REVIEW

Quorum sensing and social networking in the microbial world

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For many years, bacterial cells were considered primarily as selfish individuals, but, in recent years, it has become evident that, far from operating in isolation, they coordinate collective behaviour in response to environmental challenges using sophisticated intercellular communication networks. Cell-to-cell communication between bacteria is mediated by small diffusible signal molecules that trigger changes in gene expression in response to fluctuations in population density. This process, generally referred to as quorum sensing (QS), controls diverse phenotypes in numerous Gram-positive and Gram-negative bacteria. Recent advances have revealed that bacteria are not limited to communication within their own species but are capable of 'listening in' and 'broadcasting to' unrelated species to intercept messages and coerce cohabitants into behavioural modifications, either for the good of the population or for the benefit of one species over another. It is also evident that QS is not limited to the bacterial kingdom. The study of two-way intercellular signalling networks between bacteria and both uni- and multicellular eukaryotes as well as between eukaryotes is just beginning to unveil a rich diversity of communication pathways.

Keywords: quorum sensing; cell−cell communication; interspecies communication; inter-kingdom communication

1. INTRODUCTION

Over the last two decades, the notion that the individual cells in a bacterial population operate as autonomous units has been superseded with the realization that social interactions are common throughout the prokaryotic world. These confer on a bacterial population the ability to instigate a collective behavioural change to environmental challenges. Pivotal to such population-dependent adaptive behaviour is a change in gene expression in response to the perception and processing of chemical information in the form of diffusible signal molecules often referred to as autoinducers. This process is known as 'quorum sensing' (QS), and describes the phenomenon whereby an increase in bacterial cell population density is concomitant with an increase in the concentration of signal molecule(s) in the extracellular environment. Once the QS signal molecule concentration has reached a threshold level (at which point the population is considered to be 'quorate'), activation of a signal transduction cascade will direct the expression or repression of target genes to bring about a collective behavioural adaptation (Cámara *et al.* 2002; Williams 2007*b*; Williams *et al.* 2007). In some instances, the genes responsible for QS signal production are positively regulated, facilitating

a positive autoinductive loop driving autoinducer synthesis. The terms quorum diffusion sensing, compartment sensing and efficiency sensing have all been coined in an attempt to refine how information concerning the physiological state and spatial distribution of cells, their numbers and the specific environmental conditions being encountered will be conveyed (Redfield 2002; Winzer *et al.* 2002*b*; Hense *et al.* 2007). The term quorum diffusion sensing has recently been used to describe a threshold concentration of the QS signal eliciting a response that is achieved by a population rather than by a single cell. The number of individuals in a quorate population is therefore not fixed but will depend on the relative rates of synthesis and turnover/loss of QS signal molecule (Winzer *et al.* 2002*b*; Atkinson *et al.* 2007).

A range of chemically distinct compounds are employed as QS signal molecules, and in many bacterial species more than one compound class, along with their cognate synthase gene(s) and the associated signal transduction apparatus, form an interdependent network of regulatory units that govern how a bacterial population will respond to changes in the environment by regulating a diverse set of genes. Outside the laboratory, we must consider that in almost all environments a microbial community will contain multiple bacterial species and will normally be found in close association

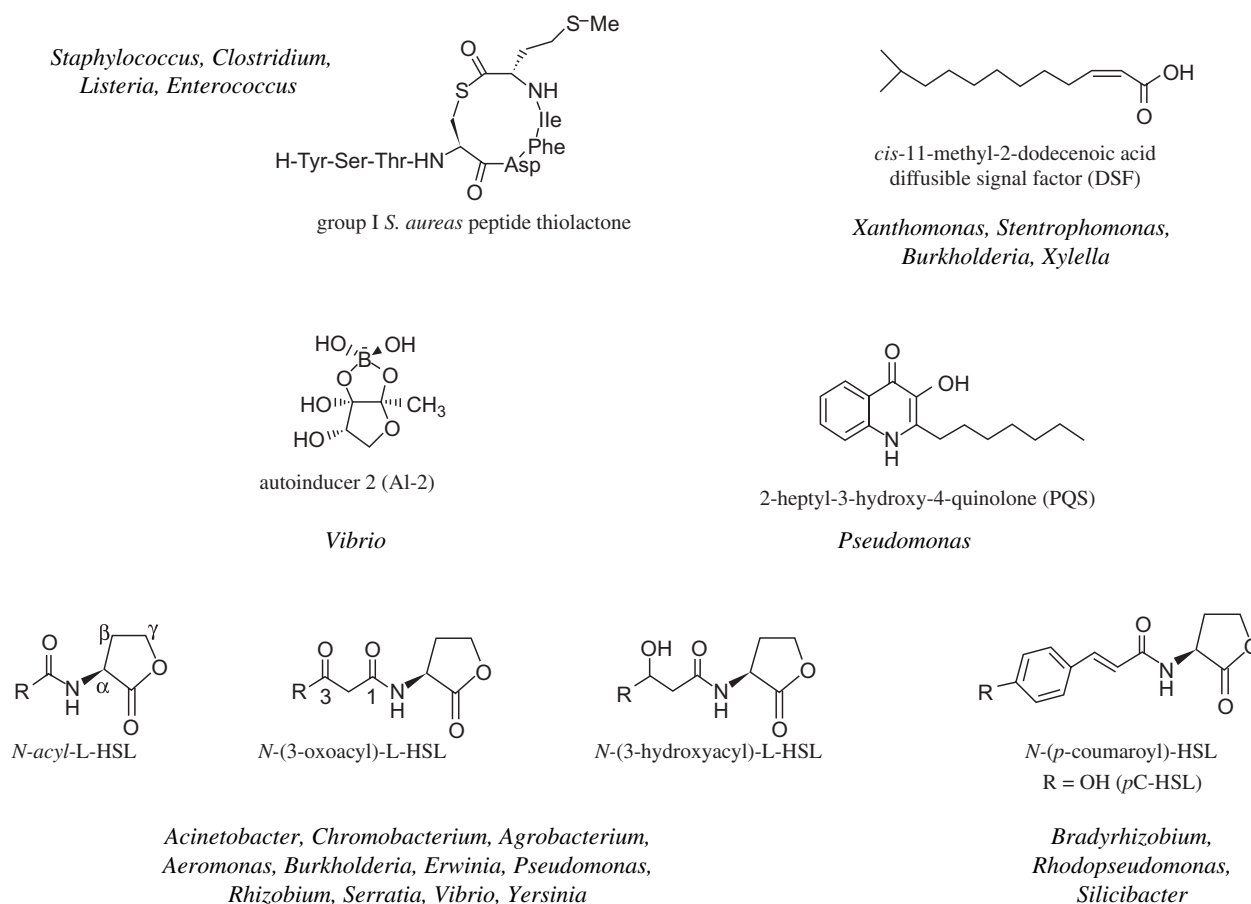*Author for correspondence (steve.atkinson@nottingham.ac.uk).

Staphylococcus, Clostridium,
Listeria, Enterococcus

group I *S. aureas* peptide thiolactone

cis-11-methyl-2-dodecenoic acid
diffusible signal factor (DSF)

Xanthomonas, Stentrophomonas,
Burkholderia, Xylella

autoinducer 2 (AI-2)

Vibrio

2-heptyl-3-hydroxy-4-quinolone (PQS)

Pseudomonas

N-acyl-L-HSL

N-(3-oxoacyl)-L-HSL

N-(3-hydroxyacyl)-L-HSL

N-(*p*-coumaroyl)-HSL
R = OH (*p*C-HSL)

Acinetobacter, Chromobacterium, Agrobacterium,
Aeromonas, Burkholderia, Erwinia, Pseudomonas,
Rhizobium, Serratia, Vibrio, Yersinia

Bradyrhizobium,
Rhodopseudomonas,
Silicibacter

Figure 1. The structures of some common QS signal molecules.

with eukaryotes. Cross-kingdom sensing of QS signal molecules may therefore constitute an adaptive survival strategy, enabling bacteria and eukaryotes to monitor their surroundings and adjust their behaviour in response to environmental challenge and population flux. This article will provide an overview of some of the key QS systems, followed by a consideration of the opportunities for crosstalk and eavesdropping between different bacterial species and between bacteria and eukaryotes.

2. PROKARYOTIC QUORUM-SENSING SYSTEMS

Several chemically distinct classes of QS signal molecules have been identified (figure 1), and in Gram-negative bacteria include the *N*-acylhomoserine lactones (AHLs), 2-alkyl-4-quinolones, γ-butyrolactones, furanones, long-chain fatty acid derivatives, fatty acid methyl esters, peptides, the 4,5-dihydroxy-2,3-pentandione (DPD) derivatives collectively referred to as autoinducer 2 (AI-2) and autoinducer 3 (AI-3, structure unknown) (Winzer *et al.* 2002*b*; Sperandio *et al.* 2003; Winzer & Williams 2003; Vendeville *et al.* 2005; Williams 2007*b*). The (AI-2)/LuxS QS system appears to be shared by both Gram-negative and Gram-positive bacteria (Winzer *et al.* 2002*a*; Winzer & Williams 2003; Vendeville *et al.* 2005) while autoinducing linear, modified or cyclic peptides and γ-butyrolactones

(e.g. A-factor) are found in Gram-positive bacteria such as the streptococci, staphylococci and streptomycetes, respectively. Yim *et al.* (2006) suggested that any number of extracellular bacterial metabolites including compounds with antibiotic activity have the potential to function as signal molecules. However, it is important to differentiate between a true signal molecule involved in cell-to-cell communication and other metabolites. The features considered to define a molecule as a QS signal include: (i) accumulation in the extracellular milieu during a specific growth stage or under certain physiological conditions, or in response to specific environmental changes, (ii) recognition by a specific cell surface or cytoplasmic bacterial receptor, and (iii) the ability to induce a cellular response that extends beyond the physiological changes required to metabolize or detoxify the molecule (Winzer *et al.* 2002*a*).

2.1. *Acylhomoserine lactone-mediated quorum-sensing systems*

AHL biosynthesis is primarily dependent on members of the LuxI family of AHL synthases (over 100 of which currently appear in the bacterial genome databases) where they are responsible for the catalysis of an amide bond between the appropriately charged acyl–acyl carrier protein (acyl-CoA derivatives can also be substrates), the source of the fatty acyl side chain, and *S*-adenosyl-methionine, the source of the homoserine lactone ring (More *et al.* 1996;

Jiang *et al.* 1998; Parsek & Greenberg 1999). Recently, Schaefer *et al.* (2008) identified an aroyl-CoA (*p*-coumaroyl-CoA) that served as an alternative source of the fatty acyl side chain and results in the formation of a novel AHL derivative, *N*-(*p*-coumaroyl)-homoserine lactone (*p*C-HSL) (figure 1) in the photosynthetic bacterium *Rhodopseudomonas palustris*. It is interesting to note that a LuxI homologue RpaI is responsible for catalysing this reaction rather than a novel enzyme specifically tailored to *p*C-HSL production. *N*-aroyl homoserine lactones may therefore be commonplace in situations where an aroyl-CoA is available as a precursor substrate to a LuxI homologue.

The response to an AHL usually takes place in the cytoplasm via the interaction between the QS signal and a transcription factor, which is usually a member of the LuxR family of transcriptional regulators (Fuqua *et al.* 1996; Swift *et al.* 2001; Cámara *et al.* 2002), although AHL signalling is sometimes transduced by a transmembrane histidine kinase sensor protein (Milton 2006). AHL–LuxR homologue complexes trigger target gene expression in response to the population size, its degree of compartmentalization and the QS signal concentration in the local environment (for reviews, see Lazdunski *et al.* 2004; Waters & Bassler 2005; Williams 2007*b*; Williams *et al.* 2007).

Two other classes of AHL synthase have been characterized. One of these, an enzyme found exclusively in *Vibrio* spp., is distinct in amino acid composition from LuxI homologues but is analogous in function and belongs to the LuxM protein family (Milton *et al.* 2001). These enzymes use the same substrates as, and coexist alongside, their LuxI counterparts in both *Vibrio fischeri* and *Vibrio anguillarum* (Hanzelka *et al.* 1999; Milton *et al.* 2001). HdtS represents a further putative class of AHL synthase that is distinct from LuxI and LuxM homologues. HdtS was originally described in *Pseudomonas fluorescens* (Laue *et al.* 2000) and more recently the homologue, Act, has been identified in *Acidithiobacillus ferrooxidans* (Rivas *et al.* 2007). When expressed in *Escherichia coli* HdtS directs the synthesis of [*N*-(3-hydroxy-7-*cis*-tetradecanoyl)homoserine lactone (3-hydroxy-C14:1HSL, which incorporates a double bond in the acyl side chain at position 7), *N*-decanoylhomoserine lactone and *N*-hexanoylhomoserine lactone (C6-HSL), the same AHLs as those produced in the *P. fluorescens* parent. Act also produces (3-hydroxy-C14:1HSL) in *E. coli* along with several shorter chain AHLs, which are apparently synthesized in small amounts (Rivas *et al.* 2007).

Often *luxI* and *luxR* orthologues are found at the same genetic locus, although a number of *luxR* 'orphans' have now been described (Fuqua 2006; Lee *et al.* 2006; Lequette *et al.* 2006; Duerkop *et al.* 2007; Ferluga *et al.* 2007). Many of these orphans are functionally similar to the *luxR* orthologues that are part of a *luxR/I* pair but may be different in size because of deletions or additions to their sequence. Alternatively, key conserved amino acid residues, which are known to be critical for successful LuxR function, may have been lost in these orphans altering or ablating their activity (Fuqua & Greenberg 2002; Fuqua 2006). There is the possibility that in some cases a LuxR

orphan might form a heterodimer with another LuxR protein, the gene for which is genetically linked to a *luxI* orthologue. For example, dimeric TraR in *Agrobacterium tumefaciens* acts as a transcriptional activator in the presence of *N*-(3-oxooctanoyl)homoserine lactone (3-oxo-C8-HSL) (Fuqua *et al.* 1996; Zhu & Winans 1999), but its activity can be repressed when it forms heterodimers with either TrlR or TraM (Luo *et al.* 2000; Chai *et al.* 2001).

AHL-mediated QS systems have been extensively characterized in bacteria such as *Vibrio* (Milton 2006), *Pseudomonas* (Williams & Cámara 2009), *Rhizobium* (Sanchez-Contreras *et al.* 2007), *Erwinia* (Barnard *et al.* 2007) *Agrobacterium* (White & Winans 2007) and *Yersinia* (Atkinson *et al.* 2006*b*). Here we will consider the last.

2.1.1. Acylhomoserine lactone-mediated quorum sensing in Yersinia. The genus *Yersinia* comprises four species that are pathogenic to animals. *Yersinia pseudotuberculosis* and *Yersinia enterocolitica* are enteropathogens responsible for a self-limiting gastroenteritis presenting as fever, diarrhoea and abdominal pain (Cover & Aber 1989; Johnson 1992; Butler 1994). *Yersinia pestis*, which presents as pneumonic, bubonic or septicaemic plague, has a biphasic lifestyle alternating between flea and mammalian hosts. *Yersinia pestis* is a highly uniform clone of *Y. pseudotuberculosis* that arose shortly before the first recorded plague pandemic, which occurred between the sixth and the eighth century AD (Bercovier *et al.* 1980; Achtman *et al.* 1999). *Yersinia ruckeri* is the only member of the yersiniae that is not pathogenic to mammals but is a major cause of morbidity and mortality in salmonid fish (Ross *et al.* 1966).

All four *Yersinia* species produce AHLs and both *luxI* and *luxR* orthologues have been identified (Throup *et al.* 1995; Atkinson *et al.* 1999; Swift *et al.* 1999; Temprano *et al.* 2001). *Yersinia pseudotuberculosis* possesses two LuxRI pairs termed YpsRI and YtbRI in which the corresponding I and R genes are convergently organized (figure 2). YpsI and YtbI are responsible for the production of at least 24 different AHLs, at least seven of which have been detected at levels considered to be capable of triggering a phenotypic change (Ortori *et al.* 2007). Both YpsI and YtbI are capable of synthesizing C6-HSL, *N*-(3-oxohexanoyl) homoserine lactone (3-oxo-C6-HSL) and *N*-3-(oxo-heptanoyl)homoserine lactone while YtbI is responsible for the synthesis of *N*-3-(hydroxy-octanonoyl)homoserine lactone, 3-oxo-C8-HSL, *N*-octanoylhomoserine lactone and *N*-3-(oxo-decanoyl)homoserine lactone (Ortori *et al.* 2007).

In *Y. pseudotuberculosis*, AHL-dependent QS controls cell-to-cell contact (aggregation) and flagellar-mediated motility (Atkinson *et al.* 1999). On soft-agar motility plates, *ypsR* and *ypsI* mutants, but not the parent strain, are motile within 24 h while, in liquid culture, motility is prematurely induced in the mutants but extensively delayed in the parent (Atkinson *et al.* 1999). The YpsRI and YtbRI systems in *Y. pseudotuberculosis* work in tandem such that YpsRI (Atkinson *et al.* 1999) negatively regulates whereas the YtbR/I system

positive and negative autoregulation
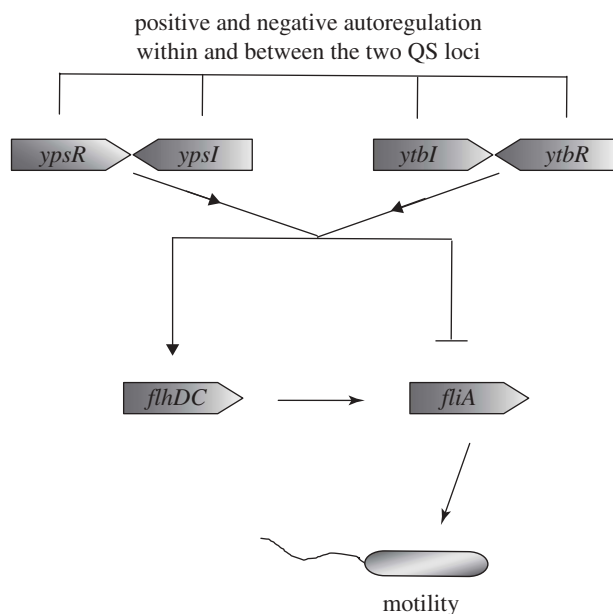within and between the two QS loci

Figure 2. The autoregulated QS circuits of *Y. pseudotuberculosis*, capable of producing up to eight AHLs at physiological concentrations, control flagella-mediated motility by regulating the expression of the motility master regulator *flhDC* and the alternative sigma factor *fliA* (adapted from Atkinson *et al.* 2008).

positively regulates swimming motility (Atkinson *et al.* 1999, 2008) (figure 2). In addition, the YpsRI system negatively autoregulates itself but positively regulates the expression of the *ytbRI* system whereas the *ytbRI* system is positively autoregulated.

It is perhaps not too surprising to find a similar range of AHLs and two pairs of *luxRI* homologues (*ypeRI* and *yepRI*) in *Y. pestis* given its close genetic relationship to *Y. pseudotuberculosis* (Swift *et al.* 1999; Isherwood 2001). However, *Y. pestis* is non-motile, and while a *Y. pestis ypeR* mutant exhibited reduced virulence in a mouse infection model (Swift *et al.* 1999), QS does not appear to be required for the transmission of plague by fleas because a triple mutant lacking *ypeI*, *yepI* and *luxS* (§2.5) retained the ability to form a biofilm in the flea foregut, a phenotype considered essential for transmissible infection (Jarrett *et al.* 2004).

Yersinia enterocolitica and *Y. ruckeri* each possess a single *luxRI* pair termed *yenRI* and *yruRI*, respectively, and exhibit similar AHL profiles to those of *Y. pseudotuberculosis* and *Y. pestis*. (Throup *et al.* 1995; Temprano *et al.* 2001; Bruhn *et al.* 2005; Atkinson *et al.* 2006*a*; Kirwan *et al.* 2006). Whether QS contributes to the virulence of these pathogens in their respective hosts is not known although tissues harvested from mice infected with *Y. enterocolitica* contain AHLs (Jacobi *et al.* 2003) and both swimming and swarming motility in these yersinia species are QS dependent (Atkinson *et al.* 2006*a*).

2.2. The Xanthomonas campestris *diffusible signal factor*

Cha *et al.* (1998) examined 106 plant-associated bacteria with representatives from seven different
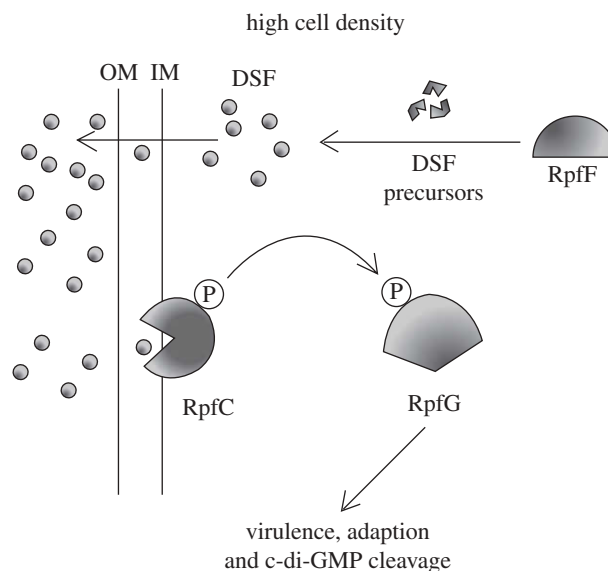
high cell density

Figure 3. *Xanthomonas campestris* DSF autoinduction. RpfC inhibits the activity of the DSF synthase RpfF at low cell density. As the cell density increases and basal DSF production allows for signal accumulation, an RpfC conformational change mediated by DSF promotes the release of RpfF to synthesize more DSF and also to initiate phosphorelay from RpfC to RpfG, which will trigger the expression of target genes in the DSF regulon (adapted from He & Zhang 2008).

genera and found that all produced AHLs except for *X. campestris*. This organism, the cause of black rot disease in a variety of cruciferous plants such as cabbages (Onsando 1992), regulates the production of extracellular enzymes such as proteases, pectinases and cellulases (Dow & Daniels 1994) using a cell-to-cell communication system that does not employ AHLs. The expression of these exo-enzymes along with the control of biofilm dispersal, toxin resistance and survival (Dow *et al.* 2003; He *et al.* 2006) is driven by a small diffusible signal factor (DSF) (Barber *et al.* 1997; Slater *et al.* 2000) chemically characterized as *cis*-11-methyl-2-dodecenoic acid (figure 1) (Wang *et al.* 2004). The *rpf* genes are responsible for DSF signal synthesis (RpfF), detection (RpfC) and signal transduction (RpfG). Interestingly, RpfG is a phosphodiesterase that cleaves the intracellular second messenger, cyclic diguanosine monophosphate (c-di-GMP). RpfG contains an HD-GYP motif, the mutation of which abolishes c-di-GMP phosphodiesterase activity and virulence factor production (Ryan *et al.* 2006). DSF-mediated intercellular signalling is therefore coupled to intracellular signalling via c-di-GMP (Ryan *et al.* 2006) (figure 3).

DSF is not unique to *X. campestris* and is also produced by *Stenotrophomonas maltophilia* together with seven other structurally related C12–C14 fatty acids (Fouhy *et al.* 2007; Boon *et al.* 2008). The *S. maltophilia rpf* locus is highly homologous to that of *X. campestris*, and the DSF signal regulates a number of virulence-associated phenotypes including swimming motility, extracellular protease and lipopolysaccharide along with antibiotic resistance. A further putative DSF signal has been identified as 12-methyltetradecanoic acid in *Xylella fastidiosa* (Colnaghi Simionato *et al.* 2007).

An *rpfF* homologue has also been isolated from *Burkholderia cenocepacia*, which is highly conserved within this genus and is responsible for the synthesis of the DSF-related signal molecule, *cis*-2-dodecenoic acid. This compound is also able to restore the biofilm and extracellular polysaccharide production phenotypes of an *X. campestris rpfF* mutant. The conservation of Rpf proteins and the relatedness of DSF structures from different bacteria indicate that cross-species signalling between Xanthomonads and *Burkholderia* spp. may well occur in nature, particularly because many of these environmental organisms are associated with plants and soil. More recently, a novel fatty acid signalling molecule, *cis*-2-decenoic acid, produced by *Pseudomonas aeruginosa* was discovered to induce dispersion of biofilms not only in *P. aeruginosa* but also in a variety of other Gram-negative and Gram-positive bacteria and the yeast *Candida albicans* (Davies & Marques 2009). *Pseudomonas aeruginosa* does not appear to possess an *rpf* cluster homologue, although there are several enoyl CoA hydratases which share some similarities to RpfF which may be responsible for DSF signal synthesis.

2.3. Autoinducer 2, CAI-1 and Vibrio cholerae

The genus *Vibrio* contains over 50 species that are found in aquatic habitats and in association with coral, seagrass, fish and shrimp (Milton 2006). Depending on the species, they can associate with marine animal tissues as commensals, symbionts or pathogens. Bioluminescent vibrios such as *V. fischeri* and *Vibrio harveyi* as well as non-luminescent species such as *V. anguillarum* and *V. cholerae* are known to possess up to three parallel QS systems employing AHLs, AI-2 and a long-chain α-hydroxyketone (CAI-1) as QS signal molecules (Milton 2006). These QS pathways converge on the σ54-dependent activator, LuxO, to control the expression of a variety of target genes that, depending on the species, are involved in symbiosis, bioluminescence, biofilm development and virulence (Milton 2006).

Vibrio cholerae, the causative agent of the diarrhoeal disease cholera, infects humans usually through the ingestion of contaminated water. On reaching the intestine, *V. cholerae* expresses a number of virulence determinants, including the toxin-coregulated pilus, which promotes adherence to intestinal epithelium, and the ADP-ribosylating exotoxin, cholera toxin, which promotes the often fatal diarrhoea necessary for transmitting the organism back into the environment. *Vibrio cholerae* uses two QS signal molecules, AI-2 and CAI-1, to regulate synergistically virulence factor expression and biofilm development (Miller *et al.* 2002; Hammer & Bassler 2003; Zhu & Mekalanos 2003). While in *V. harveyi* AI-2 is the furanosyl borate diester (2*S*,4*S*)-2-methyl-2,3,3,4-tetrahydroxytetrahydrofuran borate derived from *S*-ribosylhomocysteine via the action of LuxS, CAI-1 in *V. cholerae* is (*S*)-3-hydroxytridecan-4-one, a 13 carbon chain α-hydroxyketone derivative (Higgins *et al.* 2007). CAI-1 biosynthesis is dependent on CqsA, which is related to the pyridoxal phosphate-binding aminotransferases, which typically
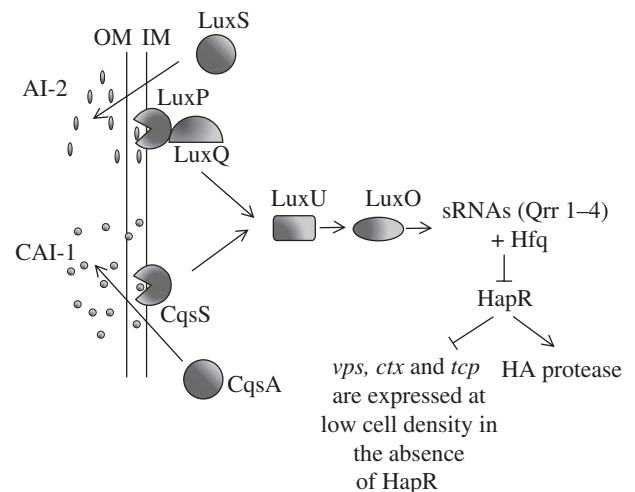

Figure 4. QS in *V. cholerae*. Two QS signals AI-2 and CAI-1 work synergistically to regulate virulence factor expression and biofilm development. At low population density in the absence of signal molecules, the absence of HapR triggers the expression of genes such as *vps, ctx* and *tcp*, which are normally repressed by HapR. As the cell density and therefore the signal molecule concentration increases HapR represses the expression of the genes responsible for colonization and promotes detachment from the intestinal epithelium to enable the organism to exit the host (adapted from Kaper & Sperandio 2005).

catalyse the condensation of amino acids and carboxylic thioesters, although the biochemical mechanism involved in CAI-1 production has yet to be elucidated.

At low population densities in the absence of QS signals, the phosphorylated sensor kinases CqsS and LuxP/Q transfer their phosphates to the phosphotransfer protein, LuxU, which in turn phosphorylates the response regulator, LuxO (figure 4). In conjunction with σ54, LuxO activates the transcription of four small RNAs (sRNA), termed Qrr1–4, which destabilize the HapR transcript. At low cell population densities, this means that no HapR protein is available so that HapR-repressed but not HapR-activated genes are expressed. At high cell population densities where CAI-1 and AI-2 are present, the CqsS and LuxP/Q phosphorelay systems, respectively, are switched from kinases to phosphatases and, as a consequence, LuxO is dephosphorylated and HapR protein can now be produced in the absence of the sRNAs. In the absence of HapR, at low population densities, biofilm exopolysaccharide (*vps*), cholera toxin (*ctx*) and toxin-coregulated pilus (*tcp*) genes are expressed, whereas, at high population densities, they are repressed by HapR, which activates the expression of the HA protease. This response presumably promotes detachment from the intestinal epithelium during the latter stages of infection when *V. cholerae* prepares to exit the host and re-enter the environment.

2.4. Agr-dependent quorum sensing and the staphylococci

In *Staphylococcus aureus*, the *agr* (accessory gene regulator) system is central to virulence gene

regulation, intracellular survival in epithelial and endothelial cells as well as biofilm development (Chan *et al.* 2004; Cheung *et al.* 2004; Novick & Geisinger 2008). *agr* is a prototypic QS system shared by a number of different Gram-positive bacteria. Although there is some strain-dependent variation in the nature of the *agr* regulon in different *S. aureus* isolates, in experimental animal infection models *agr* mutants are attenuated, highlighting a key role for this regulatory locus in staphylococcal pathogenicity (Chan *et al.* 2004; Cheung *et al.* 2004; Novick & Geisinger 2008). Furthermore, *agr* regulates all three major exotoxin classes (α-toxin, PVL and the PSMs) implicated in invasive, community-acquired methicillin-resistant *S. aureus* infections as well as the methicillin antibiotic resistance gene, *mecA* (Novick & Geisinger 2008; Queck *et al.* 2008). A functional *agr* system is also required for endosomal escape, intracellular survival and replication (Qazi *et al.* 2001) and also contributes to biofilm dispersal and antimicrobial susceptibility (Boles & Horswill 2008).

The *agr* locus consists of two adjacent but divergent transcriptional units (RNAII and RNAIII) controlled by the *agrP2* and *agrP3* promoters, respectively (Chan *et al.* 2004; Novick & Geisinger 2008) (figure 5a). The *agrP2* operon consists of four genes, *agrBDCA*, which are required for the activation of transcription from the *agrP2* and *agrP3* promoters. The P3 transcript codes for both RNAIII, a 517 nucleotide regulatory RNA, and δ-toxin. AgrA and AgrC constitute a two-component system in which AgrA is a response regulator and AgrC is a transmembrane receptor histidine kinase that is activated upon binding to a cognate autoinducing peptide (AIP). This QS signal molecule is a peptide thiolactone consisting of seven to nine amino acid residues in which the central Cys residue is covalently linked to the C-terminal amino acid carboxylate forming a cyclic thioester (figure 5b(i)) and is generated by the action of AgrB on the AgrD propeptide (Chan *et al.* 2004; Novick & Geisinger 2008). Until recently, RNAIII was considered to be the primary effector of the *agr* response, although it is now clear that the *agr* regulon can be divided into RNAIII-dependent and RNAIII-independent, AgrA-dependent, genes (Queck *et al.* 2008).

Within the staphylococci, *agr* polymorphisms occur. *Staphylococcus aureus* has four different specificity groups with distinct AIPs (figure 5b(ii)) and AgrC sensor domain sequences (Novick & Geisinger 2008). Furthermore, there are at least 20 additional *agr* groups among the coagulase-negative staphylococci (Novick & Geisinger 2008). The *agr* system is also evolutionarily conserved across the Firmicutes, which include probiotics such as *Lactobacillus plantarum* as well as human pathogens such as *Clostridium perfringens*, *Clostridium botulinum*, *Clostridium difficile*, *Listeria monocytogenes* and *Enterococcus faecalis* (Wuster & Babu 2008). Apart from *E. faecalis*, which employs an 11 membered peptide lactone, the chemical identity of the AIPs in these bacteria is not yet known, although mutation of the *agr* locus in both *Listeria* (Riedel *et al.* 2009) and *Enterococcus* (Nakayama *et al.* 2006) results in the attenuation of virulence.
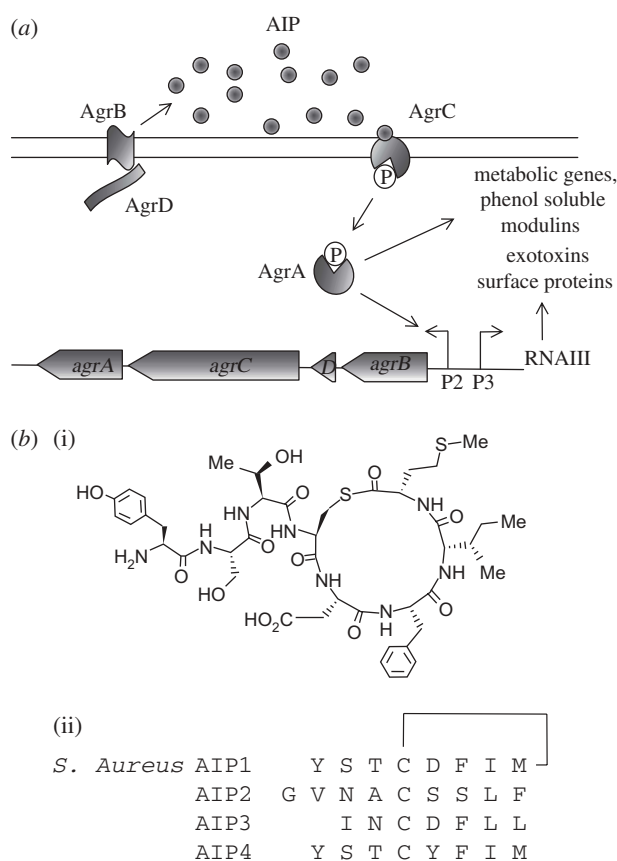

Figure 5. AIP-mediated QS in *S. aureus*. (*a*) The AIP activates the transmembrane receptor histidine kinase AgrC to trigger autophosphorylation and phosphotransfer to AgrA, which in turn drives the transcription of the *agrP2* and *agrP3* promoters. This triggers rightward and leftward transcription of the *agr* locus and RNAIII-dependent and -independent genes. (*b*)(i) Structures of AIP-1 and (ii) the linear sequences of the four *S. aureus* AIPs which constitute four different *agr* specificity groups.

2.5. Autoinducer 2 ubiquity, metabolism and quorum sensing

When *S*-ribosyl-ʟ-homocysteine (SRH) is cleaved to generate homocysteine and DPD, the latter spontaneously cyclizes to yield a number of furanones collectively known as AI-2 (Miller *et al.* 2004). The enzyme responsible for this conversion (LuxS) was originally discovered in *V. harveyi* where AI-2 is involved in the regulation of bioluminescence. Homologues of LuxS have been identified in over 50 different bacterial species (Schauder *et al.* 2001; Winzer *et al.* 2002b; Xavier & Bassler 2003; Vendeville *et al.* 2005), suggesting that AI-2-dependent QS is widespread and shared by both Gram-positive and Gram-negative bacteria. However, the LuxS-dependent cleavage of SRH is also an integral part of a metabolic pathway, the activated methyl pathway, that serves to recycle homocysteine from *S*-adenosyl methionine to maintain de novo methionine biosynthesis. The consequences of disrupting *luxS* may therefore lead to attenuation of either QS or metabolism or both depending on the organism (Winzer *et al.* 2002b;

Xavier & Bassler 2003; Vendeville *et al.* 2005; Hardie & Heurlier 2008).

The LuxS/AI-2 system has been examined in detail in *V. harveyi* and *V. cholerae* (§2.3) where AI-2, in this case a furanosyl-borate diester, activates a sophisticated receptor-mediated sensor kinase/phosphorelay system (Lenz *et al.* 2004; Neiditch *et al.* 2005). Other autoinducers and their associated sensor kinases also feed into this pathway (§2.3; Miller *et al.* 2002; Henke & Bassler 2004*a*). In vibrios, the LuxS/AI-2 system functions as a QS system (Miller *et al.* 2002; Xavier & Bassler 2003; Henke & Bassler 2004*a*,*b*; Lenz *et al.* 2004). However, despite the ubiquity of LuxS, this is not the case for all bacteria. There are currently a limited number of reports (Vendeville *et al.* 2005; Hardie & Heurlier 2008) linking the expression of a specific gene, operon or phenotype to AI-2 apart from those described for vibrios, although *Salmonella typhimurium* and *E. coli* use AI-2 to regulate the uptake and phosphorylation of AI-2 (Taga *et al.* 2001, 2003; Xavier & Bassler 2005).

In *S. typhimurium*, the *lsr* operon encodes a periplasmic binding protein LsrB, which binds a chemically distinct form of AI-2 (2*R*,4*S*)-2-methyl-2,3,3,4-tetrahydroxytetrahydrofuran and transports it into the cytoplasm whereupon it is phosphorylated by LsrK (Taga *et al.* 2003). It has been suggested that phosphorylated AI-2 relieves repression of the *lsr* operon by binding to and inhibiting the activity of the repressor LsrR, causing the subsequent upregulation of the *lsr* operon to increase the uptake of AI-2 from the environment (Taga *et al.* 2003). While provision of exogenous DPD (as a source of AI-2) restores the regulation of the *lsr* operon in a *S. typhimurium luxS* mutant, it was unable to restore a defect in biofilm formation in the same strain (De Keersmaecker *et al.* 2005). Similarly, mutation of *luxS* in *S. typhimurium* polarizes flagellar phase variation towards the more immunogenic phase 1 flagellin. While this phenotype could be complemented by the *luxS* gene supplied *in trans* it was not restored by provision of AI-2 and is, therefore, in common with the biofilm phenotype independent of QS (Karavolos *et al.* 2008). In contrast, Walters *et al.* (2006) reported that in enterohaemorrhagic *E. coli* (EHEC), the provision of exogenous AI-2 to a *luxS* mutant resulted in a gain of 62 phenotypes when compared with the *luxS* mutant using phenotype microarray analysis. The majority of these phenotypes were involved in carbon, nitrogen or phosphate metabolism. In other bacteria, LuxS and/or AI-2 have been implicated in biofilm development, flagellar-mediated motility, toxin production and virulence either as a consequence of the attenuation of QS or to a metabolic defect (for reviews, see Vendeville *et al.* 2005; Hardie & Heurlier 2008). Recently, a comparative study of genomic databases to try to establish an association between LuxS and the presence of AI-2 receptors such as LuxPQ and Lsr concluded that in most bacteria LuxS is primarily limited to a role in metabolism (Rezzonico & Duffy 2008), although the existence of as yet unidentified alternative AI-2 receptors could not be ruled out.

3. QUORUM-SENSING SIGNAL MOLECULE-MEDIATED POLYMICROBIAL INTERACTIONS

In most environments, bacteria are found in mixed communities, and therefore populations of a given QS species that can sense a signal broadcast information both to themselves and to other species sharing the same ecological niche. Different bacterial species may 'speak' the same QS 'language', some may possess sensors for specific QS signals that facilitate eavesdropping while others may manipulate the QS activities of neighbouring bacteria by degrading the QS signal molecules within their locality. Although the impact of QS on microbial community parameters, such as species diversity and function, has not been extensively investigated, Valle *et al.* (2004) reported that the exogenous provision of AHLs to an industrial wastewater treatment bioreactor containing AHL-producing bacteria generated changes in both community function (phenol degradation) and composition. Furthermore, following AHL addition, one dominant functional member of the community was transiently supplanted by another, a finding which has important implications for ecosystem functionality.

3.1. Quorum-sensing-mediated interspecies cooperative interactions

Bacteria often exist in association with plants as members of polymicrobial biofilm communities, either in the rhizosphere or *in planta*. It has been reported that more AHL-producing bacteria live within the rhizosphere in association with plants than within the bulk soil population (Danhorn & Fuqua 2007). In mixed populations, the formation, development, maturation and dispersal of biofilms are likely to be tightly regulated through cooperative inter- and intraspecies signalling. Two different bacterial species that produce the same QS signal and coexist in the same environment will detect and respond to that signal. For example, *Chromobacterium violaceum* produces the purple pigment violacein in response to C6-HSL irrespective of whether the AHL is self-generated or supplied by another C6-HSL producer (McClean *et al.* 1997). While numerous studies have investigated the role of AHL-mediated signalling in single species biofilms (for review, see Atkinson *et al.* 2007), there have been relatively few studies of signalling in multi-species biofilms, which in turn have revealed cooperative behaviour. AHL-mediated communication between *P. aeruginosa* and *B. cenocepacia* in mixed biofilms appears to be unidirectional in that the latter responds to the *P. aeruginosa* AHLs but not vice versa (Riedel *et al.* 2001). In mixed *Agrobacterium tumefaciens*/*P. aeruginosa* coculture experiments, the *A. tumefaciens* biomass decreased in coculture with the wild-type but remained constant with a *P. aeruginosa* QS mutant (An *et al.* 2006), such findings are, however, more indicative of competitive rather than cooperative interactions.

Plant beneficial bacteria such as *P. fluorescens* produce a number of antibiotics and extracellular enzymes that suppress root diseases caused by fungal root

pathogens (Dubuis & Haas 2007). These are regulated in a cell-density-dependent manner via the GacS/GacA two-component system that controls the expression of small regulatory RNAs, such as *rsmZ* and *rsmY*, which sequester RsmA/CsrA translational repressor family proteins and thereby relieve post-transcriptional repression of the exoproduct genes. Gac/Rsm signal transduction cascades occur in many different Gram-negative bacteria, and Dubuis & Haas (2007) reported that crude culture supernatant extracts taken from other plant beneficial and pathogenic pseudomonads could induce *rsmY* and *rsmZ* expression in the reciprocal host. Although the chemical identity of this putative cross-species signal molecule(s) has not yet been identified, the work suggests that it is possible for each species in a polymicrobial community to employ QS to monitor the local environment and to bring about a concerted change in behaviour that is beneficial to the community as a whole.

Sibley *et al.* (2008) developed a *Drosophila melanogaster* infection model to study polymicrobial interactions whereby a number of oropharyngeal isolates were coinoculated with *P. aeruginosa*. A number of the isolates which alone were avirulent or beneficial to the *D. melanogaster* synergistically exacerbated the pathogenicity of *P. aeruginosa* in a polymicrobial community. For example, when co-infected with *P. aeruginosa*, the oropharyngeal isolate C90 (a *Streptococcus* spp.), which alone appears to enhance *D. melanogaster* survival, significantly enhances fly killing. A number of *P. aeruginosa* QS-dependent genes, including the AHL synthase LasI, the AHL receptor RhlR, and the metalloprotease LasB, were apparently upregulated, and the authors suggested that AI-2, which is produced by C90 but not by *P. aeruginosa*, may be responsible for the change in gene expression (Sibley *et al.* 2008). As AI-2 is produced by many different bacteria, it has been suggested that AI-2 is a 'universal language' used by bacteria for interspecies communication (Bassler *et al.* 1997; Bassler 1999). However, not all AI-2 producers employ this metabolite as a QS signal molecule (§2.5) and not all bacteria synthesize AI-2 (Winzer *et al.* 2002b). As yet, there is little published data on AI-2-dependent interspecies signalling, which has been proposed to mediate interspecies communication between oral bacteria as well as biofilm community development within the human oral cavity (Kolenbrander *et al.* 2006).

3.2. Quorum-sensing-mediated signal interception and coercion

Most environments suitable for microbial growth will be populated by polymicrobial communities. While some of the interactions between species may be complementary, there is also the possibility for conflict through coercion, interception and chemical modification (reviewed by Keller & Surette 2006). LuxR homologues are most specific for their cognate AHL, but many can also be activated by other related compounds. A practical demonstration of this phenomenon is through the use of a range of bioluminescent biosensors based on the *V. fischeri* (*luxI/R*), *P. aeruginosa* (*lasI/R*) and *Aeromonas hydrophila* (*ahyI/R*) *luxR/I* homologues (pSB401, pSB1075 and pSB536, respectively) (Swift *et al.* 1997; Winson *et al.* 1998; Steindler & Venturi 2007). These plasmids contain the promoter region of the appropriate *luxI* homologue along with the response regulator fused to *luxCDABE*. While each reporter responds optimally to its cognate AHL to produce light, each also reports the presence of other signals that are structurally related to the cognate AHL (normally differing in the number of carbons in the fatty acyl chain) (Bainton *et al.* 1992a; Winson *et al.* 1998; Kirke *et al.* 2004).

Although these biosensors are engineered for *in vitro* use, they demonstrate an important point that the signal generated by one species can be detected by another species that does not synthesize the same signal molecule. This confers on one species the ability to intercept (Ahmer 2004) the signal from another, effectively using it as cue (Diggle *et al.* 2007a) to alter its behaviour as a direct response to the presence of the signal generator species. *In vivo* LuxR homologues may not be limited to recognizing AHLs. Riboflavin and its derivative lumichrome, produced from the single celled alga *Chlamydomonas*, is capable of stimulating *P. aeruginosa* LasR at the expense of the cognate AHL: *N*-(3-oxo-dodecanoylhomoserine) lactone (3-oxo-C12-HSL). Although there are no structural similarities between AHLs and riboflavin (and its derivative), amino acid substitutions in the AHL-binding domain of LasR altered responses to each class of molecule, which suggests that the AHL-binding domain of LasR is unable to discriminate between the two compounds. It is unclear from these initial observations whether this represents an example of cross-kingdom cooperation or coercion driven by the eukaryote (Rajamani *et al.* 2008).

While it is likely that cooperative interspecies interactions are beneficial in biofilms (as discussed above), there is also evidence that there may be non-cooperative interactions between species in these environments. Biofilms will develop when *P. aeruginosa* and *Burkholderia cepacia* are cocultured in flow chambers or on alginate beads in a mouse chronic lung infection model. *Burkholderia cepacia* is able to intercept and transduce the AHLs synthesized by *P. aeruginosa* but not vice versa. This was confirmed by analysing exoprotease production, which was restored to a *B. cepacia cepI* mutant when grown in the presence of some synthetic AHLs or supernatants from the AHL-producing strains *P. aeruginosa* PA01 or SH1. AHL-negative *P. aeruginosa* strains did not restore exoprotease activity. AHLs or supernatant extracts from the AHL-producing strain *B. cepacia* H111 did not restore exoprotease activity to a *P. aeruginosa lasI/rhlI* double mutant. However, the addition of spent PA01 culture supernatant or synthetic 3-oxo-C12-AHL did restore activity (Riedel *et al.* 2001). *Pseudomonas aeruginosa* may therefore have a mechanism for blocking the *B. cepacia* signals (possibly by detecting and reacting to an alternative *B. cepacia*-specific signal) to prevent *B. cepacia*-mediated coercion or alternatively is prevented from monitoring the activities of *B. cepacia*.

Escherichia coli and *Salmonella* spp. do not synthesize AHLs, but they are able to intercept and transduce AHL signals generated by other species because they possess the LuxR homologue SdiA (Ahmer 2004). *Salmonella enterica* SdiA responds to the presence of exogenous AHLs or bacterial species that produce AHLs. SdiA regulates the expression of *srgE* and the *rck* operon, so named because of its involvement in resistance to complement killing (Michael *et al.* 2001; Smith & Ahmer 2003; Ahmer 2004). In *E. coli*, several genes, including those involved in amino acid transport and metabolism and also in transcriptional regulation, have been shown to respond to C6-HSL in an SdiA-dependent manner (Van Houdt *et al.* 2006). This process of bacterial 'eavesdropping' confers on the organism the ability to intercept signals, which will provide important information about the local environment and the other species contained within. Sensing, but not synthesizing, a signal confers several advantages. For example, the organism may reduce the metabolic cost of QS signal biosynthesis and avoid conveying information about its whereabouts to other species in the immediate vicinity.

Some QS molecules exhibit biological functionalities beyond their role as signals. *Pseudomonas aeruginosa* employs 3-oxo-C12-HSL and 2-heptyl-3-hydroxy-4-quinolone (the pseudomonas quinolone signal (PQS)) as QS signals in a sophisticated gene regulatory network (Williams & Cámara 2009). However, apart from interacting with their cognate response regulator receptors (LasR and PqsR, respectively), 3-oxo-C12-HSL exhibits both antibacterial and quorum-quenching activity towards Gram-positive bacteria such as *S. aureus* (Qazi *et al.* 2006) while PQS functions as an iron chelator, a pro-oxidant and an inducer of an antioxidative stress response that shapes the population structure of *P. aeruginosa* (Diggle *et al.* 2007b; Haussler & Becker 2008). 3-oxo-C12-HSL and PQS also function as potent immune modulators (Hooi *et al.* 2004). Consequently, there is the possibility of crosstalk between the pathogen and host, which may, in return, elicit a counter-response from the host as it senses bacterial QS signal molecules. By intercepting but not synthesizing AHL signals, bacteria such as *E. coli* and *Salmonella* spp. are potentially capable of mounting a sustained stealth attack on the host while continually monitoring the local environment for potentially antagonistic AHL-producing bacteria.

In the staphylococci, the *agr* QS system has diverged such that an AIP produced by one strain may autoactivate but cross-inhibit other strains. For example, the four *S. aureus* *agr* groups can be distinguished on the basis of their ability to cross-activate or -inhibit *agr* expression, i.e. AIP-1 is an activator of AIP-1 producing group I strains but an inhibitor of *agr* groups II, III and IV. *S. epidermidis* group I strains cross-inhibit all four *S. aureus* *agr* groups but *S. aureus* AIPs have little inhibitory activity against non-group I *S. epidermidis*. In *S. aureus*, cross inhibition results in the downregulation of *agr*-dependent exotoxin synthesis. For example, exposure of an *agr* group I strain to AIP-2 or AIP-3 strains abolished production of TSST-1 and enterotoxin and attenuated

infection in a mouse subcutaneous abscess model (Mayville *et al.* 1999; McDowell *et al.* 2001). Both intragroup activation and intergroup inhibition are mediated exclusively by the same group-specific AgrC receptor (Novick & Geisinger 2008). In addition, there is also some specificity in the processing of AgrD by AgrB; AgrB1 will process AgrD1, D3 and D4 but not AgrD2 or vice versa (Novick & Geisinger 2008). This raises some interesting questions with respect to the driving force behind the evolution of the *agr* system, its consequences for staphylococcal species divergence and the contribution of *agr* interference to competitive adaptation within a specific environmental niche. The natural causes of *agr* divergence within a species are, however, at present not known, although, in an insect infection model, competitive interference between different *agr* groups has been demonstrated (Fleming *et al.* 2006).

There is some evidence to suggest that bacteria, such as *P. aeruginosa*, that do not synthesize AI-2 can nevertheless sense AI-2, which may signify that AI-2 is prompting non-cooperative behaviour. Duan *et al.* (2003) examined the differential expression of several pathogenicity-associated genes from *P. aeruginosa* following exposure to exogenous AI-2 or to AI-2 produced by an uncharacterized avirulent, oropharyngeal bacterium recovered from the sputum of cystic fibrosis patients. The expression of several pseudomonas genes was altered in these assays, suggesting that *P. aeruginosa* is capable of intercepting AI-2 so using this information as a means of monitoring its environment and the composition of the local bacterial community. There is also the possibility that AI-2-producing oropharyngeal bacteria can coerce *P. aeruginosa* into altering gene expression to bring about a change in the environment that favours the former over the latter. Further support for the notion that *P. aeruginosa* will respond to AI-2 has recently been reported by Ganin *et al.* (2008), who have shown that two AI-2 synthetic analogues are capable of inhibiting QS as determined by the reduction in pyocyanin.

4. QUORUM SENSING IN EUKARYOTES

Although QS has been the topic of intense study in prokaryotes, there is also evidence showing that QS systems are employed by eukaryotes. The dimorphic fungus *C. albicans* is a common human pathogen associated with high rates of mortality in cases of systemic candidiasis especially in immune-compromised individuals (Fridkin & Jarvis 1996; Viudes *et al.* 2002). Many fungi including *C. albicans* exhibit yeast–mycelial dimorphism and alternate between budding yeast or wheat-germ tubules and mycelia depending on environmental factors including temperature, pH, glucose levels, nitrogen source, carbon dioxide levels and cell population density (Nickerson *et al.* 2006; Romano 2008). In *C. albicans* a starting inoculum of less than 10^6 cells ml^{-1} favours mycelial growth, whereas budding yeast will be produced at a starting inoculum that is equal to or higher than 10^6 cells ml^{-1} (Hornby *et al.* 2001). This morphological shift, driven

in part by cell population density, shows striking similarities to bacterial QS and prompted Hornby *et al.* (2001) to determine whether the process was signal molecule-mediated. Organic extracts of *C. albicans*-spent culture supernatants revealed that the active signal molecule, capable of repressing the yeast to mycelium conversion (without altering the growth rate), was a 15-carbon sesquiterpine known as farnesol (3,7,11-trimethyl-2,6,10-dodecatrien-1-ol). This molecule, which is secreted extracellularly, is produced throughout growth and is often considered to be the first QS signal molecule to be characterized in a eukaryotic microbe. *Candida albicans* also produces a second autoregulatory substance that represses the yeast to mycelium conversion (Hazen & Cutler 1983). This compound is chemically distinct from farnesol but its structure remains to be elucidated. The term 'inoculum size effect' has been use to describe the link between cell morphology and cell population density and has been observed in several different fungal species and apparently controls a number of different physiological processes (for review, see Nickerson *et al.* 2006). It is therefore likely that many other as yet undiscovered QS systems operate within the fungal world.

A fascinating extension to the concept of QS in eukaryotes was proposed by Hickson *et al.* (2008), who considered how single celled organisms behave in a community as compared with the behaviour of societies of cells integral within a multicellular organism. By way of example, tumorigenesis, resulting from the migration of metastatic ovarian cancer cells, was considered in relation to bacterial cell populations establishing a multicellular biofilm community during infection. Twenty years ago the idea that individual bacterial cells behaved cooperatively was greeted with scepticism in many quarters; today, cancer cells are still considered as autonomous, independent and non-cooperative entities. The challenge will now be to reconsider whether tumours are strictly a product of a few 'unruly' individuals or whether there is a population response to tumorigenesis, with division of labour and communication occurring within the developing tumour.

5. INTER-KINGDOM COMMUNICATION

Bacteria are the most abundant and adaptive form of life on Earth and, as such, have an impact on the habitats and lifestyles of all other living organisms. We would therefore be naive to think that, over the millennia, coevolution of pro- and eukaryotes has not stimulated the development of inter-kingdom signalling pathways promoting parasitic, symbiotic or commensal relationships; the members of each biological kingdom possess hormone-like molecules responsible for cell–cell communication, a fact that is fundamental to the concept that the members of any given kingdom will respond to the signals produced by another (Fox 2004; Rumbaugh 2007). Bacterially derived signal molecules are not limited to signalling between bacterial species but also modify the behaviour of eukaryotic organisms. This is not a one-way interaction,

eukaryotes also manipulate bacterial signalling networks and therefore bacterial behaviour through molecular mimicry using cytokines, hormones and neurotransmitters. Bacterial signalling networks may also be disrupted following enzymatic degradation of the signal molecules or by the action of agonist/antagonist molecules that block QS (Bauer & Mathesius 2004; Pritchard 2006; Rasmussen & Givskov 2006; Kendall & Sperandio 2007; Williams 2007a). Earlier sections have outlined how signal interception and coercion play a key role in manipulating inter- and intraspecies communication among bacteria as does two-way signalling within and between species. Here we discuss inter-kingdom cooperative and non-cooperative behaviour.

5.1. Signal-mediated cooperative interactions between kingdoms

Symbioses across the pro- and eukaryotic divide are possible through the use of cooperative signalling systems, which undoubtedly modulate the behaviour and proliferation of the organisms involved in the relationship. There are more commensal bacterial cells in mammals than there are host cells and in humans, for example, the symbiosis that exists with the intestinal microbial flora is essential for nutrient assimilation and the development of the innate immune system (Hooper & Gordon 2001; Hughes & Sperandio 2008). Although, to date, there is little direct evidence of cooperative interactions between kingdoms, Fujiya *et al.* (2007) reported that, when the human colonic epithelial cell line Caco$_2$ was exposed to a variety of cell-free supernatants derived from probiotic bacteria (bacteria used in food supplements which are thought to be beneficial in the treatment of a number of diseases; Hong *et al.* 2005) such as *Bacillus subtilis*, the cytoprotective heat shock protein Hsp27 was induced. When over-expressed, Hsps are capable of adhering to the intestinal epithelium and protecting it from oxidative injury (Tao *et al.* 2006), a contributing factor to the maintenance of a healthy gut. Upregulation of Hsp27 was a direct result of the presence of physiologically relevant concentrations of the QS signal peptide, CFS (also known as PhrC), the competence- and sporulation-stimulating factor. In *B. subtilis* CFS, ComX and other Phr homologues regulate multiple processes including the initiation of genetic competence, sporulation, antibiotic and exopolysaccharide synthesis and the production of degradative enzymes (Lazazzera 2001; Auchtung *et al.* 2006). These data suggest that a QS signal molecule-mediated symbiosis between *B. subtilis* and its host may exist whereby bacterially driven Hsp induction can confer protection to the host gut and in return provide the bacteria with a stable and nutrient-rich niche in which to reside. Mazmanian *et al.* (2008) similarly showed that the commensal human symbiont *Bacteroides fragilis* produces a molecule known as polysaccharide A, which apparently protects animals from *Helicobacter hepaticus*-induced colitis by suppressing pro-inflammatory interleukin-17 production from intestinal immune cells.

Recently, AHLs have been shown to mimic a eukaryotic signal causing profound effects on the growth of a cancer cell line. Thymidylate synthase (TS) plays a key role in DNA biosynthesis because it generates thymidine monophosphate which upon phosphorylation is converted into thymidine triphosphate. The TS inhibitor 5-fluorouracil (5-FU) has for decades been used to treat solid tumours (Curreri *et al.* 1958), but resistance to 5-FU is frequently associated with the over-expression of TS. The expression and/or function of TS is affected by *rTS*, which codes for an antisense TS RNA and two protein subunits rTSα and rTSβ (Dolnick 1993; Dolnick & Black 1996). Elevated levels of rTSβ are concomitant with an increase in the production of a novel signal molecule and a decrease in TS protein expression levels. The structure of this signal molecule is currently unknown but the rTSβ products are known to be metabolites of methionine biosynthesis formed directly from *S*-adenosylmethionine (and an uncharacterized substrate), which might indicate that this signal shares structural similarity with AHLs (Dolnick *et al.* 2003). The possible structural relationship between AHLs and the rTS signal prompted Dolnick *et al.* (2005) to test a range of AHLs for the ability to reduce TS protein levels. Western blot analysis showed that TS expression was reduced considerably in the presence of physiologically relevant concentrations of 3-oxo-C12-HSL, which also reduced the growth of human colorectal cancer cells (H630) by 50 per cent. The fact that *N*-dodecanoylhomoserine lactone showed no effect led the authors to speculate that the response was specific and receptor-mediated. Furthermore, 3-oxo-C12-HSL significantly enhanced the efficacy of 5-FU and Taxol (Dolnick *et al.* 2005), highlighting the therapeutic potential of this bacterial QS signal molecule. Indeed, comparative structure activity relationship studies have shown that cancer growth inhibitory activity in common with QS signalling in *P. aeruginosa* requires a long acyl chain with 3-oxo substitution for maximum activity (Oliver *et al.* 2009). Whether coevolution of these two systems has created a genuine biological relationship between rTS-signal-mediated TS expression and AHL action to trigger the death of malignant cells remains to be established. However, given that *P. aeruginosa* is an opportunistic human pathogen, it seems unlikely that such a cooperative relationship exists between the host and bacterium. Indeed, immune-compromised cancer patients often develop *P. aeruginosa* lung infections, and it is therefore feasible that the bacteria use AHLs to modulate the host response through the rTS-signal pathway. However, other commensal AHL producers might be involved in two-way signalling to communicate with the host via this pathway. Given the available information, it is not yet possible to determine whether the AHL–rTS signal relationship is combative or cooperative.

5.2. Interception and coercion

Both 3-oxo-C12-HSL and PQS modulate the host response in mammals such that these QS signals may facilitate *P. aeruginosa* infections both by regulating virulence factor expression and by modifying host inflammatory and immune responses (Hooi *et al.* 2004; Cooley *et al.* 2008). In cell culture-based assays, 3-oxo-C12-HSL, depending on the concentration used, is immunosuppressive at or below $10\,\mu M$ but pro-inflammatory and pro-apoptotic at $20\,\mu M$ and above. Investigation of the 3-oxo-C12-HSL structural requirements have revealed that, in common with QS activity, immune modulatory activity required an intact homoserine lactone ring, the L-configuration and an acyl chain of 11–13 carbons (Chhabra *et al.* 2003). Interestingly, 3-oxo-C12-HSL, but not the 3-oxo-C6 or 3-oxo-C13 analogues, prevented the invasion and destruction of pancreatic islet tissue by mononuclear cells normally associated with insulitis and cumulative diabetes in genetically predisposed non-obese diabetic (NOD) mice (Pritchard *et al.* 2005), suggesting that QS molecules such as 3-oxo-C12-HSL may represent a novel source of immune modulatory compounds for the treatment of the autoimmune disease type 1 diabetes, which afflicts more than two million individuals in Europe and North America. The signalling pathways by which 3-oxo-C12-HSL exerts its immune modulatory activity have been the subject of numerous studies (for reviews, see Cooley *et al.* 2008; Jahoor *et al.* 2008) and have shown that a potential target for the intracellular binding of 3-oxo-C12-HSL in mammalian cells may be the peroxisome proliferator-activated receptors (PPARs) that belong to the nuclear hormone receptor family (Jahoor *et al.* 2008). However, it is also possible that PPARs may be activated upon 3-oxo-C12-HSL binding to an as yet unknown protein target(s). Using an affinity chromatography strategy employing immobilized 3-oxo-C12-HSL, Seabra *et al.* (2008) isolated two 3-oxo-C12-HSL-binding proteins from mouse splenocyte and human granulocyte extracts, which were identified as S100A8 and S100A9 (calprotectin). Although these proteins clearly have the capacity to bind 3-oxo-C12-HSL, in murine proliferation assays using splenocytes from wild-type and S100A9 knockout mice, no differences in immune suppression were observed, suggesting that these proteins are unlikely to be the primary targets for the immune modulatory activity of 3-oxo-C12-HSL in lymphocytes.

Upon invasion by microbial pathogens, the innate immune response is triggered by activated NF-κB, and, recently, Kravchenko *et al.* (2008) reported that 3-oxo-C12-HSL may reduce the intensity of NF-κB signalling, which would reduce the expression of inflammatory cytokines and other related immune regulators in lipopolysaccharide-induced macrophages. These observations suggest a degree of coercion levied on the host by the signal molecules produced by the invading pathogens.

While there is clearly a considerable body of literature that shows that certain QS signals modify the host immune response, there are other examples where bacterial QS signals are intercepted by other organisms leading to behavioural modification in the latter. Conversely, QS signal molecules may coerce a eukaryote into modifying its behaviour in favour of the bacteria (for reviews, see Shiner *et al.* 2005; Rumbaugh 2007;

Hughes & Sperandio 2008). A fascinating example of inter-kingdom signal interception can be seen in the relationship between *Ulva intestinalis* and biofilm-dwelling bacteria. *Ulva intestinalis* is a green microalga which occupies the inter-tidal zone and can reproduce asexually via the release of vast numbers of motile, asexual zoospores which seek and attach to a suitable surface. The zoospores discriminate between suitable and unsuitable surfaces by making a temporary attachment, which they leave rapidly if it proves to be an unsatisfactory environment for differentiation (Joint *et al.* 2007). Joint *et al.* (2000) noted that there was a positive correlation between zoospore settlement and attachment and the density of bacterial biofilms. Whether this was a property of the surface, the biofilm matrix or diffusible QS signals produced by the biofilm was unknown (Joint *et al.* 2002). Studies using parent and AHL-negative strains of *Vibrio* generated either by mutation of the AHL synthases or by introducing an AHL-degrading enzyme revealed that zoospores are preferentially attracted to AHL-producing bacterial biofilms (Joint *et al.* 2002; Tait *et al.* 2005). Zoospore swimming speeds decreased more rapidly over wild-type *Vibrio* biofilms when compared with those of an isogenic AHL-negative mutant, indicating that rather than a chemotactic response the zoospores appear to respond chemokinetically. The biological significance of AHL-mediated zoospore attachment to biofilms is unclear, although for many marine invertebrates a pre-requisite to settlement on a surface is the presence of a microbial biofilm (Joint *et al.* 2007).

The nematode *Caenorhabditis elegans* is also capable of adapting its behaviour in the presence of AHLs (Beale *et al.* 2006). Some species of *P. aeruginosa* are natural pathogens of *C. elegans*, whereas others can serve as a food source for the nematodes. When exposed to *P. aeruginosa* or an isogenic AHL-negative mutant, *C. elegans* preferentially migrated to the AHL-producing strain on standard chemotaxis plate assays. When the assays were repeated using synthetic AHLs in the absence of bacteria, the worms were significantly more likely to migrate towards the AHL than the controls. *Pseudomonas aeruginosa* induces cyanide-mediated nematode paralysis after approximately 3.0 h exposure to the bacteria. To determine whether the nematodes were capable of sensing and 'learning' to avoid this pathogen, they were exposed to *P. aeruginosa* for 1.0 h, removed prior to paralysis and placed on AHL-containing chemotaxis plates. When compared with naive worms that had not been pre-exposed to *P. aeruginosa*, the pre-exposed worms were repelled rather than attracted to the AHLs. Conversely, worms that had been pre-exposed to a non-pathogenic food source in the presence of an AHL were more likely to be attracted to synthetic AHLs (Beale *et al.* 2006). In this context, *C. elegans* appears to be capable of intercepting AHL signals and altering its behaviour to respond appropriately to the presence of what it may perceive as a pathogen or a food source. This example of cross-kingdom signal interception illustrates that this type of attractive and aversive behaviour represents an essential strategy for nematode survival given that at any time in its natural environment *C. elegans* will be exposed to a variety of potential bacterial food sources, some of which may be harmful.

AI-3, a QS signal of an as yet unknown structure, is produced by bacteria such as EHEC, which causes severe gastric infections and haemolytic–uraemic syndrome. AI-3 is involved in the regulation of virulence motility and a type III secretion system encoded by the LEE locus (Locus of Enterocyte Effacement). The mammalian neuroendocrine stress hormones adrenaline and noradrenaline can substitute for AI-3 in activating EHEC virulence gene expression and the action of all three signals can be blocked by mammalian α- and β-adrenergic receptor antagonists (Sperandio *et al.* 2003). AI-3, adrenaline and noradrenaline can all bind to the histidine sensor kinase, QseC. Upon sensing any of these signalling molecules, QseC autophosphorylates and subsequently transfers the phosphate to the response regulator, QseB, which activates a sophisticated regulatory cascade, resulting in the transcription of key virulence genes (Clarke & Sperandio 2005; Hughes & Sperandio 2008). As it is likely that AI-3, adrenaline and noradrenaline interact with the same bacterial receptor, AI-3 is likely to share some common structural features with the two catecholamines. With this in mind, it is possible that during EHEC infection the host and pathogen are involved in a signal-mediated battle involving interception and coercion. However, while bacteria clearly respond to the catecholamines, the effect of AI-3 on the host, if any, has not yet been reported.

The sesquiterpine farnesol is a QS signal molecule that represses *C. albicans* mycelial development in a density-dependent manner. It is possible that the molecule can act as an interspecies signal for morphogenesis because it will inhibit mycelial development in *Candida dubliniensis* (Henriques *et al.* 2006). There also appears to be an inter-kingdom signalling relationship between *C. albicans* and *P. aeruginosa* such that *C. albicans*-derived farnesol reduces PQS and pyocyanin production in *P. aeruginosa*. Farnesol appears to affect *pqsA* (the first gene in the PQS biosynthetic operon) transcription in the parent strain and in recombinant *E. coli* via a non-productive interaction with the LysR-type regulatory protein PqsR (Cugini *et al.* 2007). This interaction is not one-way because *P. aeruginosa* 3-oxo-C12-HSL can also inhibit *C. albicans* mycelial development (Hogan *et al.* 2004). This is a good example of how two organisms apparently coerce each other into phenotypic change using a QS signal molecule. By reducing the amount of *P. aeruginosa* pyocyanin, *C. albicans* can improve the growth conditions in its immediate environment while *P. aeruginosa* may prevent the fungus from differentiating into mycelia from the free-living yeast form, so preventing the fungus from establishing a foothold in the micro-environment in which the two organisms reside.

6. CONCLUSIONS AND FUTURE PERSPECTIVES

QS has been extensively used as a term to describe density-dependent bacterial cell–cell communication

since the early 1990s. However, the concept of individual bacteria coordinating a population-wide response to a signal or diffusible factor was first recognized over 50 years ago, initially by Lev (1954), by Jennings (1961) and by McVittie *et al.* (1964) while investigating fruiting body formation in *Myxococcus xanthus.* Genetic competence in *Streptococcus pneumoniae* and streptomycin biosynthesis and aerial mycelium formation in *Streptomyces griseus* followed as examples of other species of bacteria exhibiting multicellular behaviour in a population of individuals (Tomasz 1965; Khoklov *et al.* 1967). However, it was perhaps the discovery that bioluminescence in *V. fischeri* (Nealson *et al.* 1970) was mediated by an AHL that was subsequently shown to be employed as a QS signal by numerous other bacterial species (Bainton *et al.* 1992*a,b*) that resulted in the widespread recognition that bacteria, in general, were capable of coordinating their behaviour collectively. QS is now a well-established concept in bacteriology, is increasingly being discovered in unicellular eukaryotic microbes and has even been used to describe the behaviour of ants (Pratt *et al.* 2002), metastatic cells and even iron-impregnated particles (Taylor *et al.* 2009). While many studies have focused on unravelling the QS network operating within a single bacterial species, there has been the general realization that this approach is limited because most environments harbour multiple bacterial species and higher organisms. When we consider QS signalling networks in the context of these multi-species/multi-kingdom communities, there are several ways in which each representative group can interact. Within and between species and kingdoms there may be signal molecule-mediated cooperation or coercion. Alternatively, individuals from one group may intercept signals generated by another. There is also the intriguing possibility that different species from one kingdom may coordinate their behaviour against the individual/s from another kingdom. Irrespective of how the signals are processed, the results will either be a collective change in behaviour for the good of the community as a whole or will favour one or more species over others. These relationships are complex and the finer details of signal-mediated cross-species/kingdom communication are still to be investigated in depth, and this is reflected in the limited number of reports that have addressed the issue to date. This may be due in part to technical difficulties because by their very nature these communities are complex and difficult to standardize in the laboratory, but there is also a need for a broader cross-disciplinary approach involving collaborations between pro- and eukaryotic biologists.

Although we are only just beginning to investigate the possible benefits of exploiting QS the mechanisms underpinning bacterially driven cell–cell and cell–host communication systems constitute potential targets for novel anti-bacterials. Given that QS is not essential for the metabolic processes required for bacterial growth also means that using QS as a target for novel anti-bacterials will not impose on the bacteria the severe selective pressures that would normally lead to the development of resistance similar to that observed with conventional bactericidal agents (Rasmussen & Givskov 2006). One area that may be exploited is that of enzyme-mediated signal molecule inactivation. With respect to AHL QS signals, many bacteria produce acylase or lactonase enzymes that either hydrolyse the homoserine lactone ring or cleave the amide bond (Zhang & Dong 2004; Dong *et al.* 2007). In mammalian cells, the paraoxanase (PON) enzymes, which are a common component of serum and airway epithelia, exhibit a variety of hydrolytic activities. Both PON1 and PON2 have lactonase activity, while PON1 also has arylesterase and organophosphatase activities (Dong *et al.* 2007). Alternatively, antibodies against QS signals have been developed and shown to be capable of attenuating staphylococcal infections in experimental animal models (Park *et al.* 2007). Analogues of QS molecules such as 3-oxo-C12-HSL clearly have potential as immune modulators for treating autoimmune and cardiovascular diseases (Gardiner *et al.* 2001; Chhabra *et al.* 2003; Pritchard *et al.* 2005). Several reports have shown that plant, animal and algal cells all produce compounds that are either antagonistic or agonistic towards Gram-negative and Gram-positive bacterial QS systems (for reviews, see Bauer & Mathesius 2004; Dudler & Eberl 2006; Rasmussen & Givskov 2006; Williams 2007*b*). Both natural products and the structure-based design offer multiple opportunities (Suga & Smith 2003; Raffa *et al.* 2005) for discovery of novel inhibitors of QS. To date, a number of compounds have been synthesized which inhibit QS systems in different bacteria and include AHL and AIP analogues as well as compounds that are structurally unrelated to QS signals (Scott *et al.* 2003; Suga & Smith 2003; Glansdorp *et al.* 2004; Persson *et al.* 2005; Kaufmann *et al.* 2006; Muh *et al.* 2006; Singh *et al.* 2006; Geske *et al.* 2007; Pomianek & Semmelhack 2007; Ni *et al.* 2009).

It is now clear that both prokaryotes and eukaryotes respond to their own and each other's signals, but, at present, we can only hypothesize how significant our understanding of these relationships and their potential for exploitation might be. In the coming years, it is likely that detailed knowledge of intra- and inter-kingdom signalling systems will emerge. This will undoubtedly inform our understanding of host–pathogen relationships, the mechanisms that underlie infectious and perhaps malignant diseases and aid the design and discovery of novel therapeutic strategies.

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