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Quorum sensing, communication and cross-kingdom signalling in the bacterial world

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Although unicellular, bacteria are highly interactive and employ a range of cell-to-cell communication or 'quorum sensing (QS)' systems for promoting collective behaviour within a population. QS is generally considered to facilitate gene expression only when the population has reached a sufficient cell density and depends on the synthesis of small molecules that diffuse in and out of bacterial cells. As the bacterial population density increases, so does the synthesis of QS signal molecules and consequently, their concentration in the external environment increases. Once a critical threshold concentration is reached, a target sensor kinase or response regulator is activated, so facilitating the expression of QS-dependent target genes. Several chemically distinct families of QS signal molecules have been described, of which the *N*-acylhomoserine lactone (AHL) family in Gram-negative bacteria have been the most intensively investigated. QS contributes to environmental adaptation by facilitating the elaboration of virulence determinants in pathogenic species and plant biocontrol characteristics in beneficial species as well as directing biofilm formation and colony escape. QS also crosses the prokaryotic–eukaryotic boundary in that QS signal molecules influence the behaviour of eukaryotic organisms in both the plant and mammalian worlds such that QS signal molecules may directly facilitate bacterial survival by promoting an advantageous lifestyle within a given environmental niche.

Introduction

In recent years there has been a paradigm shift in our understanding of the unicellular bacterial world from the perspective that bacterial cells are non-co-operative to one which incorporates social interactions and multicellular behaviour. Bacteria are clearly capable of complex patterns of co-operative behaviour that result from the co-ordination of the activities of individual cells. This is primarily achieved through the deployment of small diffusible signal molecules (sometimes called 'pheromones' or 'autoinducers') which are generally considered to facilitate the regulation of gene expression as a function of cell population density. This phenomenon is termed 'quorum sensing' (QS). As the bacterial population density increases, so does the synthesis of QS signal molecules and consequently, their concentration in the external environment rises. Once a threshold concentration has been attained, activation of a signal transduction cascade leads to the induction or repression of QS target genes, often incorporating those required for QS signal molecule synthesis, so providing an auto-regulatory mechanism for amplifying signal molecule production. Consequently, the size of the 'quorum' is not fixed but will depend on the relative rates of production and loss of the signal molecule, which will, in turn, vary depending on the local environmental conditions. Apart from population sensing, QS can also be considered in the context of 'diffusion sensing' or 'compartment sensing' or even 'efficiency sensing', where the signal molecule supplies information with respect to the local environment and spatial distribution of the cells rather than, or as well as, cell population density (Redfield, 2002; Winzer *et al.*, 2002; Hense *et al.*, 2007). QS also continues to attract significant interest from the perspective of social evolution, fitness and the benefits associated with costly co-operative behaviours (Diggle *et al.*, 2007; West *et al.*, 2006).

QS signal molecules are chemically diverse and many bacteria possess several interacting QS gene regulatory 'modules' (consisting of the genes which code for the QS signal synthase and QS signal transduction machinery) employing multiple signal molecules from the same or different chemical classes and which constitute regulatory hierarchies. In general QS systems facilitate the co-ordination of population behaviour to enhance access to nutrients or specific environmental niches, collective defence against other competitor organisms or community escape where survival of the population is threatened. Although QS has primarily been studied in the context of

Abbreviations: AHL, *N*-acylhomoserine lactone; AI-2, autoinducer-2; AIP, autoinducing peptide; AQ, 2-alkyl-4-quinolone; HSL, homoserine lactone; QS, quorum sensing.

single species, the expression of QS systems may be manipulated by the activities of other bacteria within complex microbial consortia which employ different QS signals and by higher organisms. QS signal molecules also exhibit biological properties far beyond their role in co-ordinating gene expression in the producer organism. Consequently both QS systems and QS signal molecules have attracted considerable interest from the biotechnology, pharmaceutical and agricultural industries, particularly with respect to QS as a target for novel antibacterials.

QS signal molecules are chemically diverse

Most QS signals are either small (<1000 Da) organic molecules or peptides with 5–20 amino acids (Fig. 1) (Lazazzera, 2001; Chhabra *et al.*, 2005; Williams *et al.*, 2007;). Gram-negative bacteria, for example, employ *N*-acylhomoserine lactones (AHLs), 2-alkyl-4-quinolones (AQs), long-chain fatty acids and fatty acid methyl esters as well as autoinducer-2 (AI-2), a collective term for a group of interconvertible furanones derived from dihydroxypentanedione (DPD) (Fig. 1). AI-2 is also produced by some Gram-positive bacteria, although generally these organisms prefer linear, modified or cyclic peptides such as the autoinducing peptides (AIPs) made by the staphylococci (Fig. 1). The streptomycetes, however, synthesize γ-butyrolactones such as A-factor (Fig. 1), which are structurally related to the AHLs as both compound classes belong to the butanolides. QS signal molecules can also be further subdivided according to whether they interact with receptors at the cell surface (e.g. the staphylococcal AIPs) or are internalized (e.g. the AHLs, AQs, the Phr peptides of *Bacillus subtilis* and the mating pheromones of *Enterococcus faecalis*).

AHL production is widespread among Gram-negative bacteria

The screening of Gram-negative bacteria for putative AHL producers has been greatly aided by the development of simple AHL biosensors based on *lux*, *lacZ* or *gfp* reporter gene fusions (Bainton *et al.*, 1992a; Winson *et al.*, 1998; Andersen *et al.*, 2001; Shaw *et al.*, 1997) or pigment induction (e.g. violacein in *Chromobacterium violaceum*: McClean *et al.*, 1997). These assays have revealed that bacteria belonging to genera which occupy a wide variety of environmental niches – from marine and freshwater environments to soil, plants and animals, including many pathogens, symbionts, extremophiles and plant-growth-promoting bacteria – produce AHLs. Examples include species of *Acidithiobacillus*, *Acinetobacter*, *Aeromonas*, *Agrobacterium*, *Brucella*, *Burkholderia*, *Erwinia*, *Enterobacter*, *Chromobacterium*, *Hafnia*, *Mesorhizobium*, *Methylobacter*, *Paracoccus*, *Pseudomonas*, *Ralstonia*, *Rhodobacter*, *Rhizobium*, *Rhanella*, *Serratia*, *Sinorhizobium*, *Vibrio* and *Yersinia* (Williams *et al.*, 2007). Many of these bacteria produce multiple AHLs and possess more than one AHL synthase. As yet no AHL-producing *Escherichia coli* or *Salmonella* strains have been identified, although both organisms possess an AHL receptor (SdiA) of the LuxR protein class and respond to AHLs produced by other bacteria (Ahmer, 2004). Table 1 provides a summary of the known AHL-dependent QS systems and the phenotypes controlled.

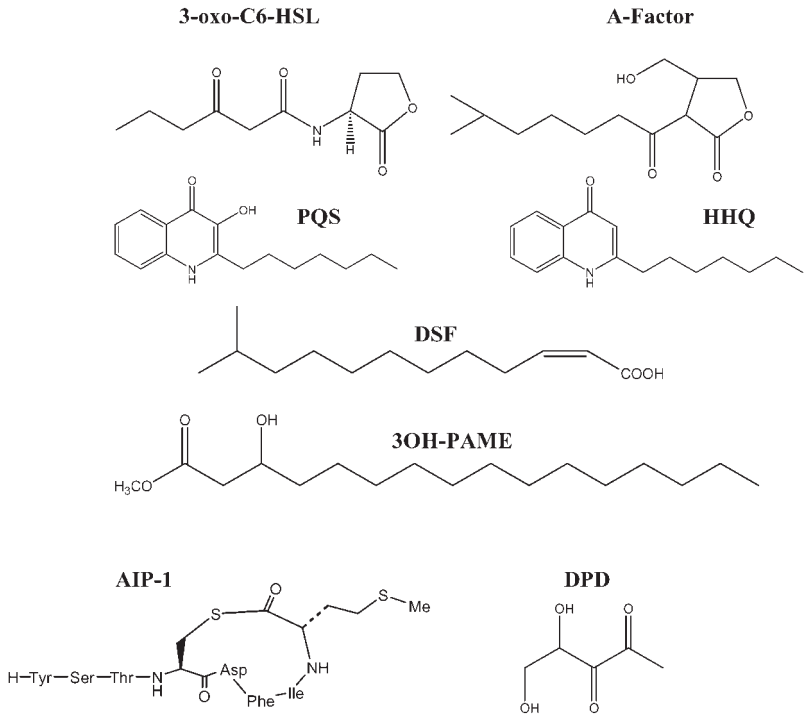

Fig. 1. Examples of the structures of some representative QS signal molecules. 3-oxo-C6-HSL, *N*-(3-oxohexanoyl)-L-homoserine lactone; A-Factor, 2-isocapryloyl-3-hydroxymethyl-γ-butyrolactone; PQS, pseudomonas quinolone signal, 2-heptyl-3-hydroxy-4(1*H*)-quinolone; HHQ, 2-heptyl-4(*1H*)-quinolone; DSF, 'diffusible factor', *cis*-11-methyl-2-dodecenoic acid; 3OH-PAME, hydroxyl-palmitic acid methyl ester; AIP-1, staphylococcal autoinducing peptide 1; DPD, the AI-2 precursor, 4,5 dihydroxy-2,3-pentanedione.

Table 1. Some examples of AHL-dependent QS systems and the phenotypes controlled

Organism	AHLs	Phenotype
Aeromonas hydrophila	C4-HSL, C6-HSL	Biofilms, exoproteases, virulence
Aeromonas salmonicida	C4-HSL, C6-HSL	Exoproteases
Agrobacterium tumefaciens	3-Oxo-C8-HSL	Plasmid conjugation
Agrobacterium vitiae	C14 : 1-HSL, 3-oxo-C16 : 1-HSL	Virulence
Acidithiobacillus ferrooxidans	3-Hydroxy-C8-HSL, 3-hydroxy-C10-HSL, C12-HSL, 3-hydroxy-C12-HSL, C14-HSL, 3-oxo-C14-HSL, 3-hydroxy-C14-HSL, 3-hydroxy-C16-HSL	Not known
Burkholderia cenocepacia	C6-HSL, C8-HSL	Exoenzymes, biofilm formation, swarming motility, siderophore, virulence
Burkholderia pseudomallei	C8-HSL, C10-HSL, 3-hydroxy-C8-HSL, 3-hydroxy-C10-HSL, 3-hydroxy-C14-HSL	Virulence, exoproteases
Burkholderia mallei	C8-HSL, C10-HSL	Virulence
Chromobacterium violaceum	C6-HSL	Exoenzymes, cyanide, pigment
Erwinia carotovora	3-Oxo-C6-HSL	Carbapenem, exoenzymes, virulence
Pantoea (*Erwinia*) *stewartii*	3-Oxo-C6-HSL	Exopolysaccharide
Pseudomonas aeruginosa	C4-HSL; C6-HSL, 3-oxo-C12-HSL	Exoenzymes, exotoxins, protein secretion, biofilms, swarming motility, secondary metabolites, 4-quinolone signalling, virulence
Pseudomonas aureofaciens	C6-HSL	Phenazines, protease, colony morphology, aggregation, root colonization
Pseudomonas chlororaphis	C6-HSL	Phenazine-1-carboxamide
Pseudomonas putida	3-Oxo-C10-HSL, 3-oxo-C12-HSL	Biofilm development
Pseudomonas fluorescens	3-Oxo-C10-HSL	Mupirocin
Pseudomonas syringae	3-Oxo-C6-HSL	Exopolysaccharide, swimming motility, virulence
Rhizobium leguminosarum bv. *viciae*	C14 : 1-HSL, C6-HSL, C7-HSL, C8-HSL, 3-oxo-C8-HSL, 3-hydroxy-C8-HSL	Root nodulation/symbiosis, plasmid transfer, growth inhibition; stationary phase adaptation
Rhodobacter sphaeroides	7-*cis*-C14-HSL	Aggregation
Serratia sp. ATCC 39006	C4-HSL, C6-HSL	Antibiotic, pigment, exoenzymes
Serratia liquefaciens MG1	C4-HSL, C6-HSL	Swarming motility, exoprotease, biofilm development, biosurfactant
Serratia marcescens SS-1	C6-HSL, 3-oxo-C6-HSL, C7-HSL, C8-HSL	Sliding motility, biosurfactant, pigment, nuclease, transposition frequency
Serratia proteamaculans B5a	3-Oxo-C6-HSL	Exoenzymes
Sinorhizobium meliloti	C8-HSL, C12-HSL, 3-oxo-C14-HSL, 3-oxo-C16 : 1-HSL, C16 : 1-HSL, C18-HSL	Nodulation efficiency, symbiosis, exopolysaccharide
Vibrio fischeri	3-Oxo-C6-HSL	Bioluminescence
Yersinia enterocolitica	C6-HSL, 3-oxo-C6-HSL, 3-oxo-C10-HSL, 3-oxo-C12-HSL, 3-oxo-C14-HSL	Swimming and swarming motility
Yersinia pseudotuberculosis	C6-HSL, 3-oxo-C6-HSL, C8-HSL	Motility, aggregation

AHL structural variation and biosynthesis

All of the AHLs which have been characterized to date consist of a homoserine lactone ring unsubstituted in the β- and γ-positions which is *N*-acylated with a fatty acyl group at the α-position (Chhabra *et al.*, 2005) (Fig. 2). Naturally occurring AHLs exhibit variable acyl chain lengths, saturation levels and oxidation states and are exemplified by compounds with 4–18-carbon acyl chains which belong to either the *N*-acyl, *N*-(3-oxoacyl) or *N*-(3-hydroxyacyl) classes (Fig. 2). Some AHLs also have unsaturation in the 5 and 7 positions in a chain of 12 or 14 carbons. Stereochemistry at the α-centre of the homoserine lactone (HSL) ring has been unequivocally established to be L for the *N*-(3-oxohexanoyl)homoserine lactone (3-oxo-C6-HSL) produced by *Erwinia carotovora* (Bainton *et al.*, 1992b) and by analogy it is extrapolated that all other natural AHLs have the same configuration. In some cases D-isomers have been synthesized and shown to lack activity (Chhabra *et al.*, 1993, 2005). The AHL lactone ring is readily hydrolysed under alkaline conditions to form the corresponding *N*-acylhomoserine compound (Fig. 2), which is inactive as a QS signal molecule (Yates *et al.*, 2002). The lactone ring of the HSL itself is completely hydrolysed at pHs above 2 whereas 70 % of *N*-propionyl-homoserine lactone (C3-HSL) is hydrolysed at pH 6 (Yates *et al.*, 2002). Consequently, a C_4 acyl chain is the minimum chain length likely to be sufficiently stable to function as a signal molecule over the pH range encountered by most bacteria. Indeed, the shortest naturally occurring AHLs so

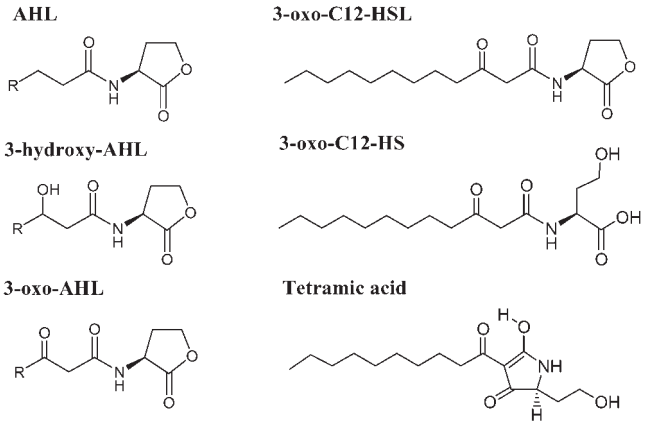

Fig. 2. The AHLs and their hydrolysis products. AHL, *N*-acylhomoserine lactone; 3-hydroxy-AHL, *N*-(3-hydoxyacyl)homoserine lactone; 3-oxo-AHL, *N*-(3-oxoacyl)homoserine lactone (R ranges from C_1 to C_{15}). The acyl side chains may also contain one or more double bonds. 3-Oxo-C12-HSL, *N*-(3-oxododecanoyl)-L-homoserine lactone; 3-oxo-C12-HS, *N*-(3-oxododecanoyl)-L-homoserine; tetramic acid, 3-(1-hydroxydecylidene)-5-(2-hydroxyethyl)pyrrolidine-2,4-dione.

far identified are C4-HSL and 3-hydroxy-C4-HSL, which are produced by *Pseudomonas aeruginosa* (Winson *et al.*, 1995) and *Vibrio harveyi* (Cao & Meighen, 1989) respectively. Apart from base-catalysed lactonolysis, 3-oxo-AHLs can also undergo an alkali-driven rearrangement reaction to form the corresponding tetramic acids, compounds with iron chelating and antibacterial activities (Fig. 2; Kaufmann *et al.*, 2005).

AHLs act by directly binding to members of the LuxR transcriptional regulator protein family such that the LuxR/AHL complex activates or represses multiple target genes including those coding for the AHL synthase (usually a member of the LuxI protein family), giving rise to a positive autoinduction circuit (Fig. 3; Salmond *et al.*, 1995; Urbanowski *et al.*, 2004; Zhang *et al.*, 2002). The genes coding for LuxR and LuxI orthologues can be found on chromosomes or on mobile genetic elements (plasmids and transposons) and are often located adjacent to each other as tandem, convergent or divergent pairs (Salmond *et al.*, 1995; Wei *et al.*, 2006). Currently, the Gram-negative bacterial genome databases contain more than 100 examples of LuxI family members. *In vitro* studies using purified LuxI family proteins have revealed that AHLs are synthesized from precursors derived from fatty acid and amino acid metabolism (Moré *et al.*, 1996; Jiang *et al.*, 1998; Parsek *et al.*, 1999). They catalyse AHL synthesis using the appropriately charged acyl carrier protein, as the main acyl chain donor (acyl-CoA derivatives can also be substrates), and *S*-adenosylmethionine as the source for the homoserine lactone moiety (Fig. 4). Since LuxI proteins are not particularly closely related it has been impossible to predict the nature of the AHL produced by a given LuxI protein from bioinformatic comparisons alone. This is further complicated by the large number of AHLs which can be synthesized via a given LuxI protein. The *Yersinia pseudotuberculosis* AHL synthase YtbI, for example, directs the synthesis of at least 24 different AHLs, although four compounds predominate (3-oxo-C6-HSL, 3-oxo-C7-HSL, 3-oxo-C8-HSL and C8-HSL) (Ortori *et al.*, 2006). Interestingly, an N-terminally truncated LuxI homologue (TslI) encoded on a plasmid in *Methylobacterium extorquens* which appears to be inactive as an AHL synthase is involved in the transcriptional control of AHL synthesis via two other LuxI-type proteins, MsaI and MlaI (Nieto Penalver *et al.*, 2006). The molecular mechanism through which TslI operates has yet to be elucidated.

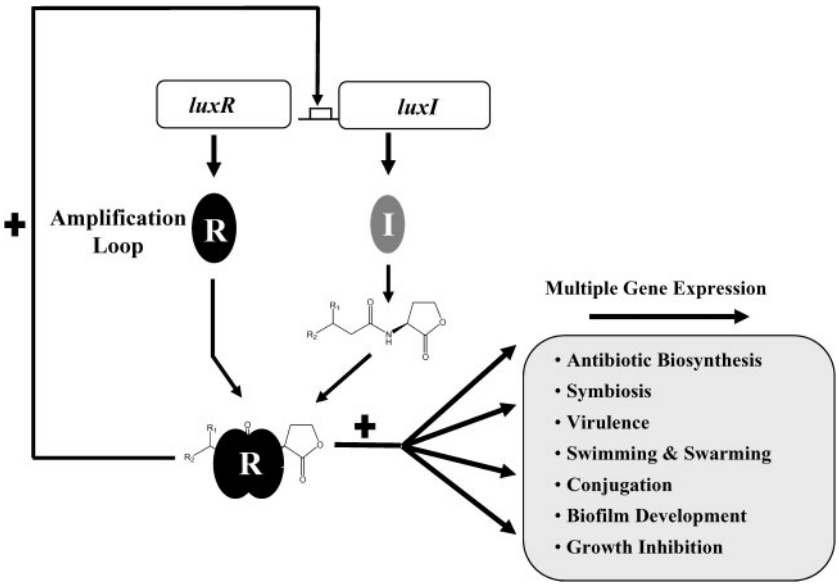

Fig. 3. AHL-mediated QS-dependent regulation of multiple target gene expression, where *luxR* and *luxI* are orthologues of the corresponding *V. fischeri* genes and code for the AHL receptor and AHL synthase respectively. In many but not all systems the AHL is an autoinducer since it drives its own production via the amplification loop leading to the AHL synthase gene.

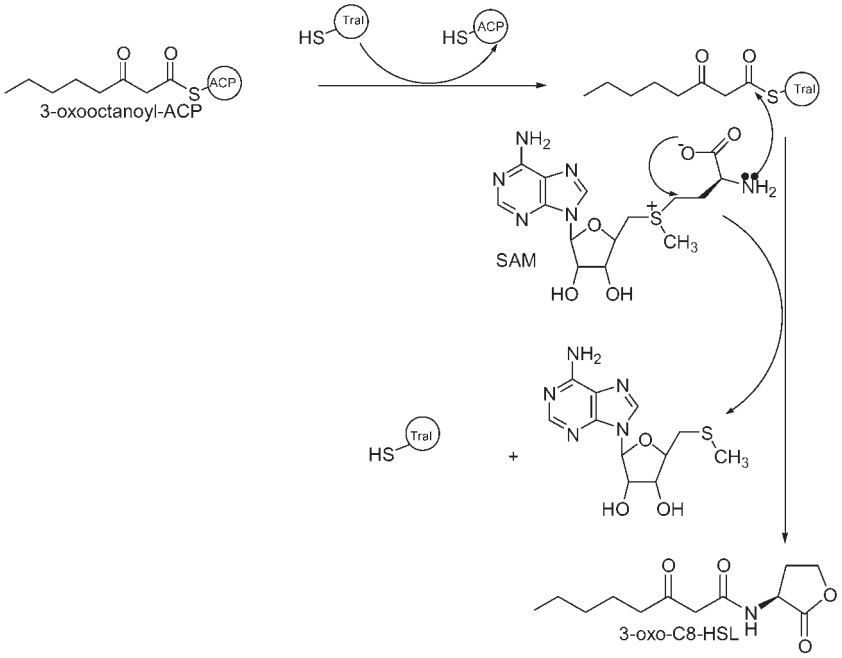

Fig. 4. The biosynthesis of AHLs via LuxI family proteins – a model of TraI-mediated biosynthesis of 3-oxo-C8-HSL. TraI catalyses the formation of an amide bond between the amino group of *S*-adenosylmethione (SAM) and the α-carbon of the fatty acid provided via the 3-oxooctanoyl-loaded acyl carrier protein (3-oxooctanoyl-ACP). Lactonization follows and the reaction products released are 3-oxo-C8-HSL and 5′-methylthioadenosine (MTA). For the LuxI orthologue, RhlI, the biosynthesis of C4-HSL is initiated by the binding of SAM and the appropriately charged ACP is subsequently bound to the complex (Parsek *et al.*, 1999). Whether this is also the case for TraI and other LuxI family proteins has not been established.

The crystal structures of EsaI and LasI from *Pantoea stewartii* and *Pseudomonas aeruginosa* respectively have been solved and both proteins revealed to belong to the GCN5-related *N*-acetyltransferase protein family (Watson *et al.*, 2002; Gould *et al.*, 2004). From the EsaI structural analysis, threonine at position 140 was shown to contribute to the specificity of the enzyme for 3-oxo-acyl-Acyl carrier proteins but not to be of such importance for LasI (Watson *et al.*, 2002; Gould *et al.*, 2004). Interestingly, the AHL substrate chain length specificity of the *Er. carotovora* synthase, ExpI, can be modified from a C_6 to a C_8 acyl chain by site-specific mutagenesis of M127T and F69L, with the former modification being critical for the change in substrate specificity and the latter for maintaining wild-type levels of enzyme activity (Brader *et al.*, 2005).

AHL biosynthesis is not exclusively dependent on LuxI homologues but can be directed by members of the LuxM protein family, which has so far only been found in the genus *Vibrio* (Milton *et al.*, 2001). LuxM proteins employ the same substrates as LuxI proteins and both AHL synthase types co-exist in both *Vibrio fischeri* and *Vibrio anguillarum* (Hanzelka *et al.*, 1999; Milton *et al.*, 2001). A third potential AHL synthase (HdtS) from *Pseudomonas fluorescens*, which does not belong to either the LuxI or LuxM families, was identified by Laue *et al.* (2000). When expressed in *E. coli*, HdtS directed the synthesis of the same AHLs [*N*-(3-hydroxy-7-*cis*-tetradecenoyl)homoserine lactone (3-hydroxy-C14 : 1HSL), *N*-decanoylhomoserine lactone (C10-HSL) and *N*-hexanoylhomoserine lactone (C6-HSL)] produced by *P. fluorescens*. HdtS is related to the lysophosphatidic acid acyltransferase protein family. An *hdtS* orthologue (termed *act*) has also been identified in the extreme acidophile *Acidithiobacillus ferrooxidans* which, when introduced into *E. coli*, also directs the synthesis of *N*-tetradecanoylhomoserine lactone (C14-HSL) together with small amounts of shorter-chain AHLs (Rivas *et al.*, 2007) Although an *in vitro* demonstration of the AHL synthase activity of HdtS and Act has yet to be undertaken, it is possible that these proteins are involved in both phospholipid and AHL biosynthesis. HdtS clearly has lysophosphatidic acid acyltransferase activity and the *hdtS* gene complements the growth defect of an *E. coli plsC* mutant (Cullinane *et al.*, 2005). However, although the production of 3-hydroxy-C14 : 1-HSL production was not reported, a *P. fluorescens hdtS* mutant still produced parental levels of C6-HSL (Cullinane *et al.*, 2005).

AHL perception

In many AHL producers, the AHL receptor gene is located adjacent to the AHL synthase gene (Fig. 3; Salmond *et al.*, 1995). This is usually a member of the LuxR family of transcriptional regulators although in some cases it may be a histidine kinase sensor protein, the activation of which induces a phosphorelay cascade leading to the transcriptional activation of the target genes (Milton, 2006). Although LuxR proteins are mostly highly AHL specific, many can be activated to different extents by closely related compounds, which explains their utility as components of AHL biosensors based on a given LuxR protein and the promoter of a target gene fused to a reporter such as *lux* (Bainton *et al.*, 1992a; Winson *et al.*, 1998). LuxR-type proteins consist of two functional domains, an N-terminal AHL-binding and a C-terminal DNA-binding domain. They reversibly bind their cognate AHLs and activate or repress gene expression by binding to specific DNA motifs containing dyad symmetry at their target promoters

(Schuster *et al.*, 2004; Urbanowski *et al.*, 2004). Depending on the organism, these related motifs have been called *lux* boxes (*V. fischeri*), *las* boxes (*P. aeruginosa*) or *tra* boxes (*Agrobacterium tumefaciens*) (Schuster *et al.*, 2004; Urbanowski *et al.*, 2004; Zhang *et al.*, 2002). X-ray structural studies have revealed that for LuxR proteins such as the dimeric TraR, each monomer binds to one half of a *tra* box via the helix–turn–helix DNA-binding motif (Zhang *et al.*, 2002). The crystal structures of the TraR (Zhang *et al.*, 2002) and LasR (Bottomley *et al.*, 2007) dimers bound to their cognate AHL [*N*-(3-oxooctanoyl)-homoserine lactone (3-oxo-C8-HSL) and *N*-(3-oxododecanoyl)homoserine lactone (3-oxo-C12-HSL) respectively] revealed that each monomer contained one deeply buried ligand and that the AHL-binding pocket of LasR is larger than that of TraR since it has to accommodate a longer acyl side chain (C_{12} instead of C_8). For both TraR and LasR, models have been proposed in which AHL binding to the nascent protein facilitates protein folding and dimerization, enabling DNA binding and transcriptional activation of the target QS genes (Zhang *et al.*, 2002; Bottomley *et al.*, 2007).

Plant–microbe interactions, *Erwinia carotovora* and AHL-dependent QS

AHLs were originally discovered in bioluminescent marine vibrios in the 1980s (Eberhard *et al.*, 1981; Cao & Meighen, 1989) but it was not until 1992 that the first paper reporting that many other bacteria including *Erwinia*, *Serratia*, *Citrobacter*, *Enterobacter* and *Pseudomonas* produced AHLs was published (Bainton *et al.*, 1992a). In the soft-rot plant pathogen *Erwinia carotovora* subsp. *carotovora* ATCC 39048, *N*-(3-oxo-hexanoyl)homoserine lactone (3-oxo-C6-HSL) was initially found to control the synthesis of a β-lactam antibiotic, 1-carbapen-2-em-3-carboxylic acid. 3-Oxo-C6-HSL synthesis in *Er. carotovora* is directed via CarI (Bainton *et al.*, 1992a, b). The gene coding for this LuxI homologue, *carI* (Swift *et al.*, 1993), is not linked to the carbapenem biosynthetic gene cluster (*carABCDEFGJH*) but both *carI* and the *car* operon are linked to adjacent genes coding for LuxR homologues (ExpR and CarR respectively) (McGowan *et al.*, 1995, 1996, 1997). In the biosynthetic cluster, CarA, B and C are absolutely required for antibiotic production, CarD and E probably supply precursors but are not essential while CarF and G confer protection against the endogenously synthesized carbapenem. The function of CarH is unknown but it is also not essential for antibiotic production (McGowan *et al.*, 1996, 1997). Intriguingly, many *Erwinia* strains are carbapenem-negative but retain the *car* operon; antibiotic biosynthesis can be restored by introducing a plasmid-borne copy of *carR* as these strains appear to contain an inactive *carR* gene (Holden *et al.*, 1998).

Er. carotovora causes the post-harvest soft rotting of many vegetable crops including potato, carrot and green pepper (Barnard *et al.*, 2007). The virulence of this plant pathogen depends on the synthesis and secretion of a number of exoenzymes including pectinases, cellulases and proteases. Expression of the genes coding for these plant cell wall degrading enzymes is controlled by QS. *Er. carotovora carI* mutants are not only carbapenem-negative but are also downregulated for exoenzyme production and their virulence is severely attenuated *in planta* (Jones *et al.*, 1993). Both virulence and antibiotic production can be restored by the exogenous provision of 3-oxo-C6-HSL. In contrast, mutation of *carR* has no impact on exoenzyme synthesis, indicating that an additional LuxR-type protein must be involved (Barnard *et al.*, 2007). Although *carI* (also called *expI*) is located adjacent to and overlaps a *luxR* gene (ExpR), mutation of *expR* exerts little effect on exoenzyme levels. Instead, a third *luxR* gene, *virR*, is required (Burr *et al.*, 2006). Unlike CarR, VirR functions as a repressor which negatively regulates exoenzyme production such that a *virR* mutation in an *Er. carotovora* AHL synthase mutant restores virulence to wild-type levels (Burr *et al.*, 2006).

The coupling of antibiotic and plant cell wall degrading enzymes via AHL-dependent QS implies that high cell population densities of *Erwinia*, having generated a substantial food resource through plant cell wall degradation, protect their investment from other bacteria by producing carbapenem antibiotic. Furthermore it has been suggested that the production of exoenzymes only at high cell population densities has contributed to the success of *Erwinia* as a plant pathogen since premature exoenzyme synthesis at low cell population densities would trigger a plant defence response and impede further development of infection (Salmond *et al.*, 1995). Such resistance to *Erwinia* infections can be achieved by inducing plant host defence responses with salicyclic acid (Palva *et al.*, 1994). Consequently, transgenic plants engineered to produce AHLs would be predicted to resist infection more readily by triggering *Erwinia* exoenzyme synthesis prematurely. Experimental exploration of this hypothesis required the availability of the AHL precursors, *S*-adenosylmethionine and the appropriately charged acyl-acyl carrier protein, and their accessibility to a recombinant AHL synthase expressed *in planta*. Such transgenic plants also offer opportunities for manipulating the behaviour of plant-beneficial as well as plant-pathogenic bacteria and for studying the impact of AHLs on the ecology of the rhizosphere.

To determine whether AHLs could be made *in planta*, Fray *et al.* (1999) introduced the *Yersinia enterocolitica* AHL synthase gene *yenI* into tobacco. In *Yersinia*, YenI directs the synthesis of a 1 : 1 mixture of 3-oxo-C6-HSL and C6-HSL (Throup *et al.*, 1995). Transgenic tobacco plants synthesizing the same two AHLs in a similar ratio were obtained, provided that the YenI protein was directed to the chloroplasts, presumably because the AHL precursors are most abundantly present in these organelles. Similarly, the *P. aeruginosa* AHL synthase gene *lasI* (responsible for the synthesis of 3-oxo-C12-HSL) has been introduced into tobacco plants alone and in combination with *yenI* (Scott *et al.*, 2006). Both transgenic plant lines produced

physiologically significant levels of 3-oxo-C12-HSL and the double transformant produced both the long- and short-chain AHLs. 3-Oxo-C6-HSL, C6-HSL and 3-oxo-C12-HSL were extracted from leaf, stem and root tissues and also detected on the surfaces of the leaves, indicating that the AHLs could freely diffuse across the plant cell plastid and plasma membranes (Fig. 5). AHLs were also detected in root exudates and in both rhizosphere and non-rhizosphere soil from transgenically grown plants, indicating that bioactive AHLs accumulate in the phytosphere and so should have an impact on communities of both plant-beneficial and plant-pathogenic bacteria (Scott *et al.*, 2006).

The AHLs produced by the transgenic tobacco plants were sufficient to induce target gene expression in several AHL biosensors and to restore biocontrol activity to a C6-HSL-deficient *Pseudomonas aureofaciens* strain. Virulence was also restored in an *Er. carotovora carI* mutant (Fray *et al.*, 1999). Since tobacco is not a natural host for *Erwinia* and high inocula are required for infection, YenI has also been introduced into potato (Toth *et al.*, 2004). In common with tobacco, the transgenic potatoes also produced both 3-oxo-C6-HSL and C6-HSL, with the stems appearing to contain higher AHL levels than the tubers. Contrary to the results anticipated, *Erwinia* strains proved to be more, rather than less, virulent on the transgenic AHL-producing potato plants, with both severity and onset of disease being greater at lower bacterial inocula than required for the control plants (Fig. 6; Toth *et al.*, 2004). Consequently, QS-dependent control of exoenzyme synthesis cannot simply be a means of avoiding plant defences but may rather be a mechanism for increasing nutrient availability at later stages of infection.

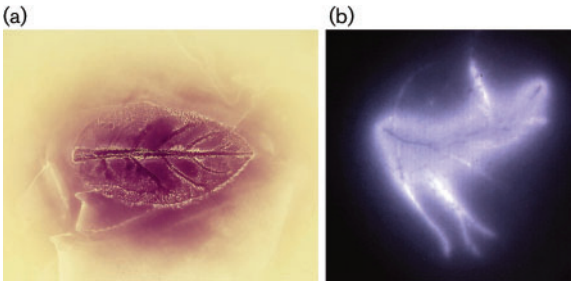

Fig. 5. Detection of AHLs on the leaves and in the roots of transgenic tobacco plants expressing the AHL synthase, YenI. (a) A transgenic tobacco leaf was placed on an agar plate overnight. The leaf was removed and the AHL biosensor, *C. violaceum* CV026, was spread over the plate. Production of the purple pigment violacein is observed where AHLs have diffused out of the leaf and into the agar. (b) The root of a transgenic tobacco plant grown in tissue culture under aseptic conditions was placed on an agar plate and overlaid with an *E. coli lux*-based AHL biosensor which emits light in response to the AHLs produced in the root. Adapted from Fray *et al.* (1999).

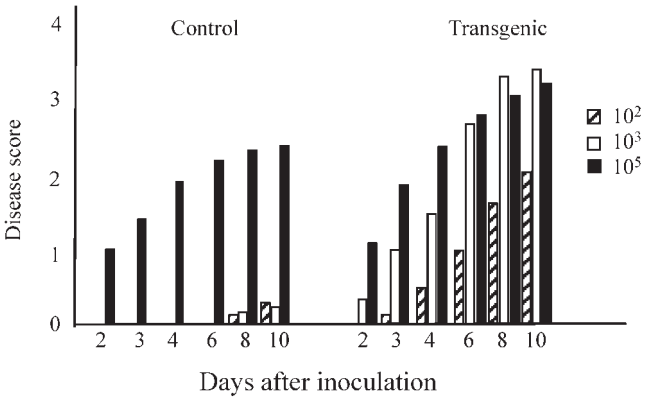

Fig. 6. Effect of *in planta* AHL production on the virulence of *Erwinia carotovora.* Potato stem disease score determined between 2 and 10 days after inoculation with 10^2, 10^3 or 10^5 c.f.u. per inoculation site on control or transgenic potatoes expressing *yenI* and producing the AHLs 3-oxo-C6-HSL and C6-HSL. A higher disease score is obtained on transgenic plants inoculated with lower numbers of bacteria compared with the control plants. Adapted from Toth *et al.* (2004).

QS and bacterial cross-talk

While the production, sensing and function of QS signal molecules within cultures of a single bacterial strain have been the subject of intensive investigations, far less attention has so far been given to QS within mixed bacterial consortia. With respect to the AHLs, species belonging to different genera which neverthless produce the same AHL molecule should be capable of cross-talk. For example, *P. aeruginosa*, *Serratia liquefaciens* and *Aeromonas hydrophila* all produce *N*-butanoylhomoserine lactone (C4-HSL) (Winson *et al.*, 1995; Eberl *et al.*, 1996; Swift *et al.*, 1997) while bacteria such as *C. violaceum* respond to other short-chain AHL-producing bacteria by producing the purple pigment violacein (McClean *et al.*, 1997). QS in bacteria such as *C. violaceum* and *A. hydrophila* is antagonized by long-chain AHLs, i.e. the latter inhibit violacein and exoenzyme production respectively (McClean *et al.*, 1997; Swift *et al.*, 1999). *P. aeruginosa* in contrast produces both long (3-oxo-C12-HSL) and short (e.g. C4-HSL) chain AHLs; however, 3-oxo-C12-HSL does not antagonize the C4-HSL-dependent activation of RhlR in *P. aeruginosa*, although RhlR is inhibited by 3-oxo-C12-HSL when it is expressed in *E. coli* (Winzer *et al.*, 2000), indicating that there must be some compartmentalization of the hierarchical *las* and *rhl* QS systems. In mixed *P. aeruginosa*/*Burkholderia cepacia* biofilm cultures *in vitro* and *in vivo* in a mouse chronic lung infection model, *B. cepacia* was shown to perceive the AHLs produced by *P. aeruginosa* but not vice versa (Riedel *et al.*, 2001). That this signalling was unidirectional was confirmed by quantifying the levels of exoproteases produced by each organism incubated with or without supernatant extracts prepared from the other, from either the wild-type or the isogenic

AHL-deficient mutant (Riedel *et al.*, 2001). Interestingly, a subset of *P. aeruginosa* virulence genes has also been reported to be upregulated by AI-2, which is not produced by *P. aeruginosa* (which lacks the AI-2 synthase, LuxS) but is made by the oropharyngeal flora and is present in sputum samples from cystic fibrosis patients infected with *P. aeruginosa* (Duan *et al.*, 2003).

Although no AHL-producing Gram-positive bacteria have so far been identified, Qazi *et al.* (2006) examined the growth response of *Staphylococcus aureus* to a range of AHLs differing in acyl chain length and the presence or absence of a 3-oxo substituent. From the data obtained, the most inhibitory compound was 3-oxo-C12-HSL, the AHL produced via LasI in *P. aeruginosa*. A reduction in acyl chain or removal of the 3-oxo moiety resulted in the reduction or complete loss of antibacterial activity. For example, C4-HSL which is produced via RhlI in *P. aeruginosa*, was inactive. When added to *S. aureus* cultures, 3-oxo-C12-HSL abolished the production of both α- and δ-haemolysins and cell wall fibronectin-binding proteins but enhanced protein A levels in a concentration-dependent manner (Qazi *et al.*, 2006). These data suggested that 3-oxo-C12-HSL may be inhibiting the *agr* QS system of *S. aureus*.

The *agr* system regulates the expression of diverse cell surface proteins and exotoxins in concert with cell population density via an AIP signal molecule. As *S. aureus* reaches stationary phase, *agr* represses genes coding for cell surface colonization proteins such as protein A and the fibronectin-binding proteins and activates expression of the genes for secreted exotoxins and tissue-degrading exoenzymes (Chan *et al.*, 2004). The *agr* locus consists of two divergent operons, controlled by the P2 and P3 promoters respectively (Chan *et al.*, 2004). The P2 operon consists of four genes, *agrBDCA*, which are all required for the activation of transcription from the P2 and P3 promoters while the P3 transcript, RNAIII, is itself the effector for the *agr* response (Chan *et al.*, 2004). AgrA and AgrC constitute a two-component system in which AgrC is the sensor kinase and AgrA is the response regulator. The system is activated through the interaction of an AIP with AgrC (Chan *et al.*, 2004). In several different experimental animal models of *S. aureus* infection, *agr* mutants exhibit significantly reduced virulence, highlighting the key role of this regulatory locus in staphylococcal pathogenicity (Chan *et al.*, 2004).

When the expression of the *agrP3* promoter and also *sarA* (which codes for a regulatory protein which, in common with *agr*, positively regulates the *agr* promoters) was examined in *S. aureus* exposed to 3-oxo-C12-HSL, the expression of both promoters was inhibited in a concentration-dependent manner. Using an *agrP3*::*blaZ* reporter gene fusion, an IC_{50} of 2 μM was calculated for 3-oxo-C12-HSL, which is within the concentration range produced by *P. aeruginosa* (Qazi *et al.*, 2006). Furthermore, 3-oxo-C12-HSL binds to *S. aureus* membranes with high affinity and may therefore exert its activity either by perturbing the processing of AgrD by AgrB to generate the AIP or by interfering with the capacity of AgrC to sense the AIP signal molecule (Qazi *et al.*, 2006). Although these hypotheses remain to be experimentally confirmed, it is also conceivable that 3-oxo-C12-HSL antagonizes the functions of other two-component signal transduction systems which modulate virulence factor production in *S. aureus* directly or indirectly via *agr* and *sarA*.

3-Oxo-AHLs such as 3-oxo-C12-HSL are readily inactivated as QS signal molecules by exposure to alkaline pHs, which generate the corresponding 3-oxo-fatty amine derivative [e.g. *N*-(3-oxo-dodecanoyl)homoserine in the case of 3-oxo-C12-HSL] (Yates *et al.*, 2002). While the ring-opened compound has no growth- or *agr*-inhibiting activity towards *S. aureus* (J. Cottam, S. R. Chhabra, S. Clarke & P. Williams, unpublished), alkali-mediated hydrolysis can also generate the tetramic acid derivative, 3-(1-hydroxydecylidene)-5-(2-hydroxyethyl)pyrrolidine-2,4-dione (Fig. 2), which has more potent growth-inhibitory properties than 3-oxo-C12-HSL (Kaufmann *et al.*, 2005).

QS and prokaryote–eukyarote interactions

Eukaryotes and prokaryotes have evolved diverse signalling mechanisms to detect and respond to each other. Although bacterial QS signal molecules have largely been considered as effectors of prokaryotic gene expression, they are bioactive molecules which can modify the behaviour of fungal, plant and animal cells (Telford *et al.*, 1998; Gardiner *et al.*, 2001; Bauer & Mathesius, 2004; Dudler & Eberl, 2006; Schuhegger *et al.*, 2006). At high concentrations (200 μM), 3-oxo-C12-HSL suppresses filamentation in *Candida albicans* (Hogan *et al.*, 2004) and in common with AQs such as PQS (2-alkyl-3-hydroxy-4-quinolone) it exhibits potent immunomodulatory activity and elicits both pro- and anti-inflammatory responses depending on the concentration and experimental model used (Telford *et al.*, 1998; Smith *et al.*, 2002; Chhabra *et al.*, 2003; Hooi *et al.*, 2004; Pritchard *et al.*, 2005). 3-Oxo-C12-HSL inhibits both mitogen-induced leukocyte proliferation and lipopolysaccharide-mediated secretion of tumour necrosis factor α (Telford *et al.*, 1998). Structure–activity studies have revealed that AHLs with an 11–13 carbon side chain and a 3-oxo- or 3-hydroxy- substituent are the optimal structures for immunosuppressive activity. Analogues lacking the homoserine lactone ring or an L-configuration at the chiral centre, or those with polar substituents, were devoid of activity (Chhabra *et al.*, 2003). These data indicated that 3-oxo-C12-HSL may have therapeutic utility in autoimmune diseases such as type 1 diabetes. In the non-obese diabetic (NOD) mouse model of type 1 insulin-dependent diabetes mellitus, destruction of the pancreatic insulin-producing β-cells and the onset of diabetes could be prevented by 3-oxo-C12-HSL (Fig. 7; Pritchard *et al.*, 2005). The mechanism of action in this context is not understood but may involve 3-oxo-C12-HSL

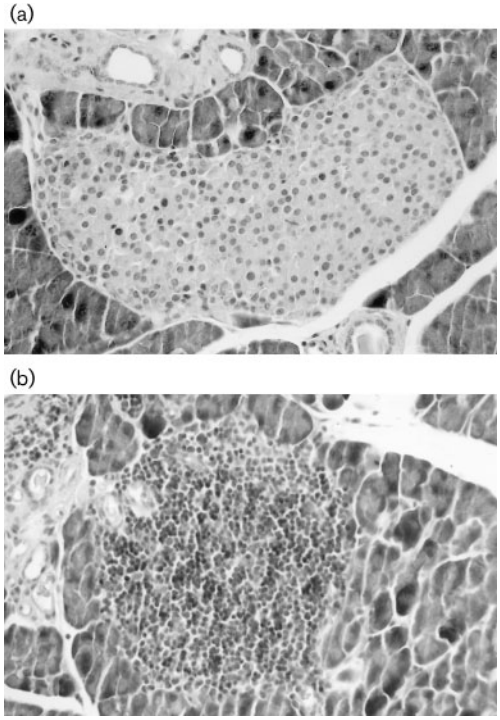

Fig. 7. Alleviation of insulitis in NOD mice by 3-oxo-C12-HSL. The morphology of the pancreatic islets in mice treated with 3-oxo-C12-HSL (a) is retained compared with the characteristic insulitis shown in (b), where the islets have been infiltrated by mononuclear cells (adapted from Pritchard *et al.*, 2005).

directing the immune response away from a T-helper-cell 1 (Th1)- to a Th2-based response (Pritchard *et al.*, 2005).

The molecular signalling pathways and mammalian receptors through which 3-oxo-C12-HSL exerts its immunomodulatory properties are not fully understood but multiple systems are likely to be involved (including mitogen-activated kinase, MAPK) depending on the cell type or immune status of the animal model employed (Pritchard, 2006; Kravchenko *et al.*, 2006; Shiner *et al.*, 2006). 3-Oxo-C12-HSL signalling in immune cells does not appear to depend on the pathogen-associated molecular recognition receptor pathways (e.g. Toll and Nod receptors) (Kravchenko *et al.*, 2006). At high concentrations, 3-oxo-C12-HSL induces apoptosis in various cell types via a mechanism that can be blocked by inhibitors of calcium signalling (Shiner *et al.*, 2006). Since these do not inhibit immunomodulation, it is likely that multiple 3-oxo-C12-HSL receptors exist. 3-Oxo-C12-HSL also influences smooth muscle contraction in blood vessels (Lawrence *et al.*, 1999) and produces a marked bradycardia in live conscious rats (Gardiner *et al.*, 2001). It is also capable of disrupting epithelial barrier integrity, a process that can partially be prevented by inhibiting p38 and p42/44 MAPK kinases (Vikstrom *et al.*, 2006). Consequently, 3-oxo-C12-HSL can be considered as a virulence factor in its own right, both functioning as a QS signal molecule controlling expression of key virulence genes and conferring on *P. aeruginosa* a competitive survival advantage in the presence of other prokaryotes and eukaryotes.

As well as promoting pathogen success, certain QS signal molecules exert beneficial effects on the host. The mechanism(s) by which probiotic bacteria such as certain bacilli and lactobacilli exert their protective effects in the gastro-intestinal tract is not well understood but is likely to involve pathogen control or exclusion as well as protection of host tissues against inflammatory responses. Interestingly, the mechanism by which a probiotic *Bacillus subtilis* strain exerts a protective effect involves a pentapeptide QS signal molecule, the competence and sporulating factor, CSF (also called PhrC). In *B. subtilis*, CSF, ComX and other Phr peptides regulate multiple processes including the initiation of genetic competence, sporulation, antibiotic and exopolysaccharide synthesis as well as the production of degradative enzymes (Lazazzera, 2001; Auchtung *et al.*, 2006). In the human colonic epithelial cell line Caco2 and in ligated mouse intestinal loops, CSF induces the expression of heat-shock-inducible protein 27 (Hsp27) (Fujiya *et al.*, 2007). Hsps confer protection against a wide variety of stresses and, when overexpressed, can protect intestinal epithelial cells from oxidative injury and so contribute to the maintenance of intestinal homeostasis (Fujiya *et al.*, 2007). This activity of CSF is within the concentration range required for QS in *B. subtilis* (10–100 nM), an important consideration given that some of observed activities of QS molecules on mammalian cells are only apparent at high, non-physiological concentrations (Pritchard, 2006). In *B. subtilis*, CSF and the other Phr peptides act intracellularly following internalization via an oligopeptide permease (Opp) by inhibiting the activity of the intracellular Rap receptor proteins (Lazazzera, 2001; Auchtung *et al.*, 2006). Fujiya *et al.* (2007) also identified a mammalian apical membrane oligopeptide transporter (OCTN2) which is required for CSF uptake. CSF and OCTN2-mediated CSF transport are both required to protect Caco2 cells against oxidant-mediated injury and loss of epithelial barrier function.

Eukaryote-mediated quorum activation and quenching

A number of studies have reported that mammalian cells, plants and algae secrete molecules which either activate or inhibit QS (Bauer & Mathesius, 2004; Dudler & Eberl, 2006). Plants and animals as well as other bacteria also express enzymes capable of chemically modifying and so inactivating QS signal molecules. For example, the AHL- and AQ-dependent QS systems of *P. aeruginosa* are induced by γ-interferon and opioids respectively (Wu *et al.*, 2005; Zaborina *et al.*, 2007) while the plant defence signal molecule salicylic acid upregulates the expression of an *Agrobacterium tumefaciens* AHL-degrading enzyme (Yuan *et al.*, 2007). Red algae produce brominated

furanones which inhibit AHL-dependent QS while plant root exudates contain compounds which stimulate both AHL and AI-2 biosensors (Bauer & Mathesius, 2004; Dudler & Eberl, 2006).

Staphylococcus aureus produces four AIP QS signal molecules which differ in primary amino acid sequence, although AIP-1 and AIP-4 differ by only one amino acid residue (McDowell *et al.*, 2001; Chan *et al.*, 2004). Both of these AIPs incorporate a C-terminal methionine which is readily oxidized during growth to form the corresponding methionine sulphoxide and inactivated (McDowell *et al.*, 2001). In a mouse skin infection model, the phagocyte NADPH oxidase, myeloperoxidase, and the inducible nitric oxide synthase both play critical roles in protecting against AIP-1-producing *S. aureus* but not against the isogenic *agr*-negative mutant (Rothfork *et al.*, 2004). Inactivation of AIP-1 *in vitro* and *in vivo* by reactive oxygen and nitrogen intermediates was shown to occur, prompting the authors to conclude that oxidant-mediated inactivation of staphylococcal AIPs can be considered as an important component of the innate immune defences against *S. aureus* infections (Rothfork *et al.*, 2004). Presumably the evolution of the hypervariable region of the *agr* locus (Dufour *et al.*, 2002), which includes *agrD* (encoding the AIP precursor protein), is in part driven by the need to evade oxidant-mediated inactivation.

An alternative biochemical strategy used for inactivating QS signal molecules such as the AHLs is enzyme-mediated inactivation (Dong *et al.*, 2007). Many bacteria possess lactonases and acylases which hydrolyse the lactone ring and cleave the amide bond respectively. Some bacteria, notably *Rhodococcus*, reduce the 3-oxo moiety of 3-oxo-AHLs to form 3-hydroxy-AHLs and so decrease the efficacy of the signal molecule for its receptor (Uroz *et al.*, 2005). The true physiological function of most of these enzymes is not known but they are found in both Gram-negative AHL producers and non-AHL producers as well as in Gram-positive bacteria. In mammalian cells and tissues, AHL inactivation has been associated with the paraoxonase (PON) enzymes which are present in serum and airway epithelia (Dong *et al.*, 2007). The PON enzymes exhibit a wide range of hydrolytic activities; PON1 for example possesses lactonase, arylesterase and organophosphatase activities. In airway epithelia, PON2 appears to be the most efficient degrader of 3-oxo-C12-HSL and consequently is considered likely to contribute to host defences against bacteria such as *P. aeruginosa* (Stoltz *et al.*, 2007). Germinating seedlings of the leguminous plant *Lotus corniculatus* have also been shown to be capable of enzymically inactivating a wide range of AHLs, although the mechanism involved has yet to be established (Delalande *et al.*, 2005).

AHL sensing by zoospores of the green alga *Ulva*

The green macroalga *Ulva intestinalis* is an inter-tidal biofouling seaweed which releases large numbers of motile zoospores into the water column which seek out and attach to a surface before differentiating into a new plant (Fig. 8). The selection of a surface by a zoospore involves the sensing of the surface followed by temporary adhesion and detachment if the surface does not offer a sufficiently favourable environment (Joint *et al.*, 2007). The presence of a bacterial biofilm is one important factor which influences zoospore attachment, and bacteria which either stimulate or inhibit zoospore settlement have been isolated from marine surfaces colonized by *Ulva* (Patel *et al.*, 2003). With respect to bacteria which encourage zoospore settlement, a positive correlation has been reported to exist between zoospore settlement and bacterial biofilm density (Joint *et al.*, 2000). This work raised the question of whether the *Ulva* zoospores were responding to the topological features and physico-chemical properties of the biofilm or to any diffusible signals released by the biofilm bacteria, or both. To explore the possibility that the zoospores were sensing QS signal molecules, Joint *et al.* (2002) used *Vibrio anguillarum* as a model marine

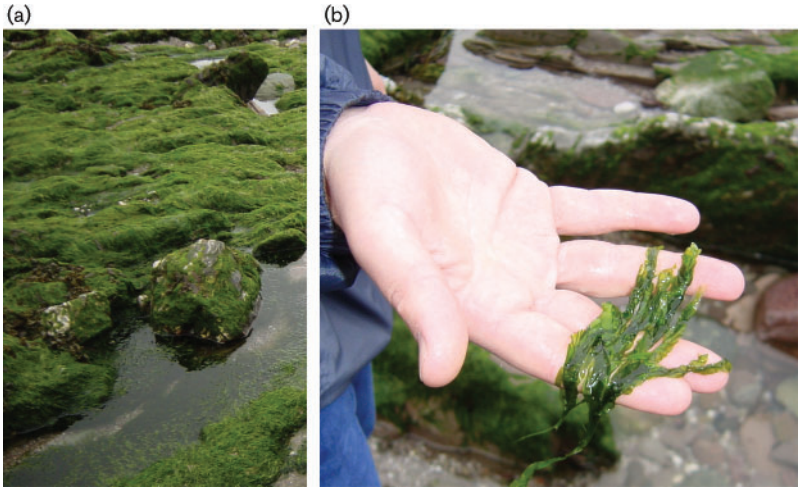

Fig. 8. *Ulva* is a common green alga characteristically found on inter-tidal rocky shores (a). An individual *Ulva* plant is shown in (b).

bacterium. *V. anguillarum* possesses a sophisticated QS regulatory system (Milton, 2006) employing both a LuxRI-type system (VanRI) and a LuxMN-type system (VanMN) and produces mainly *N*-(3-oxodecanoyl)-L-homoserine lactone (3-oxo-C10-HSL) via VanI and *N*-(3-hydroxyhexanoyl)-L-homoserine lactone (3-hydroxy-C6-HSL) and C6-HSL via VanM (Milton *et al.*, 1997, 2001). The VanRI and VanMN systems form a regulatory hierarchy where the expression of *vanR* and *vanI* are dependent on a functional *vanM*, and consequently both v*anIM* and *vanM* mutants do not produce AHLs while a *vanI* mutant continues to produce 3-hydroxy-C6-HSL and C6-HSL (Milton *et al.*, 2001).

Ulva zoospores were attracted to, and settled on, both *V. anguillarum* wild-type and *vanI* mutant biofilms and there was a positive correlation between the numbers of zoospores settling and bacterial cell density (Joint *et al.*, 2002). However, the zoospores were not attracted to biofilms of either the *V. anguillarum vanM* or *vanIM* mutants or to the wild-type strain rendered AHL-negative through the *in situ* expression of an AHL-inactivating lactonase (Joint *et al.*, 2002; Tait *et al.*, 2005). Further confirmatory evidence for zoospore AHL sensing was obtained by expressing *vanI* and *vanM* respectively in *E. coli* and by evaluating the attraction of zoospores to agarose films containing synthetic AHLs which diffuse out and generate an AHL gradient (Joint *et al.*, 2002; Tait *et al.*, 2005). While the zoospores responded to a wide range of AHLs with differing acyl chain lengths and C-3 position substitutents, they failed to settle on films containing the corresponding ring-open *N*-acylhomoserine compounds. Although these experiments suggest that zoospores preferentially respond to long-chain AHLs with 3-oxo or 3-hydroxy substituents, the differential solubility, diffusibility and degradation rates of the different AHLs in the experimental system used make it difficult to draw any firm conclusions about AHL structural preference (Tait *et al.*, 2005). However, zoospore settlement onto AHL-producing biofilms can be reduced by adding synthetic AHLs to destroy the AHL gradient. In such experiments, the zoospores were attracted to all of the AHLs tested except the shortest acyl chain compound tested (C4-HSL) (Joint *et al.*, 2002; Tait *et al.*, 2005).

Image analysis using GFP-tagged *V. anguillarum* biofilms revealed that zoospores settle directly onto the bacterial cells and in particular on microcolonies, which are sites of concentrated AHL biosynthesis (Fig. 9; Tait *et al.*, 2005). Furthermore, surface topography does not appear to play a major role in zoospore settlement on *V. anguillarum* biofilms since treatment of the biofilm with chloramphenicol or by exposure to UV light to kill the biofilm bacteria significantly reduced settlement without changing the physical integrity of the biofilm (Tait *et al.*, 2005).

Although the experiments described above used *V. anguillarum* as a model marine bacterium, AHL-producing and -degrading bacteria have recently been isolated from marine rocks colonized by *Ulva*. These include species belonging to the genera *Shewanella*, *Sulfitobacter* and *Pseudoaltermonas*, organisms not previously known to make or degrade AHLs (K. Tait, H. Williamson, P. Williams, M. Camara & I. Joint, unpublished). Interestingly, zoospore attachment experiments using these bacteria have revealed different patterns of settlement as a function of bacterial biofilm density and it is clear that the timing of both AHL production and degradation in both single- and mixed-species biofilms markedly affects zoospore attraction and hence exerts wider ecological effects across different kingdoms. (K. Tait, H. Williamson, P. Williams, M. Camara & I. Joint, unpublished).

The mechanism of zoospore attraction does not seem to involve chemotactic orientation towards AHLs but instead a chemokinesis in which spore swimming speed rapidly decreases in the presence of AHLs such that the zoospores

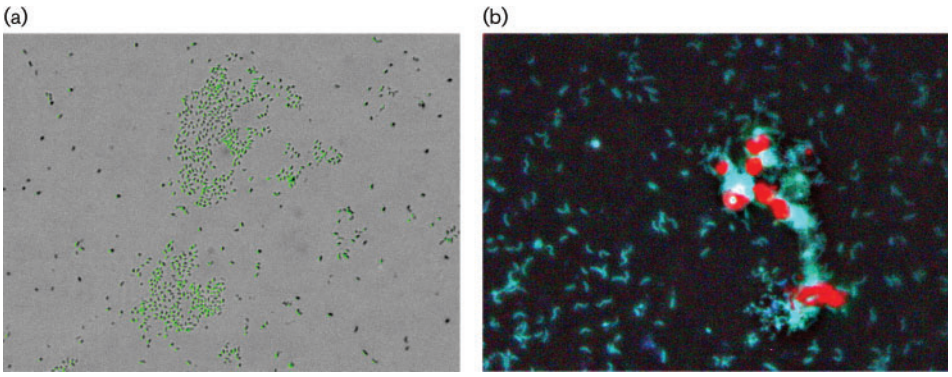

Fig. 9. Attraction of *Ulva* zoospores to AHL-producing *Vibrio anguillarum* biofilm microcolonies. (a) AHL production in biofilm microcolonies of *V. anguillarum* carrying a *gfp*-based AHL biosensor. (b) DAPI-stained biofilm showing zoospores settled on a *V. anguillarum* microcolony. Images are transmission images overlaid with the fluorescent colour image. Reproduced from Tait *et al.* (2005) with permission from Blackwell Publishing.

accumulate at the AHL source (Wheeler *et al.*, 2006). Zoospore swimming speed for example decreases more rapidly over wild-type *V. anguillarum* biofilms when compared with those of a *vanM* mutant (Wheeler *et al.*, 2006). AHL detection by zoospores causes an influx of calcium and the reduction in swimming speed may arise via calcium-dependent modulation of the flagellar beat pattern (Wheeler *et al.*, 2006). As indicated above, *Ulva* zoospores sense a wide range of AHLs (Tait *et al.*, 2005), although the chemoresponse was found to be most marked towards 3-oxo-C12-HSL (Wheeler *et al.*, 2006), an interesting finding given the broad biological activity of this AHL molecule and its capacity to modulate mammalian host cell responses, in part through calcium signalling (Shiner *et al.*, 2006).

The reason(s) why *Ulva* zoospores target bacterial biofilms as a preferred site for attachment remain(s) unclear but bacterial biofilms are clearly an important factor in biofouling. Many studies have shown that microbial biofilms influence the settlement of marine invertebrates (Joint *et al.*, 2007). Grazing organisms are assumed to exploit bacterial biofilms as food sources but this is unlikely to be a factor in algal attraction. Consequently, the presence of a bacterial biofilm may signal that the environment is benign for *Ulva* zoospores. However, given that the interaction is very specific, with zoospores settling directly on bacterial cells in the biofilm, rather than merely attaching in the vicinity, there are likely to be other reasons why zoospores prefer attaching to bacterial biofilms. For example, many green algae do not develop normal morphology when grown under axenic conditions (Provasoli & Pintner, 1980). Recently, Matsuo *et al.* (2003) have shown that differentiation in the green alga *Monostroma oxyspermum* depends on the presence of specific bacterial strains; i.e. normal morphology depends on particular bacteria and not on bacteria in general. Consequently the preference of *Ulva* zoospores for settlement on AHL-producing bacterial biofilms may facilitate a close association between the developing thallus and certain essential bacteria (Tait *et al.*, 2005).

Concluding remarks

Since the early 1990s there has been a growing appreciation of the extent, molecular mechanisms and implications of QS for biotechnology, medicine, ecology and agriculture. Given the vast number of extracellular bacterial metabolites, the chemical diversity among known QS signal molecules is also likely to represent only the 'tip of the iceberg'. Indeed it has been suggested that most low-molecular-mass organic compounds made and secreted by microbes, including many antibiotics, are likely to function as QS signalling molecules (Yim *et al.*, 2007). Much of the work to date has focused on the function of specific QS signals in a single organism, although there is growing interest in the impact and exploitation of QS in complex microbial communities. In this respect, it is interesting that the addition of AHLs to a phenol-degrading activated sludge community used in wastewater treatment results in significant shifts in community composition and phenol degradation rates (Valle *et al.*, 2004). Eukaryotes clearly respond to bacterial QS signal molecules and in some instances this work has pointed towards their potential future therapeutic exploitation (Gardiner *et al.*, 2001; Pritchard *et al.*, 2005). In pathogenic bacteria, QS represents an exciting target for novel antibacterials: the inhibition of QS in bacteria such as *P. aeruginosa* attenuates virulence and renders biofilms more susceptible to antimicrobial agents and host defences (Williams, 2002; Bjarnsholt & Givskov, 2007). The development of potent, safe QS inhibitors offers considerable scope in the battle against multi-antibiotic-resistant bacteria and chronic biofilm-centred infections.

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