Quorum Sensing and the Lifestyle of Yersinia

Steven Atkinson1, R. Elizabeth Sockett2, Miguel Cámara1 and Paul Williams1*

1Institute of Infection, Immunity and Inflammation, Centre for Biomolecular Sciences, University of Nottingham, Nottingham NG7 2UH, UK
2Institute of Genetics, University of Nottingham, Queen’s Medical Centre, Nottingham NG7 2UH, UK

Abstract
Bacterial cell-to-cell communication (‘quorum sensing’) is mediated by structurally diverse, small diffusible signal molecules which regulate gene expression as a function of cell population density. Many different Gram-negative animal, plant and fish pathogens employ N-acylhomoserine lactones (AHLs) as quorum sensing signal molecules which control diverse physiological processes including bioluminescence, swarming, antibiotic biosynthesis, plasmid conjugal transfer, biofilm development and virulence. AHL-dependent quorum sensing is highly conserved in both pathogenic and non-pathogenic members of the genus Yersinia. Yersinia pseudotuberculosis for example, produces at least eight different AHLs and possesses two homologues of the LuxI family of AHL synthases and two members of the LuxR family of AHL-dependent response regulators. In all Yersinia species so far examined, the genes coding for LuxR and LuxI homologues are characteristically arranged convergently and overlapping. In Y. pseudotuberculosis AHL-dependent quorum sensing is involved in the control of cell aggregation and swimming motility, the latter via the flagellar regulatory cascade. This is also the case for swimming and also swarming motility in Yersinia enterocolitica. However the role of AHL-dependent quorum sensing in Yersinia pestis remains to be determined.

Introduction
Until relatively recently, cell-to-cell communication was rarely considered to constitute a major mechanism for facilitating bacterial adaptation to an environmental challenge. However, it is now clear that diverse bacterial genera communicate using specific, extracellular signal molecules, which facilitate the coordination of gene expression in a multi-cellular fashion. Signalling can be linked to specific environmental or physiological conditions and is employed by bacteria to monitor their cell population density. The term quorum sensing is commonly used to describe the phenomenon whereby the accumulation of a diffusible, low molecular weight signal molecule (sometimes called an ‘autoinducer’) enables individual bacterial cells to sense when the minimum number or quorum of bacteria has been achieved for a concerted response to be initiated (Williams et al., 2000; Swift et al., 2001; Cámara et al., 2002). The term ‘autoinducer’ implies a positive feedback or autoregulatory mechanism of action. However this is frequently not the case and therefore the term can be misleading and will be avoided here (Cámara et al., 2002). The accumulation of a diffusible signal molecule also indicates the presence of a diffusion barrier, which ensures that more molecules are produced than lost from the micro-habitat (Winzer et al., 2002b; Redfield, 2002). This could be regarded as a type of ‘compartment sensing’, where signal molecule accumulation is both the measure for the degree of compartmentalization and the means to distribute this information among the entire population. Similarly, diffusion of quorum sensing signal molecules between spatially separated bacterial sub-populations may convey information about their physiological state, their numbers, and the individual environmental conditions encountered.

At the molecular level, quorum sensing requires a synthase together with a signal transduction system for producing and responding to the signal molecule respectively. The latter usually involves a response regulator and/or sensor kinase protein (Swift et al., 2001; Cámara et al., 2002). While quorum sensing systems are ubiquitous in both Gram-negative and Gram-positive bacteria, there is considerable chemical diversity in the nature of the signal molecules involved which range from post-translationally modified peptides to quinolones, lactones and furanones (Cámara et al., 2002). In addition, siderophores, which previously were considered only in the context of iron transport, may also function in the producer organism as signal molecules capable of controlling genes unrelated to iron acquisition (Lamont et al., 2002). While there is, as yet, no clear cut evidence for a molecularly conserved quorum sensing system throughout the bacterial kingdom, the LuxS protein and the furanone generated from the ribosyl moiety of S-ribosylhomocysteine (termed AI-2 for autoinducer-2) have been suggested to fulfill such a role (Bassier, 2002). However, many bacteria (e.g. the pseudomonads) do not possess luxS and hence do not produce AI-2 (Winzer et al., 2002a). Furthermore, as LuxS is a key metabolic enzyme in the activated methyl cycle responsible for recycling S-adenosylmethionine (SAM) (Winzer et al., 2002b; Winzer et al., 2002a) phenotypes associated with mutation of luxS are often not a consequence of a defect in cell-to-cell communication but the result of the failure to recycle SAM metabolites (Winzer et al., 2002b; Winzer et al., 2002a). To date, only in Vibrio harveyi is there any direct experimental data to support the function of LuxS as a quorum sensing signal molecule synthase. Thus although Yersinia spp. possess a luxS gene and produce AI-2 (unpublished data) this does not constitute evidence for the presence of a quorum sensing system based on AI-2. Here we examine the nature and contribution of N-acylhomoserine lactone (AHL)-mediated quorum sensing to the lifestyle of Yersinia spp.

*For correspondence: paul.williams@nottingham.ac.uk
© Horizon Scientific Press. Offprints from www.cimb.org
N-Acylhomoserine lactone-dependent quorum sensing

The most intensively investigated family of quorum sensing signal molecules in Gram-negative bacteria are the AHLs. These are produced by many different bacterial genera including Aeromonas, Agrobacterium, Brucella, Burkholderia, Chromobacterium, Erwinia, Enterobacter, Pseudomonas, Rhizobium, Serratia, Vibrio and Yersinia although not in all strains and species of the same genus (Swift et al., 2001). AHLs consist of a homoserine lactone ring covalently linked via an amide bond to an acyl side chain (Fig. 1). To date, naturally occurring AHLs with chain lengths between 4 and 18 carbons have been identified which may be saturated or unsaturated and with or without a hydroxy-, oxo- or no substituent on the carbon at the 3 position of the N-linked acyl chain (Fig. 1). AHLs are usually synthesized by enzymes belonging to the LuxI family, which employ the appropriately charged acyl acyl-carrier protein (acyl-ACP) as the major acyl chain donor while \( \text{S-adenosyl methionine (SAM)} \) provides the homoserine lactone moiety (Jiang et al., 1998). Recent data derived from the crystal structure of EsaI from \( \text{Pantoea Stewartii} \) indicates that LuxI proteins share structural similarities with eukaryotic \( N \)-acyltransferases which employ similar fatty acid precursors as substrates (Watson et al., 2002). Once synthesized, AHLs either move across the bacterial cell envelope by simple diffusion in the case of AHLs with a short acyl side chain, or are pumped out of the cell if the AHLs have a long, e.g. C12 acyl side chain (Pearson et al., 1999).

Apart from the AHL synthase, the second regulatory component of an AHL-dependent quorum sensing circuit is usually a member of the LuxR family of transcriptional regulators (Swift et al., 2001; Câmara et al., 2002; Fuqua et al., 1996). AHLs bind to and activate LuxR proteins such that the LuxR/AHL complex is responsible for either activating or repressing target structural genes (Swift et al., 2001; Câmara et al., 2002; Zhang et al., 2002). The \( V. \ \text{fischeri} \) LuxR protein for example, consists of an N-terminal, cytoplasmic membrane-associated regulatory domain that binds AHLs and a cytoplasmic C-terminal domain that binds DNA. LuxR is considered to activate transcription as a dimer and a number of studies have identified regions within the protein required for multimerization (Choi and Greenberg, 1992a), AHL binding (Shadel et al., 1990; Slock et al., 1990; Hanzelka and Greenberg, 1995), DNA binding, transcriptional activation (Choi and Greenberg, 1991; Choi and Greenberg, 1992b) and autorepression (Choi and Greenberg, 1991). The \( V. \ \text{fischeri} \) LuxR protein appears unable to associate with DNA until a conformational change has been induced via an interaction between the N-terminal AHL binding-domain and the cognate AHL molecule(s) (Choi and Greenberg, 1992b). However, among the growing family of LuxR homologues, it is now clear that there are variations between their respective mechanisms of transcriptional activation (Luo and Farrand, 1999). In addition, certain LuxR homologues function as repressors rather than activators (e.g. EsaR) (von Bodman et al., 1998). To date the structure of only one LuxR homologue has been solved, that of \( \text{TraR} \) from \( \text{Agrobacterium tumefaciens} \) (Zhang et al., 2002). These data revealed that \( \text{TraR} \) forms a dimer and confirmed that the amino-terminal domain contains the AHL-binding region. However, the cognate AHL, \( N \)-3-(oxooctanoyl)homoserine lactone (3-oxo-C8-HSL) is completely buried within the protein and it appears that the AHL associates with nascent, actively folding \( \text{TraR} \) during protein synthesis. However this \( \text{‘co-translational’} \) mechanism may prove to be unique to \( \text{TraR} \) and does not appear to be representative of all LuxR homologues.

The DNA sequence of the LuxR binding site has been determined and is called the \( \text{lux} \) box. This is a 20 nucleotide inverted repeat, which, in \( V. \ \text{fischeri} \) is situated within the intergenic region between \( \text{luxR} \) and \( \text{luxI} \), and is required for the primary regulation of \( \text{lux} \) gene expression. This DNA element has features that are conserved with the binding sites of other LuxR-type proteins and share the consensus RNSTGYAXGATNXTRCASRT where \( N = A, T, C \text{ or G}; R = G \text{ or A}; S = C \text{ or G}; Y = C \text{ or T} \text{ and X = N} \text{ or a gap in the sequence.} \) (Stevens and Greenberg, 1997). In \( \text{Pseudomonas aeruginosa} \) for example, \( \text{lux box-like} \) elements have been identified in the promoter regions of many different target structural genes (Whiteley and Greenberg, 2001) and purified LuxR proteins such as \( \text{TraR} \) (from \( A. \ \text{tumefaciens} \)) and \( \text{ExpR} \) (from \( E. \ \text{chrysanthemi} \)) have been shown to bind \( \text{in vitro} \) to \( \text{lux} \) box-type sequences (Zhu and Winans, 1999; Nasser et al., 1998; Reverchon et al., 1998).

---

Fig. 1. Structures of AHLs produced by \( Y. \ \text{pseudotuberculosis} \). (A) \( N \)-hexanoyl homoserine lactone. (B) \( N \)-(3-oxohexanoyl)homoserine lactone. (C) \( N \)-octanoyl homoserine lactone. (D) \( N \)-(3-oxodecanoyl)homoserine lactone.
AHL-controlled multicellular behaviour in Gram-negative bacteria includes a variety of physiological processes such as bioluminescence, swarming, swimming and sliding motility, antibiotic biosynthesis, biofilm maturation, plasmid conjugal transfer and the production of virulence determinants in animal, fish and plant pathogens (for reviews see Swift et al., 1999; Swift et al., 2001; Williams, 2002).

The search for AHL-mediated quorum sensing in Yersinia

Because of their relative hydrophobicities AHLs can readily be concentrated from culture supernatants by partitioning into organic solvents such as dichloromethane or ethyl acetate. However, since they lack good chromophores, relatively high concentrations are required for detection via their ultra violet spectroscopic properties following separation by HPLC. As a consequence, a number of sensitive bioassays have been developed which facilitate detection of AHL production by bacterial colonies on plates, in microtitre plate assays, on thin layer chromatograms (TLC) and in fractions collected after HPLC-based separation (Bainton et al., 1992; Throup et al., 1995; McClean et al., 1997; Shaw et al., 1997; Winson et al., 1998). For example, Throup et al. (1995) constructed a recombinant AHL reporter plasmid which coupled luxR and the luxI promoter region from Vibrio fischeri to the luxCDABE structural operon of Photobacterium luminescens. When introduced into E. coli, this lux-based AHL reporter (termed pSB401) is dark but responds to the presence of exogenous AHLs by emitting light. The advantage of this AHL reporter compared with earlier versions employing luxAB alone (Bainton et al., 1992; Swift et al., 1993) is that it does not require the addition of a long chain fatty aldehyde (which is required by the luciferase) since the inclusion of the luxCDE genes provides for in situ synthesis of the aldehyde.

Using this AHL bioassay, cell free supernatants from several species of Yersinia including isolates of Yersinia enterocolitica belonging to serotypes O:3, O:8, O:9, O:10K, O:1(2a, 3), a non-typeable strain along with Yersinia pseudotuberculosis serotype III pIB1, Yersinia frederiksenii, Yersinia kristensenii, and Yersinia intermedia were screened. All of these strains induced bioluminescence in the reporter offering preliminary evidence that Yersinia spp. were AHL producers (Throup et al., 1995). Subsequently, Yersinia pestis (Swift et al., 1999) and Yersinia ruckeri (unpublished data) were also confirmed as AHL-producers using the same strategy.

AHL-mediated quorum sensing in Y. enterocolitica

Although E. coli [pSB401] incorporates LuxR from V. fischeri, the cognate AHL for which is N-(3-oxohexanoyl)homoserine lactone (3-oxo-C6-HSL), it responds, with differing sensitivities, to a variety of AHLs (Winson et al., 1998). While a positive bioassay result confirms the presence of AHLs, it does not indicate either which AHL or indeed how many different AHLs may be present in the sample. To characterize unequivocally the AHLs present in spent Y. enterocolitica cultures supernatants, they were first concentrated by solvent extraction and subjected to preparative HPLC. The fractions found to be positive in the AHL bioassay were subjected to high-resolution tandem mass spectrometry (MS-MS) which revealed the presence of two AHLs in an approximately 50:50 ratio. These AHLs were identified as 3-oxo-C6-HSL and N-hexanoyl homoserine lactone (C6-HSL) respectively (Fig. 1) (Throup et al., 1995) and are easily separated and visualized by thin layer chromatography (TLC) as purple spots on a white background using the Chromobacterium violaceum CV026 biosensor in an agar overlay (see Fig. 2 for an example of this TLC overlay originally described by McClean et al., 1997).

Since AHL synthesis depends on the presence of an AHL synthase, a plasmid-based in trans complementation strategy in which a Y. enterocolitica chromosomal gene library was transformed into E. coli [pSB401] was devised (Swift et al., 1993; Throup et al., 1995). This approach yielded a highly bioluminescent clone indicative of an insert carrying a gene whose product was capable of inducing the AHL reporter fusion. This clone was subsequently shown to be producing both 3-oxo-C6-HSL and C6-HSL and to contain two convergently transcribed and overlapping open reading frames (Fig. 3). The first of these genes was termed yenl, the product of which exhibited homology to the LuxI protein family while the product of the second, termed yenR belongs to the LuxR protein family. TLC in conjunction with the CV026 biosensor showed that inactivation of yenl by deletion mutagenesis abolished AHL production in Y. enterocolitica confirming the function of yenl as an AHL synthase.

In V. fischeri lux expression is governed by a positive feedback loop as a consequence of its induction by LuxR activated by 3-oxo-C6-HSL and C6-HSL and to contain two convergently transcribed and overlapping open reading frames (Fig. 3). The first of these genes was termed yenl, the product of which exhibited homology to the LuxI protein family while the product of the second, termed yenR belongs to the LuxR protein family. TLC in conjunction with the CV026 biosensor showed that inactivation of yenl by deletion mutagenesis abolished AHL production in Y. enterocolitica confirming the function of yenl as an AHL synthase.

![Fig. 2. TLC chromatogram of the AHLs present in cell-free supernatants of Y. pseudotuberculosis grown with shaking in LB at 22°C and detected using the C. violaceum CV026 AHL biosensor. Lane A, 3-oxo-C6-HSL; 1.4 x 10^{-4} mol; lane B from top, AHL standards, N-pentanoylhomoserine lactone (C5-HSL) (5.4 x 10^{-5} mol), C6-HSL (5 x 10^{-4} mol), C7-HSL (4.7 x 10^{-4} mol) and C8-HSL (2.2 x 10^{-4} mol); lane C, Y. pseudotuberculosis supernatant extract from cultures grown at 22°C showing the presence of 3-oxo-C6-HSL, C6-HSL, C7-HSL and C8-HSL. It should be noted that CV026 does not respond to long chain AHLs and the size of the spots are not equivalent to the concentration of AHL present.](image-url)
Such multicellular behaviour has been implicated in bacterial migration over solid surfaces and motility is a flagellum-dependent process unable to swim or swarm (unpublished data). Swarming mutants of Y. enterocolitica 90/54, in contrast to the 10460 parental strain, grew into a biofilm and may be involved in quorum sensing in controlling plasmid-borne functions of the type III secretion system (Kapperud et al., 1985; Cornish et al., 1987; Pepe and Miller, 1993; Pierson and Falkow, 1993). Although Y. enterocolitica strains 10460 and 90/54 are not isogenic, these data do provide preliminary evidence linking quorum sensing with the virulence plasmid even though the loss of motility and swarming are both linked to flagellin expression/function given that flagellar biosurfactant production. For mutants defective in AHL production, swarming can be restored by the provision of either the appropriate AHL signal molecule(s) or a biosurfactant. In addition, although such mutants do not swim or swarm, this suggests that AHL-mediated quorum sensing in this organism is directly linked to flagellin expression/function given that flagellar biosurfactant production is required for both swimming and swarming motility. In the Y. enterocolitica 90/54 mutant, swimming could not be restored by providing an exogenous surfactant and since the mutant also failed to swim, this suggests that AHL-mediated quorum sensing in this organism is directly linked to flagellin expression/function given that flagellar biosurfactant production is required for both swimming and swarming motility. When analysed by SDS-PAGE the 90/54 mutant fails to produce flagellin when compared to the parent and using RT-PCR the expression of fliC was examined in the Y. enterocolitica parent and Y. enterocolitica 90/54 mutant. These data suggested that quorum sensing regulates motility at the level of fliC expression (unpublished observations). Furthermore, given that motility is required to initiate host cell invasion by Y. enterocolitica (Young et al., 2000), it is possible that AHL-mediated quorum sensing may also turn out to play a role in this context.

AHL-mediated quorum sensing in Y. pseudotuberculosis

TLC analysis of Y. pseudotuberculosis spent culture supernatants grown at 22°C (2°C) extracted with dichloromethane using both C. violaceum CV026 (Fig. 2) and E. coli pSB401 revealed the presence of at least three AHLs, the chemical identities of which were subsequently confirmed using HPLC and MS as 3-oxo-C6-HSL, C6-HSL and N-octanoyl-l-homoserine lactone (C8-HSL) (Fig. 2). However, when grown to stationary phase at 37°C in LB medium, Y. pseudotuberculosis appears to produce extremely low levels of these AHLs. Since temperature plays an important role in the regulation of virulence genes in Y. pseudotuberculosis (Kapperud et al., 1985; Cornish et al., 1987; Pepe and Miller, 1993; Pierson and Falkow, 1993; Cornish and Wolf-Watz, 1997; Bleves and Cornil, 2000), AHL production was examined in stationary phase cultures grown for 24 h at 28°C and 22°C (Yates et al., 2002). By comparing the AHL profiles of cultures grown at the three different temperatures, AHL levels were found to be markedly dependent on growth temperature. The higher levels appeared to be greater after growth at 22°C, substantially reduced at 28°C and almost undetectable after growth at 37°C. Initially this suggested that either the expression or function of the corresponding AHL synthase(s) or substrate availability might be temperature dependent, implying that quorum sensing was temperature dependent.
dependent. However, it subsequently became clear that there was a simple physico-chemical explanation. This is because the AHL ring is highly susceptible to pH-dependent lactonolysis. Ring opened compounds are unable to activate LuxR-type proteins and therefore do not activate AHL biosensors. By simply acidifying spent culture supernatants to below pH 2.0, lactonolysis can be reversed and the AHLs recovered from cultures grown at 37°C (Yates et al., 2002). In LB, which is unbuffered, the growth of *Y. pseudotuberculosis* rapidly renders the pH alkaline as a consequence of the metabolism of amino acids and the release of ammonia. The rate of pH-dependent lactonolysis is further enhanced by raising the incubation temperature. Thus, these parameters must be taken into consideration for any study which seeks to quantitate AHL levels in culture supernatants. Furthermore, the AHL profile is also dependent on the structure of the AHLs present. In the context of lactonolysis, the longer the acyl side chain of an AHL then the more stable it will be towards increases in pH and temperature such as those encountered *in vivo* in mammalian hosts. Indeed the data obtained by Yates et al. (2002) revealed that to be functional under physiological conditions in mammalian tissue fluids, AHLs require an acyl side chain of at least 4 carbons in length. This explains why homoserine lactone itself cannot function as a quorum sensing signal molecule as it rapidly undergoes lactonolysis at pHs above 2 (Yates et al., 2002). This in turn suggests that the production of a range of AHL signal molecules with different acyl side chain lengths provides a pathogen with greater versatility in adapting to changing environments.

**Y. pseudotuberculosis produces multiple short and long chain AHLs**

Using the same trans-complementation strategy described by (Throup et al., 1995) i.e. the introduction of a genomic library into the AHL biosensor *E. coli* [pSB401], Atkinson et al (1999) cloned a luxI homologue from *Y. pseudotuberculosis* by screening for bioluminescent clones. This approach facilitated the identification of a LuxI homologue, YpsI and a LuxR homologue, YpsR. Deletion insertion mutations were introduced into both ypsI and ypsR and an analysis of the AHL profiles of both mutants revealed that after growth at 37°C, the mutants produced the same three AHLs (3-oxo-C6-HSL, C6-HSL and C8-HSL) as the wild type. However, the ypsI mutant produced no 3-oxo-C6-HSL at 28°C while the ypsR mutant produced reduced levels of 3-oxo-C6-HSL and C6-HSL and no C8-HSL could be detected (Atkinson et al., 1999). These data suggested that *Y. pseudotuberculosis* possessed an additional AHL synthase, the expression of which was likely therefore to be controlled by YpsR.

To locate this second putative AHL synthase, the same complementation strategy used above was employed but using genomic DNA prepared from the Y. *pseudotuberculosis* ypsI mutant (Atkinson et al., 1999). This approach yielded a clone containing two genes termed ytbI and ytbR which when translated, exhibited homology to YpsI and YpsR and were therefore additional members of the LuxR and LuxI protein families. When expressed in *E. coli*, YtbI is responsible for the production of C6-HSL and C8-HSL and a molecule we tentatively identified as, N-heptanoylhomoserine lactone (C7-HSL) while YpsI generates 3-oxo-C6-HSL and C6-HSL (Atkinson et al., 1999 and unpublished data). These data suggest that there is a difference in the AHLs made via YtbI and YpsI in an *E. coli* genetic background and those made in *Y. pseudotuberculosis* since in the latter homologous background, YtbI can clearly produce all three major AHLs in the absence of YpsI (Atkinson et al., 1999). This finding was unexpected since the AHL profile of recombinant LuxI proteins from other bacteria expressed in *E. coli* has previously corresponded closely with the AHL profile in the original organism (Atkinson et al., 1999). Notwithstanding our observations with respect to pH-dependent lactonolysis, these data indicate that YtbI is able to vary the AHL profile produced in *Y. pseudotuberculosis* in a temperature-dependent manner and in the absence of YpsI. AHL production in *Y. pseudotuberculosis* is abolished when both ypsI and ytbI are mutated but not after deletion of both ypsR and ytbR indicating that, as in *Y. enterocolitica*, AHL production is not dependent on the presence of a functional YpsR or YtbR protein (Buckley, 2002).

Much of the analysis of AHL production in *Y. pseudotuberculosis* was undertaken using AHL biosensors which preferentially respond to short chain (C4-C8) AHLs (Fig. 2). Recently, we have re-investigated AHL production in *Y. pseudotuberculosis* using an AHL biosensor (pSB1075) based on the LuxR homologue, LasR from *Pseudomonas aeruginosa*, the cognate AHL for which is 3-oxo-C12-HSL. In pSB1075, we have incorporated lasR and the lasI promoter fused to luxCDAEB (Winson et al., 1998). When *E. coli* [pSB1075] was used as an overlay in conjunction with a TLC system optimized for the chromatography of long chain AHLs employing reverse phase RP-2 TLC plates and 45% v/v methanol in water as the mobile phase (Yates et al., 2002), dichloromethane extracts of the Y. *pseudotuberculosis* wild type were found to contain three long chain AHLs. These were tentatively identified as N-(3-oxododecanoyl)homoserine lactone (3-oxo-C10-HSL), N-(3-oxododecanoyl)homoserine lactone (3-oxo-C12-HSL) and N-(3-oxotetradecanoyl)homoserine lactone (3-oxo-C14-HSL). None of these AHLs were made by the ypsI/ytbI double mutant nor by the ytbI mutant suggesting that YtbI was the synthase responsible for their synthesis. This was confirmed by demonstrating that *E. coli* expressing ytbI but not ypsI also produces the long chain AHLs (Buckley, 2002).

**AHL-mediated quorum sensing controls motility and cell aggregation in Y. pseudotuberculosis**

When grown in LB at 28°C or 37°C, *Y. pseudotuberculosis* ypsR mutant cells aggregate, a phenomenon which does not occur at 22°C or in either the parent or ypsR mutant at any temperature (Atkinson et al., 1999) (Fig. 4). This phenotype implied the involvement of surface components which were likely to be regulated via AHL-dependent quorum sensing. SDS-PAGE of cell surface extracts prepared from parent, ypsI and ypsR mutants revealed that the expression of numerous proteins was affected. In particular, a prominent protein of approximately 42 kDa was substantially up-regulated.
motility. These data indicate that the ytbR/I locus may be an activator of motility while the ypsR/I locus functions to repress motility. The two quorum sensing circuits also appear to be linked since YpsR appears to be involved in the control of ytbI expression since ypsR mutants fail to produce the YtbI-generated AHL, C8-HSL during growth at 28°C.

Since ypsl/ytbl and ypsR/ytbR double mutants are non-motile while the ypsR and ypsl single mutants exhibit early motility in batch culture when compared to the wild type, it is likely that the quorum sensing circuitry controls the expression of key regulatory genes involved in the control of swimming motility. In enteric bacteria, flagellar gene transcription is arranged as a hierarchy with genes in three regulatory levels or classes which are responsible for producing and maintaining functional flagella and the chemotaxis apparatus (MacNab, 1996). FliD and FliC activate the class II structural genes encoding the basal body and hook proteins, along with an activator FliA (αF) and a repressor FliM (anti-αF) of class III genes. Class III genes encode the proteins which constitute the mature flagellar filament, chemotactic sensors and motor and are transcribed from FliA-dependent promoters in a temperature dependent manner (MacNab, 1996). Although the flhDC master regulatory system was once considered to be exclusively involved in regulating the production of flagella for swimming motility (Fraser and Hughes, 1999) it is now known to play a more global role in regulating diverse physiological processes (Pearson et al., 2000; Tan et al., 1999). In Y. enterocolitica (Young et al., 1999) and P. mirabilis (Fraser and Hughes, 1999), fliDC is required for flagellin production, swimming motility and swarming motility.

An analysis of the Y. pseudotuberculosis flhDC and fliA promoter/operator region revealed the presence of putative lux-box like elements. By employing reporter gene fusions in single and double ypsR/I and ytbR/I mutants we have established that, for example flhDC expression is repressed by YpsR and YtbR at 37°C. In addition, Ytb appears to be involved as an activator of flhDC expression at temperatures below 28°C (unpublished data).

In a Y. enterocolitica flhDC mutant (Bleves et al., 2002) noted an increase in Yop expression and a loss of the temperature dependency usually associated with Yop expression at 37°C. Since the Y. pseudotuberculosis quorum sensing circuitry appears to control flhDC, this implies the existence of a regulatory link between type III secretion and motility. However such a link has to be demonstrated experimentally.
biofilm development is associated with the expression of components of the hms locus (Jarrett et al., 2004). However, under the conditions tested and using a triple Y. pestis mutant defective for ypel, yepl and luxS, Jarrett et al (2004) were unable to establish a link between the quorum sensing system of Y. pestis and the hms locus. The relationship between the hms locus and quorum sensing has yet to be characterized in Y. pseudotuberculosis but once tested may stimulate further investigations into the differential control of biofilm formation in both yersinia species.

The biological significance of long chain AHLs produced by Y. pseudotuberculosis

Although only C6-HSL, 3-oxo-C6-HSL and C8-HSL were initially identified and fully chemically characterized (Atkinson et al., 1999), it is also clear, as indicated above that Y. pseudotuberculosis produces three long chain AHLs, 3-oxo-C10-HSL, 3-oxo-C12-HSL and 3-oxo-C14-HSL. These AHLs which are also produced via recombinant YtbI expressed in E. coli are not produced by recombinant YpsI. They are present in spent supernatants prepared from ypsI mutants but absent from spent culture supernatants prepared from ytbI mutants or ypsi ytbI double mutants, the latter of which make no detectable AHLs (Buckley, 2002). While the significance of this finding in the context of quorum sensing dependent gene regulation in Y. pseudotuberculosis is not yet clear, it is worth noting that 3-oxo-C12-HSL (which is also produced via Lasl in P. aeruginosa; (Passador et al., 1993; Pearson et al., 1994) possesses potent, dose dependent, pro-inflammatory, immune modulatory and vasorelaxant properties (Telford et al., 1998; Lawrence et al., 1999; Smith et al., 2001; Chhabra et al., 2003). For example 3-oxo-C12-HSL modulates both T and B cell functions, blocks lipopolysaccharide-stimulated production of tumour necrosis factor alpha (TNF-α) by peritoneal macrophages and mediates switching of the T-helper-cell response from the antibacterial Th-1 response (characterized by IL-12 and gamma interferon production) to a Th-2 response. In addition, 3-oxo-C12-HSL also exerts a pharmacological effect on the cardio-vascular system suggesting that host cardiovascular function may be modulated, or influenced, by bacterial quorum sensing molecules. In isolated porcine coronary arteries, 3-oxo-C12-HSL caused a concentration dependent relaxation effect on thromboxane mimetic-induced contractions (Lawrence et al., 1999) and also induces a marked bradycardia in live conscious rats (Gardiner et al., 2001). Structure activity studies have indicated that the immune modulatory properties of AHLs are optimal in compounds with 11 to 13 acyl chain carbons and a 3-oxo substituent (Chhabra et al., 2003). Short chain (4 to 8 acyl carbons) AHLs have little activity in this context. Thus, 3-oxo-C12-HSL and to a lesser extent 3-oxo-C10-HSL and 3-oxo-C14-HSL may contribute to virulence and tissues to maximize the provision of nutrients via the bloodstream while down regulating host defence mechanisms.

Quorum sensing in Y. pestis and Y. ruckeri

Given the close genetic relationship between Y. pseudotuberculosis and Y. pestis, it is perhaps not surprising to find two pairs of LuxRI homologues, the genes for which have been termed ypeRI and yepRI in the latter (Isherwood, 2001; Swift et al., 1999). In addition, preliminary studies on Y. ruckeri, which is responsible for enteric red mouth disease in salmonid fish, have also revealed the presence of a luxRI pair, termed yukRI (unpublished data). Each of these Yersinia LuxRI and LuxI homologues share significant homology with each other and each genetic locus is organized similarly in that the two genes are convergently transcribed and overlapping by either 8 or 20 bp in the respective host species (Fig. 3). Neither the genetic nor physiological consequences of such a gene arrangement are clear since LuxR and LuxI homologues in different organisms can be transcribed in tandem, divergently or convergently (Salmond et al., 1995; Swift et al., 1999).

Using AHL biosensors such as C. violaceum CV026, initial cross streak analysis of Y. pestis isolates taken from a range of environmental and geographic sources revealed that AHL production is conserved in this Yersinia species. Subsequent analysis indicated that Y. pestis, in common with Y. pseudotuberculosis produces C6-HSL, 3-oxo-C6-HSL and C8-HSL (Swift et al., 1999). Furthermore, YpeI (which is more closely related to YpsI and YenI than YtbI), directs the synthesis of C6-HSL and 3-oxo-C6-HSL suggesting that the second Y. pestis LuxI homologue, YpeI is responsible for C8-HSL synthesis. Sequence analysis indicates that YpeI is more closely related to YtbI than YpsI which is consistent with the nature of the respective AHLs produced. Whether YpeI in Y. pestis is also responsible for producing additional AHLs including compounds with long (C10-C14) acyl side chains is not yet known. For Y. ruckeri, preliminary TLC analysis of the recombinant YukI expressed in E. coli suggests that this fish pathogen also produces C6-HSL, 3-oxo-C6-HSL and C8-HSL (unpublished data). Indeed although analysis of the translated sequence for a given LuxI homologue provides little information on the nature of the corresponding AHL(s) produced, the presence of a threonine at position 143 in the carboxy terminal region relative to the V. fischeri LuxI protein (Watson et al., 2002) is characteristic of synthases which direct the synthesis of 3-oxo-substituted AHLs. This observation appears to hold for all six Yersinia LuxI homologues all of which are capable of 3-oxo-C6-HSL production.

As yet, the target structural genes controlled via the yukRI locus in Y. ruckeri have not been identified and no mutants have been constructed. However in Y. pestis, both ypel and ypeR have both been mutated. Despite the observation that quorum sensing is a key regulator of motility in both Y. pseudotuberculosis and Y. enterocolitica, Y. pestis does not exhibit any motility under the conditions tested at all temperatures even though the genome contains one full set of flagella genes plus a second partial set (Parkhill et al., 2001; Isherwood, 2001).
of Y. pestis virulence gene expression in parent, ypeL and ypeFR mutants grown at 28°C and 37°C did not reveal any differences with respect to V-antigen, pH 6 antigen, the coagulase/fibrinolytic protein Pla or lipopolysaccharide profile (Swift et al., 1999). However in a mouse infection model an increase in time to death was observed for mice challenged with the mutant when compared to the parent. For example at a challenge dose of approximately 10^6 cfu the mean time to death increased from 5.4 days for mice challenged with Y. pestis GB to 6.7 days for mice challenged with the ypeR mutant implying a possible role for AHL-dependent quorum sensing in the pathogenesis of Y. pestis infections (Swift et al., 1999).

Conclusions
AHL-dependent quorum sensing is clearly highly conserved within the genus Yersinia. Together with temperature, bacterial cell population density is at least one additional environmental parameter which the organism must sense during adaptation to life in different ecological niches. In Y. pseudotuberculosis and Y. enterocolitica, motility depends on the integration of both parameters. In Y. pestis, the identity of the target structural genes regulated via AHL-dependent quorum sensing is not yet apparent and may reflect the recent gene/promoter loss or rearrangement as a consequence of its recent divergence from the Y. pseudotuberculosis infection route to the insect vector route. For all three species of human pathogenic yersiniae, genome wide microarray analysis should rapidly facilitate mapping of the quorum sensing regulons and the subsequent definition of their contribution to survival and virulence.

Acknowledgements
Work in the authors’ laboratories has been supported by grants and studentships from the Wellcome Trust, Biotechnology and Biological Sciences Research Council, UK and the Medical Research Council, UK which are gratefully acknowledged.

References


Yersinia pestis from an infectious biofilm in the flea vector. J. Infect. Dis. 190, 783–792.


Further Reading

Caister Academic Press is a leading academic publisher of advanced texts in microbiology, molecular biology and medical research. Full details of all our publications at caister.com

• MALDI-TOF Mass Spectrometry in Microbiology
  Edited by: M Kostrzewa, S Schubert (2016)
  www.caister.com/malditof

• Aspergillus and Penicillium in the Post-genomic Era
  Edited by: RP Vries, IB Gelber, MR Andersen (2016)
  www.caister.com/aspergillus2

• The Bacteriocins: Current Knowledge and Future Prospects
  Edited by: RL Dorfl, SM Roy, MA Riley (2016)
  www.caister.com/bacteriocins

• Omics in Plant Disease Resistance
  Edited by: V Bhadauria (2016)
  www.caister.com/oppdr

• Acidophiles: Life in Extremely Acidic Environments
  Edited by: R Quatrini, DB Johnson (2016)
  www.caister.com/acidophiles

• Climate Change and Microbial Ecology: Current Research and Future Trends
  Edited by: J Marxsen (2016)
  www.caister.com/climate

• Biofilms in Bioremediation: Current Research and Emerging Technologies
  Edited by: G Lear (2016)
  www.caister.com/bioem

• Microalgae: Current Research and Applications
  Edited by: MN Tsialogiou (2016)
  www.caister.com/microalgae

• Gas Plasma Sterilization in Microbiology: Theory, Applications, Pitfalls and New Perspectives
  Edited by: H Shintani, A Sakudo (2016)
  www.caister.com/gasplasma

• Virus Evolution: Current Research and Future Directions
  Edited by: SC Weaver, M Denison, M Roossinck, et al. (2016)
  www.caister.com/virosevol

• Arboviruses: Molecular Biology, Evolution and Control
  Edited by: N Vasikakis, DJ Gubler (2016)
  www.caister.com/arbo

• Shigella: Molecular and Cellular Biology
  Edited by: WD Picking, WL Picking (2016)
  www.caister.com/shigella

• Aquatic Biofilms: Ecology, Water Quality and Wastewater Treatment
  Edited by: AM Romani, H Guasch, MD Balaguer (2016)
  www.caister.com/aquaticbiofilms

• Alphaviruses: Current Biology
  Edited by: S Mahalingam, L Herrero, B Herring (2016)
  www.caister.com/alphha

• Thermophilic Microorganisms
  Edited by: F Li (2015)
  www.caister.com/thermophile

• Flow Cytometry in Microbiology: Technology and Applications
  Edited by: MG Wilkinson (2015)
  www.caister.com/flow

• Probiotics and Prebiotics: Current Research and Future Trends
  Edited by: K Venema, AP Carmon (2015)
  www.caister.com/probiotics

• Epigenetics: Current Research and Emerging Trends
  Edited by: BP Chadwick (2015)
  www.caister.com/epigenetics2015

• Corynebacterium glutamicum: From Systems Biology to Biotechnological Applications
  Edited by: A Burkovski (2015)
  www.caister.com/cory2

• Advanced Vaccine Research Methods for the Decade of Vaccines
  Edited by: F Bagnoli, R Rappuoli (2015)
  www.caister.com/vaccines

• Antifungals: From Genomics to Resistance and the Development of Novel Agents
  Edited by: AT Coste, P Vandeputte (2015)
  www.caister.com/antifungals

• Bacteria-Plant Interactions: Advanced Research and Future Trends
  www.caister.com/bacteria-plant

• Aeromonas
  Edited by: J Graf (2015)
  www.caister.com/aeromonas

• Antibiotics: Current Innovations and Future Trends
  Edited by: S Sánchez, AL Demain (2015)
  www.caister.com/antibiotics

• Leishmania: Current Biology and Control
  Edited by: S Adak, R Datta (2015)
  www.caister.com/leish2

• Acanthamoeba: Biology and Pathogenesis (2nd edition)
  Author: NA Khan (2015)
  www.caister.com/acionthamoeba2

• Microarrays: Current Technology, Innovations and Applications
  Edited by: Z He (2014)
  www.caister.com/microarrays2

• Metagenomics of the Microbial Nitrogen Cycle: Theory, Methods and Applications
  Edited by: D Marco (2014)
  www.caister.com/n2