The social behaviours of bacterial pathogens

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Introduction

The term quorum sensing (QS) is used to describe communication between bacterial cells, whereby a coordinated population response is controlled by diffusible signal molecules produced by individuals.

Sources of data: Studies on QS-mediated signalling processes in bacteria have revealed the existence of intricate regulatory networks to enable bacterial populations to fine tune their responses to environmental changes and increase their chances of survival, using complex signalling pathways.

Areas of agreement: A population of bacteria invading a host may benefit from the coordinated release of virulence determinants and in vitro studies have shown that QS regulates virulence factor production in many species of bacteria.

Areas of controversy: However, the role of QS in vivo is less well understood, but has been demonstrated to be important in several pathogenic organisms.

Growing points and areas timely for developing research: There is a growing interest in blocking bacterial cell–cell communication as a means to control infections. This review discusses QS from a pathogenic perspective and discusses the potential of QS as an anti-pathogenic target.

Keywords: quorum sensing/Pseudomonas aeruginosa
At the heart of tackling, the huge challenge posed by infectious microorganisms is the overwhelming need to understand their nature. Although often viewed as simple creatures, the study of microbial development has shown that they are capable of a multitude of behaviours. Crucially, this is not just at the level of the individual cell. One of the most remarkable discoveries in microbiology in the past 30 years has been the fact that bacterial cells communicate with each other in a process commonly known as quorum sensing (QS). This enables the coordination of a population of cells, resulting in a hitherto unrecognized sophistication in the behaviour of bacteria. Unravelling the complexities of bacterial social behaviours enables a deeper understanding of their pathogenic mechanisms and offers new and promising opportunities for future antimicrobial agents. The progress made in this field will be discussed in this review and illustrated using the behaviour of the important human pathogen *Pseudomonas aeruginosa* in the cystic fibrosis (CF) lung environment.

**What is quorum sensing?**

The history of bacterial cell-to-cell communication dates back several decades. While working on the marine bioluminescent bacterium *Vibrio fischeri* which naturally illuminates the light organs of squid, Nealson and colleagues noticed that bioluminescence only occurred at a threshold population density of bacterial cells and that spent culture supernatants could induce luminescence at lower population densities. It was hypothesized that small diffusible molecules (autoinducers) were emitted and that the concentration of autoinducer could be sensed by bacterial cells and that this conveyed information about population density. The autoinducer was later purified and identified as N-(3-oxohexanoyl)-homoserine lactone (3-oxo-C6-HSL) which is a member of the N-acyl homoserine lactone (AHL) family of molecules.2

Although initially thought to be a phenomenon restricted to a few marine *Vibrio* species, it was later shown that the production of the β-lactam antibiotic, 1-carbapen-2-em-3-carboxylic acid (carbapenem) by the terrestrial plant pathogen *Erwinia carotovora* was also regulated by AHLs.3 Since then, many Gram-negative species have been shown to possess AHL-signalling systems that regulate a wide variety of different phenotypes.2 Signalling is not restricted to Gram-negative bacteria; a number of Gram-positive organisms have been shown to employ small, modified oligopeptides as extracellular signalling molecules. These peptides activate gene expression by interacting with two-component histidine protein kinase signal transduction systems. For example, in *Staphylococcus aureus*, the expression of a number of cell
density-dependent virulence factors is regulated by the global regulatory locus \textit{agr} (accessory gene regulator).\textsuperscript{2} In fact, a lexicon of compounds have now been described as QS signal molecules in both Gram-negative and Gram-positive bacteria\textsuperscript{2} (Fig. 1). For this short review, we focus primarily on AHL QS systems found in Gram-negative bacteria.

The process of cell-to-cell signalling using small molecules was termed ‘Quorum sensing’ because it is a similar process to how decisions in a law court are made only when a threshold number of members (a quorum) are present.\textsuperscript{4} Autoinduction by AHL molecules occurs when an AHL signal binds to its cognate receptor inside the cell, usually at a threshold concentration of the signal which correlates to the number of bacterial cells present. Once the signal-receptor complex is formed, it binds to the promoter region of the signal synthase gene. This results in an autoinduction of signal synthesis. It is important to note that the process of signal autoinduction also occurs...
in QS systems where AHLs are not the signal molecule. In addition to increased signal production, the signal-receptor complex regulates the expression of a suite of genes, resulting in a change in phenotypic behaviour. An example of a QS-circuit is depicted in Figure 2.

AHL-dependent QS has been shown to regulate a number of diverse behaviours and phenotypes in Gram-negative bacteria. Although many of these behaviours can theoretically be performed by individual bacterial cells working in isolation, they become more effective when the bacteria work as a group. These include antibiotic production, exotoxin production, virulence and motility.2 One of the most complex social behaviours performed by microbes is the development of biofilms. In the formation of biofilms, cells abandon the isolation of the planktonic mode of growth and group together to form organized ‘slime-cities’. These complicated structures often contain channels for the import of nutrients and the disposal of waste products and they may even contain specialist cells, which appear to have specific roles within the biofilm.5 Medically, biofilms are of huge importance as they are capable of forming in the lungs of chronically ill patients and often colonize medical devices such as catheters and prosthetic valves. This is especially problematic as they are often resistant to desiccation and treatment with biocides and antibiotics. QS appears to play a role in the development of biofilms as in several species of Gram-negative
bacteria, disruption of the QS system has been shown to affect biofilm formation and differentiation.6

**Quorum sensing and virulence**

**QS and virulence factor production**

The virulence of an organism often depends on its ability to produce and release toxins that can damage a host. However, production of extracellular metabolites may lead to detection of the bacteria and ultimately destruction by the host immune system. Therefore, an effective strategy for bacterial cells to adopt is to avoid producing and releasing toxins until their numbers are sufficient when a simultaneous toxin release can overwhelm a host immune response. By using several animal host models, including nematodes and mice, QS-dependent control of virulence determinants and virulence itself has now been demonstrated in several human pathogenic organisms. These include *Burkholderia pseudomallei*, the causative agent of melioidosis,7 *B. mallei*,8 *B. cenocepacia*,9 *V. cholerae*10 and *S. aureus*.11

Perhaps, the most studied QS system is that of *P. aeruginosa*. *Pseudomonas aeruginosa* is a Gram-negative bacterium, capable of causing disease in plants, animals and humans.12 It is a major source of nosocomial infections and is a leading cause of mortality in CF patients.13 As an opportunistic human pathogen, *P. aeruginosa* can colonize a wide variety of anatomical sites. This is because the organism produces an arsenal of extracellular virulence factors which are capable of causing extensive tissue damage and bloodstream invasion which consequently promotes systemic dissemination. Many of these exoproducts are regulated in a cell density-dependent manner via QS. *Pseudomonas aeruginosa* possesses two AHL-dependent QS systems. These are termed the las and rhl systems, comprising the LuxRI homologues, LasRI14 and RhlRI,15 respectively. LasI directs the synthesis of primarily N-(3-oxododecanoyl)-L-homoserine lactone (3-oxo-C12-HSL) and together with the transcriptional regulator LasR regulates the production of virulence factors including elastase, the LasA protease, alkaline protease and exotoxin A.2 RhlI directs the synthesis of N-butanoyl-L-homoserine lactone (C4-HSL)16 which activates RhlR and in turn RhlR/C4-HSL induces the production of rhamnolipid, elastase, LasA protease, hydrogen cyanide, pyocyanin, siderophores and the cytotoxic lectins PA-I and PA-II.15–18 The las and the rhl systems are organized in a hierarchical manner such that the las system exerts transcriptional control over both rhlR and rhlI17 (Fig. 3). In addition to AHL-dependent QS, *P. aeruginosa* also produces over
50 2-alkyl-4(1H)-quinolones (AQs), some of which were originally identified from their antibacterial properties, although the biological function of many of these is not known. One of these compounds, 2-heptyl-3-hydroxy-4(1H)-quinolone was discovered to function as a diffusible signal molecule and termed the pseudomonas quinolone signal (PQS; Fig. 1). Subsequently, PQS was shown to regulate *P. aeruginosa* virulence gene expression and to function as an integral component of the QS network since PQS production is modulated by both the *las* and the *rhl* systems (Fig. 3). This demonstrates the complexity of some QS systems. The importance of QS for the virulence of *P. aeruginosa* in acute infections has been confirmed in a number of animal models including mice, nematodes and insects.

**QS in vivo: the cystic fibrosis lung**

While *P. aeruginosa* can cause severe infections in burn wounds and intubated patients, it is particularly problematic in the lungs of patients with CF where it causes chronic infections which result in extensive tissue damage, reduction in lung function and ultimately death. It has been demonstrated that *P. aeruginosa* QS genes are expressed in the lungs of CF patients and that sputum from the CF lung often contains QS signal molecules suggesting that QS virulence is important during pathogenesis in CF.

In CF infections, *P. aeruginosa* is more resistant to antibiotic treatment than is predicted by susceptibility tests on planktonic cells and it is thought that the added resistance of *P. aeruginosa* is due, in part, to...
a ‘biofilm’ lifestyle within the lung.\textsuperscript{26} Here, the cells adhere to a surface and secrete a polymeric matrix that binds and protects them from antibiotic therapy and the host immune response.\textsuperscript{27} QS has been shown to play a role in the development of \textit{P. aeruginosa} biofilms \textit{in vitro}. Inactivation of QS results in the formation of flatter, less structured biofilms than those seen in the corresponding wild type. Furthermore, when QS is disrupted, the biofilms formed are often more susceptible to treatment with biocides and antibiotics.\textsuperscript{28}

However, \textit{in vivo}, the role of QS in biofilm formation is less clear. Recent observations suggest that QS-deficient \textit{P. aeruginosa} mutants are often isolated from sputum samples.\textsuperscript{29,30} These mutants mostly carry mutations in the \textit{lasR} regulator gene which impairs the response to signal molecules. There are a number of plausible explanations for this. First, QS may not be important for growth in this environment and therefore Darwinian selection results in a loss of QS over time. Secondly, it has been suggested that a \textit{lasR} mutation confers a growth advantage on particular carbon and nitrogen sources which may give a selective advantage over QS-positive strains.\textsuperscript{31}

Another plausible explanation for the prevalence of \textit{lasR} mutants is that bacteria can ‘cheat’ or ‘freeload’ on QS cooperating populations.\textsuperscript{32} In \textit{P. aeruginosa}, many QS-regulated products are released into the extracellular environment and benefit not only the producing cell but also its neighbours. Mutants that do not respond to QS signals do not incur the cost of producing these ‘public goods’ but gain the benefit of production by neighbours.\textsuperscript{33,34} Put another way, \textit{lasR} cheaters have a social fitness advantage over QS-positive strains. This cooperation and cheating principle has been demonstrated for QS \textit{in vitro}\textsuperscript{33,34} but also for the production of other public goods which in turn influence the virulence of an infection.\textsuperscript{35,36} Social cheating could reconcile why \textit{lasR} mutants are repeatedly found in clinical isolates from CF patients and yet QS-deficient mutants of \textit{P. aeruginosa} have reduced virulence when grown in monocultures.\textsuperscript{37} A detailed ecological survey of CF lung infections investigating the extent of QS-positive and negative strains will help us to unravel the secrets of this problematic organism within this environment and may provide clues as to why CF infections become chronic and persistent over time.

**Bacterial cross-talk**

Much of the work on QS signalling systems has focused on studying signalling between single species (intraspecies) bacterial populations and communication between \textit{P. aeruginosa} cells using AHLs is a likely example of intraspecies signalling. Because different bacterial species
may make similar signal molecules, more recently researchers have begun to focus on interactions between bacterial species (interspecies) and even between bacteria and eukaryotic (interkingdom) organisms sharing a similar environment. This has been commonly called ‘cross talk’.

Within a medical context, much of this research has focused on studying organisms that are commonly found together in the CF lung. *Pseudomonas aeruginosa* and *B. cenocepacia* often occur together in the lungs of people with CF, where they are associated with high morbidity and mortality.13,39 *Burkholderia cenocepacia* has been shown to up-regulate the production of virulence determinants in response to AHLs produced by *P. aeruginosa*, although this does not appear to happen the other way round. The term ‘cross talk’ is suggestive of a cooperative venture between two or more species; however, we must be careful with our interpretation of certain behaviours. In this case, it suggests that *B. cenocepacia* uses *P. aeruginosa* AHLs as an environmental cue to alter its behaviour rather than there being true signalling between the two bacterial species. *Pseudomonas aeruginosa* pays the cost of producing AHLs, possibly for intraspecies signalling, but appears to gain no benefit from *B. cenocepacia* in return. Understanding the true nature of these interactions is important if we are to fully understand the complex ecology of environments such as the CF lung. Cross-talk using QS signals is not limited to just Gram-negative bacteria. *Pseudomonas aeruginosa* and *S. aureus* also co-exist in the CF lung, where AHLs from the former can influence the expression of virulence determinants in the latter40 and AQs can induce the formation of *S. aureus* small colony variants which increases the resistance of *S. aureus* to antibiotics.41

In addition to controlling gene expression in bacterial populations, AHLs have also been found to be directly recognized by eukaryotic cells and even influence the behaviour of eukaryotic organisms. AHLs have been shown in several different studies to have immunomodulatory effects, influencing the production of cytokines that in turn determines the type of immune response elicited upon infection.42,43 Furthermore, AHLs can also have cardiovascular effects by inducing relaxation of blood vessels.44 If we put these two effects into the context of infection, it becomes apparent that bacteria have the power to influence immune responses, probably to their benefit, and stimulate the delivery of nutrients for their survival by increasing the blood supply.

QS signalling systems are quite vulnerable and can be degraded or ‘quenched’ by other bacteria, eukaryotic cells or even eukaryotic organisms. This degradation can be dependent upon conditions in the surrounding environment or due to the action of certain enzymes. AHL
signals are rendered biologically inactive in alkaline environments and, therefore, in certain environmental niches, signalling may be ineffective. A number of bacteria co-existing with AHL producers have been found to produce enzymes which can degrade these signal molecules. This has been shown in soil, where Bacillus strains produce lactonase enzymes responsible for this activity. There are also organisms which have the ability to synthesize as well as degrade QS molecules. One such organism is P. aeruginosa in which a number of enzymes have been shown to have the ability to inactivate AHLs. Whether this is their primary role has yet to be elucidated.

In clinical settings, P. aeruginosa has been shown to co-exist and suppress the growth of the fungus Candida albicans. However, it is reasonable to assume that eukaryotic organisms such as C. albicans will have developed mechanisms to protect themselves against bacterial damage. In fact, C. albicans has been shown to down-regulate the production of virulence factors by P. aeruginosa by producing the sesquiterpene compound farnesol. It was demonstrated that addition of farnesol to P. aeruginosa cultures resulted in a reduction in the levels of the PQS QS signal molecule and subsequently a reduction in the production of the phenazine pyocyanin. Farnesol is not unique to C. albicans and it is likely that many other organisms are capable of modulating the virulence of P. aeruginosa using this or related compounds.

It is now known that human cells synthesize enzymes which are able to degrade AHLs possibly as a strategy to prevent their damage by bacterial pathogens and this forms the basis for novel antimicrobial therapies against problematic organisms such as P. aeruginosa.

Quorum sensing as a target for therapy

It should not be a surprise that bacteria acquire resistance to antibiotics as most antimicrobial agents are derived from microorganisms themselves, the products of microbial warfare in a competitive unicellular environment. Treatment with antibiotics creates a natural selection for resistant genotypes, which consequently spread through a population. Often the genes for antibiotic resistance are carried on mobile plasmids that can be horizontally transferred from resistant to non-resistant cells, increasing the spread of resistance through a population. In the medical arms race with microbes, the supply of novel antibiotics has been plentiful but is starting to run dangerously low and the scientific challenge is compounded by social problems. General Practitioners must be sparing with the prescription of antibiotics in order to reduce the emergence of resistance. However, resistance can spread between bacterial taxa, between patients and between nations, so global
inconsistencies in treatment practices can lead to a widespread resistance on a global scale. There is now, therefore, a clear need to design novel antimicrobial treatments. Progressive routes to antimicrobial therapy include approaches that do not kill the cells or impair their essential functions but more specifically inhibit the mechanisms of pathogenesis (i.e. disabling rather than destroying). Such anti-pathogenic agents are less likely to pose harsh selective pressures for the development of resistant mutants. The basic strategy is to down-regulate virulence, which will assist the immune system in eradicating the invading bacteria.

QS systems regulate bacterial pathogenesis and therefore represent novel targets for such therapies. There has recently been much interest in the discovery of QS inhibitors (QSI). The first naturally occurring agents found to possess such QS antagonistic activity were brominated furanones produced by the Australian macro-alga Delisea pulchra. Biofilms grown in the presence of synthetic furanone derivatives were rendered susceptible to antimicrobial killing with the antibiotic tobramycin and detergent SDS in contrast to the untreated biofilms. Further reports of QSI compounds derived from food, herbal and fungal sources have been published and their potential therapeutic role investigated in biofilm and pulmonary murine models. A recent study identified three compounds that efficiently inhibited the synthesis of molecules required for the activation of the P. aeruginosa AQ regulatory pathway. This limited P. aeruginosa infection in mice, without affecting bacterial viability and such compounds provide a starting point for the design and development of novel anti-pathogenic agents targeted at important human pathogens.

Conclusions

Microbes display an extraordinary level of social differentiation, organization and coordination. Much of this is orchestrated by QS communication systems which allow cells to sense their population density and coordinate an appropriate response. There are several species of bacteria where it has been demonstrated that QS is important for the full virulence of the organism. The molecular mechanisms governing QS systems are being increasingly understood, but there are still many questions that need to be addressed. For example, what is the role of QS in vivo? In only a handful of well-studied organisms (e.g. P. aeruginosa) has this been partially addressed and the results suggest a role for QS in virulence. Given this, does QS provide an attractive target for novel antibacterial agents? Proof of principle studies in animal models has been encouraging and a future challenge will be to take these studies
into human clinical trials. Another area that is growing in maturity is understanding social evolution in microorganisms. As a population of pathogenic bacteria may evolve during an infection, cooperation and conflict among bacterial cells may ultimately have a profound influence on the outcome of an infection. Therefore, understanding the conditions that favour cooperation will enable us to better understand the evolution of virulence over the course of an infection. Only by understanding the complicated ecology of bacterial environments can we continue to develop successful treatments against these problematic organisms.

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**References**


