Bacterial conversations: talking, listening and eavesdropping. An introduction

Natural selection operates at the level of the individual, with the retention of mutations that benefit that individual. However, current understanding from theoretical ecology suggests that the most important level of organization for a species is the population. That is, communities are more important than the individual, at least in the present day, in maintaining ecological stability. If the processes that drive evolution operate at the level of an individual, but the success is determined at the population level, then evolutionary development can only be effective if individuals are able to interact with other individuals of the same species to ensure the success of the population.

One obvious requirement for concerted action by a population is the need for an individual to be aware of and respond to other individuals of the same species that are growing in the vicinity. For many years it was considered that bacteria were simple organisms, with little capacity for the types of interaction seen in higher organisms. The ability to interact was thought to be confined to metabolic consortia, where one bacterium released a compound as a result of its metabolism that was then the substrate for another species. This second species may or may not release a metabolite that could be used by a third species. This simple mechanism results in stable consortia of different species but it is basically an altruistic process where the activity of one organism results in the success of another species. It does nothing to ensure the success of other individuals of the same species. In that sense, metabolic consortia do not provide a mechanism for intraspecies cooperation that would be required to ensure the survival of a species; it does not provide selective pressure for the development of new traits. On one hand, it might be argued that consortia can never be long-lived phenomena in bacteria because of the relative ease with which one bacterium can incorporate genetic information from another bacterium. Thus, although lateral gene transfer could result in one individual from a consortium acquiring essential genes from another member of the consortium, this might make the other members of the consortium redundant. However, on the other hand, it can be argued that a population of bacteria can afford to maintain much greater genetic diversity than any single bacterium. This will be particularly important for bacterial populations trying to adapt to many diverse environments, such as those found in the rhizosphere for example. For selection to act in such microbial populations, it will be essential to have an efficient mechanism of horizontal gene transfer and this in turn will require a mechanism of bacteria recognizing when they are in close proximity to potential mating partners.

Work in the last decade has shown that bacteria can communicate with each other, in the sense that gene expression of the whole population can be simultaneously regulated. The process of communication is called ‘quorum sensing’. It is population-density-dependent and involves the production of signalling molecules that coordinate gene expression but that can diffuse between cells, hence affecting the whole population. The term ‘quorum sensing’ encapsulates the essence of the process—that is, it is population-density-dependent and sufficient bacteria have to be present for the process to be effective. It is analogous to the legal context of a quorum, when an appropriate number of voting members must be present before a decision can be taken. Quorum sensing allows individuals within a microbial population to cooperate by acting in concert—behaviour that begins to have attributes that are similar to those of a multicellular organism. Quorum sensing is now known to be a widespread phenomenon responsible for modulating many different activities in diverse Gram-negative and Gram-positive bacteria found in association with higher organisms, in soils and both marine and fresh water environments. It is no coincidence that many of the genes associated with quorum-sensing regulation are located on plasmids that are potentially transmissible, or are associated with pathogenicity or symbiosis islands that are potentially highly transmissible. In several cases, the gene transfer systems are regulated by these quorum-sensing regulatory systems and in nature it is most likely that conjugation usually occurs when there are populations of bacteria present in ecological niches.

It is known that quorum sensing also regulates a wide range of other physiological processes and involves a variety of different signal molecules. Activities under quorum-sensing control include secondary metabolite production, motility, symbiosis, nodulation, conjugal plasmid transfer, biofilm maturation and virulence in numerous bacterial genera. A number of chemically distinct families of quorum-sensing signal molecules have been identified. The most intensively investigated are the N-acylhomoserine lactone (AHL) family in Gram-negative bacteria and the peptide autoinducers of Gram-positive bacteria. Quorum sensing contributes to environmental adaptation in many bacteria in a number of different ways. For example, in Pseudomonas, AHLs are involved in the elaboration of virulence determinants in pathogenic species, and AHLs are thought to be important in biofilm maturation.

The level to which quorum sensing is central to the success of bacteria is now becoming clear. The...
proportion of genes that are controlled by quorum-sensing regulatory networks has been estimated to be between 5 and 25% of the bacterial genomes sequenced to date. In recent years, there has been an almost exponential growth in the number of publications relating to quorum sensing. It is therefore appropriate for this issue of the Philosophical Transactions of the Royal Society to try to review what we currently know about communication between bacteria.

The first example of AHL-dependent quorum sensing came from the bioluminescent bacteria that inhabit the light organs of deep-sea fish and squid. The amount of light produced by *Vibrio fisheri* was not correlated with the number of bacteria present but there was a threshold in cell concentration above which light was produced. Nealson *et al.* (1970) demonstrated that light production was activated by the production of an autoinducing substance. In this issue, Williams *et al.* (2007) describe how understanding of quorum sensing has developed since those first observations. Quorum sensing depends on the synthesis of small molecules that diffuse in and out of bacterial cells and that increase in concentration as the bacterial population density increases. At a critical threshold concentration, a target sensor kinase or response regulator is activated, resulting in gene expression. Such concerted action benefits the population by, for example, maintaining biofilm mode of growth or providing collective defence against competitors. Williams *et al.* (2007) also highlight the growing evidence that quorum sensing crosses the prokaryotic–eukaryotic boundary—a recurring theme throughout the other contributions to this issue.

In recent years, the involvement of quorum sensing in plant and animal diseases has received particular attention. The evidence is strong because virulence is greatly reduced in mutants of pathogenic bacteria that are defective in quorum sensing. Three papers deal with the involvement of quorum sensing in essential interactions of bacteria with plants and with plant diseases. White & Winans (2007) describe the involvement of quorum sensing in virulence of *Agrobacterium tumefaciens*, which induces the formation of crown gall tumours at wound sites on host plants; this is achieved by directly transforming plant cells. The bacteria benefit because the infected plant tissue produces novel nutrients, called opines, that the colonizing bacteria use as nutrients. Almost all of the genes that are required for virulence and all of the opine uptake and utilization genes are carried on highly transmissible large tumour-inducing plasmids. The consequence of this system is that once an *A. tumefaciens*-infected plant starts to release opines, the pathogenicity plasmid is then transferred to related but non-pathogenic agrobacteria. In this way, the pathogenicity genes can be rapidly spread horizontally and there is not a requirement on all individuals in the population to maintain this character. Legume-nodulating *Rhizobium* spp. are phylogenetically very close to *Agrobacterium* spp. and so it is no surprise that, as reviewed by Sanchez-Contreras *et al.* (2007), the transfer of rhizobial plasmids carrying genes required for this symbiosis can also be under the control of quorum sensing. An interesting twist to the regulation of plasmid transfer between rhizobia is that sometimes potential recipients (lacking appropriate nodulation genes) can induce plasmid transfer. The resulting acquisition of the capacity to nodulate a specific legume greatly increases the selection and spread of this character. Thus, the populations of both rhizobia and agrobacteria can maintain a larger gene pool than individuals and quorum sensing can act as a mechanism to facilitate horizontal transfer and hence selection of useful characters that need not be maintained by all individuals in the population. Quorum sensing also influences many other aspects of the interaction between rhizobia and legumes, including the nodulation process. In addition, legumes may actively interfere with rhizobial quorum sensing, and it appears that they are also able to respond to the quorum-sensing signalling molecules produced by bacteria. In another example of plant interactions, Barnard *et al.* (2007) show that in the disease caused by plant soft-rotting bacteria, *E. coli*, quorum sensing controls the production of cell-wall degrading enzymes, as well as virulence determinants and secondary metabolites, such as carbapenem antibiotic production.

Much work has been done on the role of quorum sensing in human pathogens and the field has been extensively reviewed recently (Williams 2007). Gram-positive bacteria, such as staphylococci and enterococci are important human pathogens but they have a different quorum-sensing system to Gram-negative bacteria that involves oligopeptides as signal molecules. Dunny (2007) describes how the transfer of the antibiotic resistance plasmid pCF10 is controlled by quorum sensing. Moreover, the regulatory circuit that is controlled by the quorum-sensing peptide signal is also involved in both maintenance of the plasmid and in virulence of the bacterium. Dunny (2007) also suggests this system may be a useful bacterial model to study the complexity of biological evolution. We are familiar with the role of antibiotics in controlling bacterial populations by causing death of the cells but Yim *et al.* (2007) suggest that antibiotics can also act as signalling molecules. This is based on the hypothesis that the majority of low-molecular-weight organic compounds secreted by microbes play roles in cell-to-cell signalling. Since many antibiotics can modulate gene transcription at low concentrations, it is proposed that their primary activity is in cell-to-cell signalling not growth inhibition in order to sustain microbial communities in the environment and the interactions of these populations with surrounding organisms.

With the widespread increase in antibiotic resistant bacteria and the progressive ineffectiveness of antibiotics as therapeutic agents, there is an urgent need to develop alternative procedures to neutralize pathogenic bacteria. Many bacterial pathogens use quorum-sensing mechanisms to synchronize the processes leading to infection and survival in the host. There is a developing expectation that a better understanding of quorum sensing offers the best hope for future, novel opportunities to combat the bacteria that cause human, animal and plant diseases. Dong *et al.* (2007) suggest one such promising disease-control strategy by quenching the signal molecules involved in quorum sensing, i.e. quorum quenching through enzymatic inactivation of
quorum-sensing signal molecules. Such mechanisms occur widely in many prokaryotic and eukaryotic organisms and appear to play important roles in microbe–microbe and pathogen–host interactions. In an investigation of the types of compounds that are capable of blocking quorum sensing, Bjarnsholt & Givskov (2007) have found a number of naturally occurring compounds. These are very effective in blocking quorum sensing and show great promise in attenuating, but not killing, the infecting bacteria and hence providing the host defences with an opportunity to deal with the bacterium whose virulence is blocked. This strategy could be a very attractive alternative to antibiotics, particularly with the emergence of multi-antibiotic resistant bacteria.

There are wider issues concerned with quorum sensing, which are increasingly seen to have implications for organisms other than bacteria alone. Evidence is accumulating that animals and plants may ‘listen’ to bacterial signals and use these signals in complex ways. Perhaps this should not be surprising since bacteria are ubiquitous and have interacted closely with eukaryotic organisms for a long time—between 1 and 2 billion years. If bacteria can communicate with each other, it is not difficult to see that eukaryotes could benefit by intercepting those signals. Joint et al. (2007) discuss one such example where the motile reproductive stages (zoospores) of the green seaweed, Ulva, detect signal molecules produced by bacterial biofilms and use that information to select the site for permanent attachment. When the zoospore detects a quorum-sensing signal, swimming rate is reduced and this results in accumulation of cells at the source of the AHL. The detection of AHLs results in calcium influx into the zoospore. This is the first example of a calcium signalling event in a eukaryote in response to bacterial quorum-sensing molecules.

Other examples reveal the potential to develop a broader biotechnological focus of quorum sensing and to apply that knowledge to the natural environment—applications that are far from the current focus on the attenuation of virulence in human and plant pathogens. Representatives of the bacterial genera that produce quorum-sensing signals are abundant in the environment and in bioprocessing plants such as activated sludge. In an example of biodegradation of phenolic waste, Manefield & Whiteley (2007) show that the addition of quorum-sensing signal molecules could induce population shifts in a consortium of phenol-degrading organisms that resulted in a more balanced community and better maintenance of phenol degradation.

Finally, we return to the question of the role of quorum sensing in evolution. Diggle et al. (2007) show that, from an evolutionary perspective, intraspecies quorum-sensing signalling is readily explained by models such as kin selection. However, it is less easy to explain communication between species and they suggest that it is most likely that the signal molecules are being used as chemical ‘cues’. That is, the use of signal molecules by a species other than the one which produced it cannot readily be described as signalling but the signals are likely to be used as cues, or for the coercion of other cells.

REFERENCES


