene encoding one such AHL-lactonase showed enhanced resistance to infection by *Ecc* (Dong *et al.* 2004). In a complementary study, *Eca* carrying the *aiiA* gene was used to show the usefulness of this approach in preventing infection by erwinias (Smadja *et al.* 2004). In this investigation, the *aiiA* gene significantly reduced the levels of 3-oxo-C6-HSL detected and correspondingly reduced the severity of the tuber soft rot. Of course, these studies not only highlight the potential of quorum sensing as a target for therapeutics but also reinforce the importance of AHLs in *E. carotovora* virulence. It is important to note that in these situations, as in the case of *expI* mutants, *Erwinia* is still able to colonize and grow in its hosts, suggesting that other events that occur early in the infection process are still able to take place in the absence of the quorum sensing signal.

21. CONCLUDING COMMENTS

Quorum sensing plays an important role in the regulation of production of secondary metabolites and virulence factors in *Erwinia* and other soft-rotting bacteria. However, quorum sensing is just one component of an extremely complicated regulatory hierarchy that allows bacteria to titrate and respond to external signals. The challenge now is to determine what position quorum sensing occupies in the global regulatory hierarchy and to elucidate its true physiological and evolutionary function.

Work in the authors' laboratory is funded by the BBSRC. S.D.B. was supported by a BBSRC studentship. R.E.M. is supported by the Gates Cambridge Trust and the UK Overseas Research Student Award Scheme. Authors would like to thank Louise Woodley for critical reading of the manuscript.

REFERENCES

- Alfano, J. R. & Collmer, A. 2004 Type III secretion system effector proteins: double agents in bacterial disease and plant defense. *Annu. Rev. Phytopathol.* **42**, 385–414. (doi:10.1146/annurev.phyto.42.040103.110731)
- Andersson, R. A., Koiv, V., Norman-Setterblad, C. & Pirhonen, M. 1999 Role of RpoS in virulence and stress tolerance of the plant pathogen *Erwinia carotovora* ssp. *carotovora*. *Microbiology* **145**, 3547–3556.
- Andersson, R. A., Eriksson, A. R., Heikinheimo, R., Mae, A., Pirhonen, M., Koiv, V., Hyytiäinen, H., Tuikkala, A. & Palva, E. T. 2000 Quorum sensing in the plant pathogen *Erwinia carotovora* sp. *carotovora*: the role of *expR*(Ecc). *Mol. Plant Microbe Interact.* **13**, 384–393.
- Arevalo-Ferro, C., Hentzer, M., Reil, G., Gorg, A., Kjelleberg, S., Givskov, M., Riedel, K. & Eberl, L. 2003 Identification of quorum-sensing regulated proteins in the opportunistic pathogen *Pseudomonas aeruginosa* by proteomics. *Environ. Microbiol.* **5**, 1350–1369. (doi:10.1046/j.1462-2920.2003.00532.x)
- Babb, K., von Lackum, K., Wattier, R. L., Riley, S. P. & Stevenson, B. 2005 Synthesis of autoinducer 2 by the Lyme disease spirochete, *Borrelia burgdorferi*. *J. Bacteriol.* **187**, 3079–3087. (doi:10.1128/JB.187.9.3079-3087.2005)
- Bainton, N. J., Stead, P., Chhabra, S. R., Bycroft, B. W., Salmond, G. P. C., Stewart, G. S. A. B. & Williams, P. 1992a *N*-(3-oxohexanoyl)-L-homoserine lactone regulates carbapenem antibiotic production in *Erwinia carotovora*. *Biochem. J.* **288**, 997–1004.
- Bainton, N. J. *et al.* 1992b A general role for the *lux* autoinducer in bacterial cell signalling: control of antibiotic synthesis in *Erwinia*. *Gene* **116**, 87–91. (doi:10.1016/0378-1119(92)90633-Z)
- Baker, C. S., Morozov, I., Suzuki, K., Romeo, T. & Babitzke, P. 2002 CsrA regulates glycogen biosynthesis by preventing translation of *glgC* in *Escherichia coli*. *Mol. Microbiol.* **44**, 1599–1610. (doi:10.1046/j.1365-2958.2002.02982.x)
- Barras, F., van Gijsegem, F. & Chatterjee, A. K. 1994 Extracellular enzymes and pathogenesis of soft-rot *Erwinia*. *Annu. Rev. Phytopathol.* **32**, 201–234.
- Basset, A., Tzou, P., Lemaitre, B. & Boccard, F. 2003 A single gene that promotes interaction of a phytopathogenic bacterium with its insect vector, *Drosophila melanogaster*. *EMBO Rep.* **4**, 205–209. (doi:10.1038/sj.embor.embor730)
- Bassler, B. L., Wright, M. & 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. (doi:10.1111/j.1365-2958.1994.tb00422.x)

- Bell, K. S. *et al.* 2004 Genome sequence of the enterobacterial phytopathogen *Erwinia carotovora* subsp. *atroseptica* and characterization of virulence factors. *Proc. Natl Acad. Sci. USA* **101**, 11 105–11 110. (doi:10.1073/pnas.0402424101)
- Brencic, A. & Winans, S. C. 2005 Detection of and response to signals involved in host–microbe interactions by plant-associated bacteria. *Microbiol. Mol. Biol. Rev.* **69**, 155–194. (doi:10.1128/MMBR.69.1.155-194.2005)
- Burgess, N. A., Kirke, D. F., Williams, P., Winzer, K., Hardie, K. R., Meyers, N. L., Aduse-Opoku, J., Curtis, M. A. & Camara, M. 2002 LuxS-dependent quorum sensing in *Porphyromonas gingivalis* modulates protease and haemagglutinin activities but is not essential for virulence. *Microbiology* **148**, 763–772.
- Burr, T., Barnard, A. M. L., Corbett, M. J., Pemberton, C. L., Simpson, N. J. L. & Salmond, G. P. C. 2006 Identification of the central quorum sensing regulatory of virulence in the enteric phytopathogen, *Erwinia carotovora*: the VirR repressor. *Mol. Microbiol.* **59**, 113–125. (doi:10.1111/j.1365-2958.2005.04939.x)
- Byers, J. T., Lucas, C., Salmond, G. P. C. & Welch, M. 2002 Nonenzymatic turnover of an *Erwinia carotovora* quorum-sensing signaling molecule. *J. Bacteriol.* **184**, 1163–1171. (doi:10.1128/jb.184.4.1163-1171.2002)
- Chapon-Herve, V., Akrim, M., Latifi, A., Williams, P., Lazdunski, A. & Bally, M. 1997 Regulation of the *xcp* secretion pathway by multiple quorum-sensing modulons in *Pseudomonas aeruginosa*. *Mol. Microbiol.* **24**, 1169–1178. (doi:10.1046/j.1365-2958.1997.4271794.x)
- Chatterjee, A., Cui, Y., Liu, Y., Dumenyo, C. K. & Chatterjee, A. K. 1995 Inactivation of *rsmA* leads to overproduction of extracellular pectinases, cellulases, and proteases in *Erwinia carotovora* ssp. *carotovora* in the absence of the starvation/cell density-sensing signal, *N*-(3-oxohexanoyl)-L-homoserine lactone. *Appl. Environ. Microbiol.* **61**, 1959–1967.
- Chatterjee, A., Cui, Y., Hasegawa, H., Leigh, N., Dixit, V. & Chatterjee, A. K. 2005 Comparative analysis of two classes of quorum-sensing signaling systems that control production of extracellular proteins and secondary metabolites in *Erwinia carotovora* subspecies. *J. Bacteriol.* **187**, 8026–8038. (doi:10.1128/JB.187.23.8026-8038.2005)
- Chen, X., Schauder, S., Potier, N., Van Dorsselaer, A., Pelczar, I., Bassler, B. L. & Hughson, F. M. 2002 Structural identification of a bacterial quorum-sensing signal containing boron. *Nature* **415**, 545–549. (doi:10.1038/415545a)
- Corbett, M. J. *et al.* 2005 Identification of a new quorum-sensing-controlled virulence factor in *Erwinia carotovora* subsp. *atroseptica* secreted via the type II targeting pathway. *Mol. Plant Microbe Interact.* **18**, 334–342.
- Coulthurst, S. J., Kurz, C. L. & Salmond, G. P. 2004 *luxS* mutants of *Serratia* defective in autoinducer-2-dependent ‘quorum sensing’ show strain-dependent impacts on virulence and production of carbapenem and prodigiosin. *Microbiology* **150**, 1901–1910. (doi:10.1099/mic.0.26946-0)
- Coulthurst, S. J., Barnard, A. M. L. & Salmond, G. P. C. 2005 Regulation and biosynthesis of carbapenem antibiotics in bacteria. *Nat. Rev. Microbiol.* **3**, 295–306. (doi:10.1038/nrmicro1128)
- Coulthurst, S. J., Lilley, K. S. & Salmond, G. P. C. 2006 Genetic and proteomic analysis of the role of *luxS* in the enteric phytopathogen, *Erwinia carotovora*. *Mol. Plant Pathol.* **7**, 31–45. (doi:10.1111/j.1364-3703.2005.00314.x)
- Cox, A. R., Thomson, N. R., Bycroft, B. W., Stewart, G. S., Williams, P. & Salmond, G. P. 1998 A pheromone-independent CarR protein controls carbapenem antibiotic synthesis in the opportunistic human pathogen *Serratia marcescens*. *Microbiology* **144**, 201–209.
- Cui, Y., Chatterjee, A., Liu, Y., Dumenyo, C. K. & Chatterjee, A. K. 1995 Identification of a global repressor gene, *rsmA*, of *Erwinia carotovora* ssp. *carotovora* that controls extracellular enzymes, *N*-(3-oxohexanoyl)-L-homoserine lactone, and pathogenicity in soft-rotting *Erwinia* spp. *J. Bacteriol.* **177**, 5108–5115.
- Cui, Y., Mukherjee, A., Dumenyo, C. K., Liu, Y. & Chatterjee, A. K. 1999 *rsmC* of the soft-rotting bacterium *Erwinia carotovora* ssp. *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.
- Cui, Y., Chatterjee, A. & Chatterjee, A. K. 2001 Effect of the two-component system comprising GacA and GacS of *Erwinia carotovora* ssp. *carotovora* on the production of global regulatory *rsmB* RNA, extracellular enzymes, and harpin_{Ecc}. *Mol. Plant Microbe Interact.* **14**, 516–526.
- Cui, Y., Chatterjee, A., Hasegawa, H., Dixit, V., Leigh, N. & Chatterjee, A. K. 2005 ExpR, a LuxR homolog of *Erwinia carotovora* ssp. *carotovora*, activates transcription of *rsmA*, which specifies a global regulatory RNA-binding protein. *J. Bacteriol.* **187**, 4792–4803. (doi:10.1128/JB.187.14.4792-4803.2005)
- Delepelaire, P. 2004 Type I secretion in gram-negative bacteria. *Biochim. Biophys. Acta* **1694**, 149–161. (doi:10.1016/j.bbamcr.2004.05.001)
- Derzelle, S., Duchaud, E., Kunst, F., Danchin, A. & Bertin, P. 2002 Identification, characterization, and regulation of a cluster of genes involved in carbapenem biosynthesis in *Photobacterium luminescens*. *Appl. Environ. Microbiol.* **68**, 3780–3789. (doi:10.1128/AEM.68.8.3780-3789.2002)
- Dong, Y. H., Zhang, X. F., Xu, J. L. & Zhang, L. H. 2004 Insecticidal *Bacillus thuringiensis* silences *Erwinia carotovora* virulence by a new form of microbial antagonism, signal interference. *Appl. Environ. Microbiol.* **70**, 954–960. (doi:10.1128/AEM.70.2.954-960.2004)
- Dubey, A. K., Baker, C. S., Suzuki, K., Jones, A. D., Pandit, P., Romeo, T. & Babitzke, P. 2003 CsrA regulates translation of the *Escherichia coli* carbon starvation gene, *cstA*, by blocking ribosome access to the *cstA* transcript. *J. Bacteriol.* **185**, 4450–4460. (doi:10.1128/JB.185.15.4450-4460.2003)
- Dubey, A. K., Baker, C. S., Romeo, T. & Babitzke, P. 2005 RNA sequence and secondary structure participate in high-affinity CsrA–RNA interaction. *RNA* **11**, 1579–1587. (doi:10.1261/rna.2990205)
- Eriksson, R. B. A., Andersson, R. A., Pirhonen, M. & Palva, E. T. 1998 Two-component regulators involved in the global control of virulence in *Erwinia carotovora* sp. *carotovora*. *Mol. Plant Microbe Interact.* **11**, 743–752.
- Federle, M. J. & Bassler, B. L. 2003 Interspecies communication in bacteria. *J. Clin. Invest.* **112**, 1291–1299. (doi:10.1172/JCI200320195)
- Filloux, A. 2004 The underlying mechanisms of type II protein secretion. *Biochim. Biophys. Acta* **1694**, 163–179. (doi:10.1016/j.bbamcr.2004.05.003)
- Fineran, P. C., Slater, H., Everson, L., Hughes, K. & Salmond, G. P. 2005 Biosynthesis of tripyrrole and beta-lactam secondary metabolites in *Serratia*: integration of quorum sensing with multiple new regulatory components in the control of prodigiosin and carbapenem antibiotic production. *Mol. Microbiol.* **56**, 1495–1517.
- Gerrata, B., Stapon, A. & Townsend, C. A. 2003 Inhibition and alternate substrate studies on the mechanism of carbapenem synthetase from *Erwinia carotovora*. *Biochemistry* **42**, 7836–7847. (doi:10.1021/bi034361d)
- Ham, J. H., Cui, Y., Alfano, J. R., Rodriguez-Palenzuela, P., Rojas, C. M., Chatterjee, A. K. & Collmer, A. 2004 Analysis of *Erwinia chrysanthemi* EC16 *pelE::uidA*,

- pelL::uidA*, and *hrpN::uidA* mutants reveals strain-specific atypical regulation of the Hrp Type III secretion system. *Mol. Plant Microbe Interact.* **17**, 184–194.
- Hammer, B. K. & Bassler, B. L. 2004 Quorum sensing controls biofilm formation in *Vibrio cholerae*. *Mol. Microbiol.* **51**, 1521. (doi:10.1111/j.1365-2958.2004.03939.x)
- Harris, S. J., Shih, Y.-L., Bentley, S. D. & 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. (doi:10.1046/j.1365-2958.1998.00825.x)
- He, S. Y., Lindeberg, M., Chatterjee, A. K. & Collmer, A. 1991 Cloned *Erwinia chrysanthemi* *out* genes enable *Escherichia coli* to selectively secrete a diverse family of heterologous proteins to its milieu. *Proc. Natl Acad. Sci. USA* **88**, 1079–1083. (doi:10.1073/pnas.88.3.1079)
- He, S. Y., Nomura, K. & Whittam, T. S. 2004 Type III protein secretion mechanism in mammalian and plant pathogens. *Biochim. Biophys. Acta* **1694**, 181–206. (doi:10.1016/j.bbamcr.2004.03.011)
- Henke, J. M. & Bassler, B. L. 2004a Quorum sensing regulates type III secretion in *Vibrio harveyi* and *Vibrio parahaemolyticus*. *J. Bacteriol.* **186**, 3794–3805. (doi:10.1128/JB.186.12.3794-3805.2004)
- Henke, J. M. & Bassler, B. L. 2004b Three parallel quorum-sensing systems regulate gene expression in *Vibrio harveyi*. *J. Bacteriol.* **186**, 6902–6914. (doi:10.1128/JB.186.20.6902-6914.2004)
- Henke, J. M. & Bassler, B. L. 2004c Bacterial social engagements. *Trends Cell Biol.* **14**, 648–656. (doi:10.1016/j.tcb.2004.09.012)
- Hinton, J. C. D. & Salmond, G. P. C. 1987 Use of *TnphoA* to enrich for extracellular mutants of *Erwinia carotovora* subspecies *carotovora*. *Mol. Microbiol.* **1**, 381–386. (doi:10.1111/j.1365-2958.1987.tb01946.x)
- Holden, M. T. G., McGowan, S. J., Bycroft, B. W., Stewart, G. S. A. B., Williams, P. & 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**, 495–508.
- Holeva, M. C., Bell, K. S., Hyman, L. J., Avrova, A. O., Whisson, S. C. & Birch, P. R. J. 2004 Use of a pooled transposon mutation grid to demonstrate roles in disease development for *Erwinia carotovora* subsp. *atroseptica* putative Type III secreted effector (DspE/A) and Helper (HrpN) proteins. *Mol. Plant Microbe Interact.* **17**, 943–950.
- Hugouvieux-Cotte-Pattat, N., Condemine, G., Nasser, W. & Reverchon, S. 1996 Regulation of pectinolysis in *Erwinia chrysanthemi*. *Annu. Rev. Microbiol.* **50**, 213–257. (doi:10.1146/annurev.micro.50.1.213)
- Jones, S. *et al.* 1993 The *lux* autoinducer regulates the production of exoenzyme virulence determinants in *Erwinia carotovora* and *Pseudomonas aeruginosa*. *EMBO J.* **12**, 2477–2482.
- Kazemi-Pour, N., Condemine, G. & Hugouvieux-Cotte-Pattat, N. 2004 The secretome of the plant pathogenic bacterium *Erwinia chrysanthemi*. *Proteomics* **4**, 3177–3186. (doi:10.1002/pmic.200300814)
- Kim, S. Y., Lee, S. E., Kim, Y. R., Kim, C. M., Ryu, P. Y., Choy, H. E., Chung, S. S. & Rhee, J. H. 2003 Regulation of *Vibrio vulnificus* virulence by the LuxS quorum-sensing system. *Mol. Microbiol.* **48**, 1647–1664. (doi:10.1046/j.1365-2958.2003.03536.x)
- Li, R., Stapon, A. & Townsend, C. A. 2000 Three unusual reactions mediate carbapenem and carbapenam biosynthesis. *J. Am. Chem. Soc.* **122**, 9296–9297. (doi:10.1021/ja001723l)
- Liu, M. Y. & Romeo, T. 1997 The global regulator CsrA of *Escherichia coli* is a specific mRNA-binding protein. *J. Bacteriol.* **179**, 4639–4642.
- Liu, Y., Murata, H., Chatterjee, A. & Chatterjee, A. K. 1993 Characterization of a novel regulatory gene *aepA* that controls extracellular enzyme production in the phytopathogenic bacterium *Erwinia carotovora* ssp. *carotovora*. *Mol. Plant Microbe Interact.* **6**, 299–308.
- Liu, M. Y., Yang, H. & Romeo, T. 1995 The product of the pleiotropic *Escherichia coli* gene *csrA* modulates glycogen biosynthesis via effects on mRNA stability. *J. Bacteriol.* **177**, 2663–2672.
- Liu, Y., Cui, Y., Mukherjee, A. & 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. (doi:10.1046/j.1365-2958.1998.00924.x)
- Liu, Y., Jiang, G., Cui, Y., Mukherjee, A., Ma, W. L. & Chatterjee, A. K. 1999 *kdgR_{Ecc}* negatively regulates genes for pectinases, cellulase, protease, harpin_{Ecc}, and a global RNA regulator in *Erwinia carotovora* subsp. *carotovora*. *J. Bacteriol.* **181**, 2411–2421.
- Lupp, C. & Ruby, E. G. 2004 *Vibrio fischeri* LuxS and AinS: comparative study of two signal synthases. *J. Bacteriol.* **186**, 3873–3881. (doi:10.1128/JB.186.12.3873-3881.2004)
- Lyon, W. R., Madden, J. C., Levin, J. C., Stein, J. L. & Caparon, M. G. 2001 Mutation of *luxS* affects growth and virulence factor expression in *Streptococcus pyogenes*. *Mol. Microbiol.* **42**, 145–157. (doi:10.1046/j.1365-2958.2001.02616.x)
- Mäe, A., Montesano, M., Koiv, V. & Palva, E. T. 2001 Transgenic plants producing the bacterial pheromone *N*-acyl-homoserine lactone exhibit enhanced resistance to the bacterial phytopathogen *Erwinia carotovora*. *Mol. Plant Microbe Interact.* **14**, 1035–1042.
- McGowan, S. J. & Salmond, G. P. C. 1999 Regulation of carbapenem antibiotic and exoenzyme synthesis by chemical signalling. In *SGM Symp. 57: microbial signalling and communication* (eds R. England, G. Hobbs, N. Bainton & D. McL. Roberts), pp. 161–175. Cambridge, UK: Cambridge University Press.
- McGowan, S. J. *et al.* 1995 Carbapenem antibiotic production in *Erwinia carotovora* is regulated by CarR, a homologue of the LuxR transcriptional activator. *Microbiology* **141**, 541–550.
- McGowan, S. J., Sebaihia, M., Porter, L. E., Stewart, G. S. A. B., Williams, P., Bycroft, B. W. & Salmond, G. P. C. 1996 Analysis of bacterial carbapenem antibiotic production genes reveals a novel β -lactam biosynthesis pathway. *Mol. Microbiol.* **22**, 415–426. (doi:10.1046/j.1365-2958.1996.00125.x)
- McGowan, S. J., Sebaihia, M., O'Leary, S., Kardia, K. R., Williams, P., Stewart, G. S. A. B., Bycroft, B. W. & 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 β -lactam resistance mechanism. *Mol. Microbiol.* **26**, 545–556. (doi:10.1046/j.1365-2958.1997.6001974.x)
- McGowan, S. J. *et al.* 2005 Carbapenem antibiotic biosynthesis in *Erwinia carotovora* is regulated by physiological and genetic factors modulating the quorum sensing-dependent control pathway. *Mol. Microbiol.* **55**, 526–545. (doi:10.1111/j.1365-2958.2004.04397.x)
- Miller, M. T., Bachmann, B. O., Townsend, C. A. & Rosenzweig, A. C. 2002a The catalytic cycle of β -lactam synthetase observed by X-ray crystallographic snapshots. *Proc. Natl Acad. Sci. USA* **99**, 14 752–14 757. (doi:10.1073/pnas.232361199)
- Miller, M. B., Skorupski, K., Lenz, D. H., Taylor, R. K. & Bassler, B. L. 2002b Parallel quorum sensing systems converge to regulate virulence in *Vibrio cholerae*. *Cell* **110**, 303–314. (doi:10.1016/S0092-8674(02)00829-2)

- Miller, M. T., Garratana, B., Stapon, A., Townsend, C. A. & Rosenzweig, A. C. 2003 Crystal structure of carbapenam synthetase (CarA). *J. Biol. Chem.* **278**, 40 996–41 002. (doi:10.1074/jbc.M307901200)
- Miller, S. T., Xavier, K. B., Campagna, S. R., Taga, M. E., Semmelhack, M. F., Bassler, B. L. & Hughson, F. M. 2004 *Salmonella typhimurium* recognizes a chemically distinct form of the bacterial quorum-sensing signal AI-2. *Mol. Cell* **15**, 677–687. (doi:10.1016/j.molcel.2004.07.020)
- Minogue, T. D., Wehland-von Trebra, M., Bernhard, F. & von Bodman, S. B. 2002 The autoregulatory role of EsaR, a quorum-sensing regulator in *Pantoea stewartii* ssp. *stewartii*: evidence for a repressor function. *Mol. Microbiol.* **44**, 1625–1635. (doi:10.1046/j.1365-2958.2002.02987.x)
- Minogue, T. D., Carlier, A. L., Koutsoudis, M. D. & von Bodman, S. B. 2005 The cell density-dependent expression of stewartan exopolysaccharide in *Pantoea stewartii* ssp. *stewartii* is a function of the *rcsA* gene. *Mol. Microbiol.* **56**, 189–203. (doi:10.1111/j.1365-2958.2004.04529.x)
- Molina, L., Rezzonico, F., Defago, G. & Duffy, B. 2005 Autoinduction in *Erwinia amylovora*: evidence of an acyl-homoserine lactone signal in the Fire Blight pathogen. *J. Bacteriol.* **187**, 3206–3213. (doi:10.1128/JB.187.9.3206-3213.2005)
- Mukherjee, A., Cui, Y., Lui, Y. & Chatterjee, A. 1997 Molecular characterization and expression of the *Erwinia carotovora* *hrpN_{Ecc}* gene, which encodes an elicitor of the hypersensitive reaction. *Mol. Plant Microbe Interact.* **10**, 462–471.
- Mukherjee, A., Cui, Y., Ma, W., Liu, Y., Ishihama, A., Eisenstark, A. & 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.
- Mukherjee, A., Cui, Y., Ma, Y., Liu, Y. & 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. (doi:10.1046/j.1462-2920.2000.00093.x)
- Murata, H., Chatterjee, A., Lui, Y. & Chatterjee, A. K. 1994 Regulation of the production of the extracellular pectinase, cellulase and protease in the soft rot bacterium *Erwinia carotovora* ssp. *carotovora*: evidence that *aepH* of *E. carotovora* ssp. *carotovora* 71 activates gene expression in *E. carotovora* ssp. *carotovora*, *E. carotovora* ssp. *atroseptica*, and *Escherichia coli*. *Appl. Environ. Microbiol.* **60**, 3150–3159.
- Nachin, L. & Barras, F. 2000 External pH: an environmental signal that helps to rationalize *pel* gene duplication in *Erwinia chrysanthemi*. *Mol. Plant Microbe Interact.* **13**, 882–886.
- Nasser, W., Bouillant, M. L., Salmond, G. & 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. (doi:10.1046/j.1365-2958.1998.01022.x)
- Ohtani, K., Hayashi, H. & Shimizu, T. 2002 The *luxS* gene is involved in cell–cell signalling for toxin production in *Clostridium perfringens*. *Mol. Microbiol.* **44**, 171–179. (doi:10.1046/j.1365-2958.2002.02863.x)
- Pemberton, C. L. *et al.* 2005 Novel quorum-sensing controlled genes in *Erwinia carotovora* subsp. *carotovora*: identification of a fungal elicitor homologue in a soft-rotting bacterium. *Mol. Plant Microbe Interact.* **18**, 343–353.
- Pirhonen, M., Flego, D., Heikinheimo, R. & Palva, E. T. 1993 A small diffusible signal molecule is responsible for the global control of virulence and exoenzyme production in the plant pathogen *Erwinia carotovora*. *EMBO J.* **12**, 2467–2476.
- Qin, Y., Luo, Z. Q., Smyth, A. J., Gao, P., Beck von Bodman, S. & 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. (doi:10.1093/emboj/19.19.5212)
- Rantakari, A., Virtaharju, O., Vahamiko, S., Taira, S., Palva, E. T., Saarilahti, H. T. & Romantschuk, M. 2001 Type III secretion contributes to the pathogenesis of the soft-rot pathogen *Erwinia carotovora*: partial characterisation of the *hrp* gene cluster. *Mol. Plant Microbe Interact.* **14**, 962–969.
- Redfield, R. J. 2002 Is quorum sensing a side effect of diffusion sensing? *Trends Microbiol.* **10**, 365–370. (doi:10.1016/S0966-842X(02)02400-9)
- Reeves, P. J. *et al.* 1993 Molecular cloning and characterization of 13 *out* genes from *Erwinia carotovora* subspecies *carotovora*: genes encoding members of a general secretion pathway (GSP) widespread in Gram-negative bacteria. *Mol. Microbiol.* **8**, 443–456. (doi:10.1111/j.1365-2958.1993.tb01589.x)
- Riedel, K., Ohnesorg, T., Krogfelt, K. A., Hansen, T. S., Omori, K., Givskov, M. & Eberl, L. 2001 *N*-Acyl-L-homoserine lactone-mediated regulation of the *lip* secretion system in *Serratia liquefaciens* MG1. *J. Bacteriol.* **183**, 1805–1809. (doi:10.1128/JB.183.5.1805-1809.2001)
- Rivet, M. M. 1998 Investigation into the regulation of exoenzyme production in *Erwinia carotovora* subspecies *carotovora*. Ph.D. thesis, University of Warwick, UK.
- Sandkvist, M. 2001 Type II secretion and pathogenesis. *Infect. Immun.* **69**, 3523–3535. (doi:10.1128/IAI.69.6.3523-3535.2001)
- Schauder, S. & Bassler, B. L. 2001 The languages of bacteria. *Genes Dev.* **15**, 1468–1480. (doi:10.1101/gad.899601)
- Schauder, S., Shokat, K., Surette, M. G. & Bassler, B. L. 2001 The LuxS family of bacterial autoinducers: biosynthesis of a novel quorum-sensing signal molecule. *Mol. Microbiol.* **41**, 463–476. (doi:10.1046/j.1365-2958.2001.02532.x)
- Schuster, M., Lostroh, C. P., Ogi, T. & Greenberg, E. P. 2003 Identification, timing, and signal specificity of *Pseudomonas aeruginosa* quorum-controlled genes: a transcriptome analysis. *J. Bacteriol.* **185**, 2066–2079. (doi:10.1128/JB.185.7.2066-2079.2003)
- Sebaihia, M. 1999 Quorum sensing and carbapenem antibiotic production in *Erwinia carotovora* subspecies *carotovora*. Ph.D. thesis, University of Warwick, UK.
- Shih, Y., Harris, S. J., Borner, G., Rivet, M. M. & 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. (doi:10.1046/j.1462-2920.1999.00060.x)
- Slater, H., Crow, M., Everson, L. & Salmond, G. P. 2003 Phosphate availability regulates biosynthesis of two antibiotics, prodigiosin and carbapenem, in *Serratia* via both quorum-sensing-dependent and -independent pathways. *Mol. Microbiol.* **47**, 303–320. (doi:10.1046/j.1365-2958.2003.03295.x)
- Sleman, M. C. & Schofield, C. J. 2004 Carboxymethylproline synthase (CarB), an unusual carbon–carbon bond-forming enzyme of the crotonase superfamily involved in carbapenem biosynthesis. *J. Biol. Chem.* **279**, 6730–6736. (doi:10.1074/jbc.M311824200)
- Smadja, B., Latour, X., Faure, D., Chevalier, S., Dessaux, Y. & Orange, N. 2004 Involvement of *N*-acylhomoserine lactones throughout plant infection by *Erwinia carotovora* subsp. *atroseptica* (*Pectobacterium atroseptica*). *Mol. Plant Microbe Interact.* **17**, 1269–1278.

- Sperandio, V., Mellies, J. L., Nguyen, W., Shin, S. & 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**, 15 196–15 201. (doi:10.1073/pnas.96.26.15196)
- Sperandio, V., Torres, A. G., Giron, J. A. & Kaper, J. B. 2001 Quorum sensing is a global regulatory mechanism in enterohemorrhagic *Escherichia coli* O157:H7. *J. Bacteriol.* **183**, 5187–5197. (doi:10.1128/JB.183.17.5187-5197.2001)
- Strocher, U. H., Paton, A. W., Ogunniyi, A. D. & Paton, J. C. 2003 Mutation of *luxS* of *Streptococcus pneumoniae* affects virulence in a mouse model. *Infect. Immun.* **71**, 3206–3212. (doi:10.1128/IAI.71.6.3206-3212.2003)
- Sun, J., Daniel, R., Wagner-Dobler, I. & Zeng, A. P. 2004 Is autoinducer-2 a universal signal for interspecies communication: a comparative genomic and phylogenetic analysis of the synthesis and signal transduction pathways. *BMC Evol. Biol.* **4**, 36. (doi:10.1186/1471-2148-4-36)
- Surette, M. G., Miller, M. B. & 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. (doi:10.1073/pnas.96.4.1639)
- Swift, S. *et al.* 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. (doi:10.1111/j.1365-2958.1993.tb00923.x)
- Taga, M. E., Semmelhack, J. L. & Bassler, B. L. 2001 The LuxS-dependent autoinducer AI-2 controls the expression of an ABC transporter that functions in AI-2 uptake in *Salmonella typhimurium*. *Mol. Microbiol.* **42**, 777–793. (doi:10.1046/j.1365-2958.2001.02669.x)
- Taga, M. E., Miller, S. T. & Bassler, B. L. 2003 Lsr-mediated transport and processing of AI-2 in *Salmonella typhimurium*. *Mol. Microbiol.* **50**, 1411–1427. (doi:10.1046/j.1365-2958.2003.03781.x)
- Thomson, N. R., Cox, A., Bycroft, B. W., Stewart, G. S. A. B., Williams, P. & 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. (doi:10.1046/j.1365-2958.1997.5981976.x)
- Thomson, N. R., Nasser, W., McGowan, S. J., Sebahia, M. & Salmond, G. P. C. 1999 *Erwinia carotovora* has two KdgR-like proteins belonging to the IclR family of transcriptional regulators: identification and characterization of the RexZ activator and the KdgR repressor of pathogenesis. *Microbiology* **145**, 1531–1545.
- Thomson, N. R., Crow, M. A., McGowan, S. J., Cox, A. & Salmond, G. P. 2000 Biosynthesis of carbapenem antibiotic and prodigiosin pigment in *Serratia* is under quorum sensing control. *Mol. Microbiol.* **36**, 539–556. (doi:10.1046/j.1365-2958.2000.01872.x)
- Toth, I. K. & Birch, P. R. J. 2005 Rotting softly and stealthily. *Curr. Opin. Plant Biol.* **8**, 424–429. (doi:10.1016/j.pbi.2005.04.001)
- Toth, I. K., Thorpe, C. J., Bentley, S. D., Mulholland, V., Hyman, L. J., Perombelon, M. C. & Salmond, G. P. C. 1999 Mutation in a gene required for lipopolysaccharide and enterobacterial common antigen biosynthesis affects virulence in the plant pathogen *Erwinia carotovora* subsp. *atroseptica*. *Mol. Plant Microbe Interact.* **12**, 499–507.
- Toth, I. K., Bell, K. S., Holeva, M. C. & Birch, P. R. J. 2003 Soft rot erwiniae: from genes to genomes. *Mol. Plant Pathol.* **4**, 17–30. (doi:10.1046/j.1364-3703.2003.00149.x)
- Toth, I. K., Newton, J. A., Hyman, L. J., Lees, A. K., Daykin, M., Ortori, C., Williams, P. & Fray, R. G. 2004 Potato plants genetically modified to produce *N*-acylhomoserine lactones increase susceptibility to soft rot erwiniae. *Mol. Plant Microbe Interact.* **17**, 880–887.
- Unlu, M., Morgan, M. E. & Minden, J. S. 1997 Difference gel electrophoresis: a single gel method for detecting changes in protein extracts. *Electrophoresis* **18**, 2071–2077.
- Valverde, C., Lindell, M., Wagner, E. G. & Haas, D. 2004 A repeated GGA motif is critical for the activity and stability of the riboregulator RsmY of *Pseudomonas fluorescens*. *J. Biol. Chem.* **279**, 25 066–25 074. (doi:10.1074/jbc.M401870200)
- Van Houdt, R., Aertsen, A., Moons, P., Vanoirbeek, K. & Michiels, C. W. 2006 *N*-acyl-L-homoserine lactone signal interception by *Escherichia coli*. *FEMS Microbiol. Lett.* **256**, 83–89. (doi:10.1111/j.1574-6968.2006.00103.x)
- Vendeville, A., Winzer, K., Heurlier, K., Tang, C. M. & Hardie, K. R. 2005 Making 'sense' of metabolism: autoinducer-2, LuxS and pathogenic bacteria. *Nat. Rev. Microbiol.* **3**, 383–396. (doi:10.1038/nrmicro1146)
- Venturi, V., Venuti, C., Devescovi, G., Lucchese, C., Friscina, A., Degrassi, G., Aguilar, C. & Mazzucchi, U. 2004 The plant pathogen *Erwinia amylovora* produces acyl-homoserine lactone signal molecules *in vitro* and *in planta*. *FEMS Microbiol. Lett.* **241**, 179–183. (doi:10.1016/j.femsle.2004.10.015)
- Von Bodman, S. B., Majerczak, D. R. & 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. (doi:10.1073/pnas.95.13.7687)
- Von Bodman, S. B., Ball, J. K., Faini, M. A., Herrera, C. M., Minogue, T. D., Urbanowski, M. L. & Stevens, A. M. 2003a The quorum sensing regulators EsaR and ExpR_{Ecc} homologues within the LuxR family, retain the ability to function as activators of transcription. *J. Bacteriol.* **185**, 7001–7007. (doi:10.1128/JB.185.23.7001-7007.2003)
- Von Bodman, S. B., Bauer, W. D. & Coplin, D. L. 2003b Quorum sensing in plant-pathogenic bacteria. *Annu. Rev. Phytopathol.* **41**, 455–482. (doi:10.1146/annurev.phyto.41.052002.095652)
- Wagner, V. E., Bushnell, D., Passador, L., Brooks, A. I. & Iglewski, B. H. 2003 Microarray analysis of *Pseudomonas aeruginosa* quorum-sensing regulons: effects of growth phase and environment. *J. Bacteriol.* **185**, 2080–2095. (doi:10.1128/JB.185.7.2080-2095.2003)
- Watson, W. T., Minogue, T. D., Val, D. L., von Bodman, S. B. & Churchill, M. E. 2002 Structural basis and specificity of acyl-homoserine lactone signal production in bacterial quorum sensing. *Mol. Cell* **9**, 685–694. (doi:10.1016/S1097-2765(02)00480-X)
- Wei, B. L., Brun-Zinkernagel, A. M., Simecka, J. W., Prüß, B. M., Babitzke, P. & Romeo, T. 2001 Positive regulation of motility and *flhDC* expression by the RNA-binding protein CsrA of *Escherichia coli*. *Mol. Microbiol.* **40**, 245–256. (doi:10.1046/j.1365-2958.2001.02380.x)
- Welch, M., Todd, D. E., Whitehead, N. A., McGowan, S. J., Bycroft, B. W. & Salmond, G. P. C. 2000 *N*-acyl homoserine lactones binding to the CarR receptor determines quorum-sensing specificity in *Erwinia*. *EMBO J.* **19**, 631–641. (doi:10.1093/emboj/19.4.631)
- Welch, M., Dutton, J. M., Glansdorp, F. G., Thomas, G. L., Smith, D. S., Coulthurst, S. J., Barnard, A. M. L., Salmond, G. P. C. & Spring, D. R. 2005 Structure–activity relationships of *Erwinia carotovora* quorum sensing signalling molecules. *Bioorg. Med. Chem. Lett.* **15**, 4235–4238. (doi:10.1016/j.bmcl.2005.06.066)
- Wen, Z. T. & Burne, R. A. 2004 LuxS-mediated signaling in *Streptococcus mutans* is involved in regulation of acid and

- oxidative stress tolerance and biofilm formation. *J. Bacteriol.* **186**, 2682–2691. (doi:10.1128/JB.186.9.2682-2691.2004)
- Whitehead, N. A. 1999 Analysis of the quorum sensing system of *Erwinia carotovora* subspecies *carotovora*. Ph.D. thesis, University of Cambridge, UK.
- Whitehead, N. A., Barnard, A. M. L., Slater, H., Simpson, N. J. L. & Salmond, G. P. C. 2001 Quorum-sensing in Gram-negative bacteria. *FEMS Microbiol. Rev.* **25**, 365–404. (doi:10.1111/j.1574-6976.2001.tb00583.x)
- Whitehead, N. A., Harris, A. K. P., Williams, P. & Salmond, G. P. C. 2002 Quorum sensing in Gram-negative bacteria: global regulons controlled by cell-density-dependent chemical signalling. In *SGM Symp. 61: signals, switches, regulons and cascades: control of bacterial gene expression* (eds D. A. Hodgson & C. M. Thomas), pp. 251–287. Cambridge, UK: Cambridge University Press.
- Winzer, K., Sun, Y. H., Green, A., Delory, M., Blackley, D., Hardie, K. R., Baldwin, T. J. & Tang, C. M. 2002a Role of *Neisseria meningitidis luxS* in cell-to-cell signaling and bacteremic infection. *Infect. Immun.* **70**, 2245–2248. (doi:10.1128/IAI.70.4.2245-2248.2002)
- Winzer, K. *et al.* 2002b LuxS: its role in central metabolism and the *in vitro* synthesis of 4-hydroxy-5-methyl-3(2H)-furanone. *Microbiology* **148**, 909–922.
- Xavier, K. B. & Bassler, B. L. 2005 Regulation of uptake and processing of the quorum-sensing autoinducer AI-2 in *Escherichia coli*. *J. Bacteriol.* **187**, 238–248. (doi:10.1128/JB.187.1.238-248.2005)
- Yang, C.-H., Gavilanes-Ruiz, M., Okinaka, Y., Vedel, R., Berthuy, I., Boccara, M., Chen, J. W., Perna, N. T. & Keen, N. T. 2002 *hrp* genes of *Erwinia chrysanthemi* 3937 are important virulence factors. *Mol. Plant Microbe Interact.* **15**, 472–480.
- Yao, Y., Martinez-Yamout, M. A., Dickerson, T. J., Brogan, A. P., Wright, P. E. & Dyson, H. J. 2006 Structure of the *Escherichia coli* quorum sensing protein SdiA: activation of the folding switch by acyl homoserine lactones. *J. Mol. Biol.* **355**, 262–273. (doi:10.1016/j.jmb.2005.10.041)
- Yates, E. A. *et al.* 2002 *N*-acylhomoserine lactones undergo lactonolysis in a pH-, temperature-, and acyl chain length-dependent manner during growth of *Yersinia pseudotuberculosis* and *Pseudomonas aeruginosa*. *Infect. Immun.* **70**, 5635–5646. (doi:10.1128/IAI.70.10.5635-5646.2002)