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Bacterial Quorum Sensing (QS): Advances and Promises of an Emerging Research Area and Complex, Dynamics Changes in Environment

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A B S T R A C T

Quorum Sensing is the regulation of gene expression in response to fluctuations in cell population density. Quorum-sensing bacteria produce and release chemical signaling molecules called Self-Conductors whose concentration increases with cell density. The detection of the minimum threshold excitatory concentration of the autoantibody leads to a change in gene expression. Gram-positive and Gram-negative bacteria use quorum-sensing communication circuits to regulate a wide range of physiological activities. These processes include symbiosis, virulence, competence, conjugation, antibiotic production, motility, sporulation, and biofilm formation. In general, gram-negative bacteria use acylated homoserine lactone as an Auto-Inducer and gram-positive bacteria use treated oligo-peptides for communication. Recent advances in this field indicate that cell-to-cell communication via self-conductors occurs both within and between bacterial species. In addition, growing evidence suggests that bacterial autoinducers elicit specific responses from host organisms. Although the nature of the chemical signals, signal transduction mechanisms, and target genes controlled by the bacterial quorum sensing system are different, in all cases the ability to communicate with each other allows bacteria to coordinate gene expression, and thus behavior, of entire communities. Presumably, this process gives the bacteria some of the qualities of a higher organism. Thus, the development of quorum sensing systems in bacteria could be one of the first steps in the development of multicellularity. This review highlights how we can build on the relatively new and rapidly growing field of microbial communication or quorum sensing (QS). We now have in-depth knowledge of how bacteria use QS signaling to communicate with each other and coordinate their activities. There have been extraordinary advances in QS genetics, genomics, biochemistry, and diversity of signaling systems. We are beginning to understand the link between QS and bacterial sociability. This platform puts us on the threshold of a new era where researchers can advance the development of new drugs to treat dangerous infectious diseases, while also using bacteria to understand social biology.

Introduction

Bacterial QS involves self-produced extracellular chemical signals, which can accumulate in the local environment to levels required to activate the transcription of specific genes. The first clues to QS emerged in the late 1960s and early 1970s, when researchers showed that genetic capacity in *Streptococcus pneumoniae* and luminescence in two marine bacterial species required production of Extracellular molecules (Papenfort *et al.,* 2016). Cell-to-cell signaling via these molecules has been proposed as a form of chemical communication. The 1980s brought two landmark discoveries: (i) the luminescence (lux) genes of the marine bacterium *Vibrio fischeri* were identified, and the genes necessary for what is now known as control Minimal luminescence, luxI and luxR, have been shown to control transcription of the lux gene; and (ii) the QS signal of *V. fischeri* was identified as N-3-oxohexanoy-L-homoserine lactone (3OC6-HSL). The luxI gene encodes an auto-inducible fusion required for 3OC6-HSL production, and luxR encodes the 3OC6-HSL-responsive transcriptional activator of the lux gene. The common interest remains silent for another decade. By the 1990s, DNA sequencing and comparative sequence analysis had become routine in the laboratory, and gene pairs exhibiting similarity to luxR and luxI began to attract the curiosity of some researchers (Engebrecht *et al.,* 1984). This led to an explosion of findings that other bacterial species control genes for conjugation, exoenzyme production and antibiotic synthesis with systems like LuxI-LuxR. A common theme emerged; The LuxI homologs that catalyzed the synthesis of an acylated lactone homoserine (AHL) and the LuxR homologues both showed specificity for their cognate AHL (Vadakkan *et al.,* 2018a).

The convergence of these findings led to the concept of QS; that diffuse AHLs act as a proxy for cell density and allow a bacterial species to produce expensive extracellular public goods only when there is sufficient biomass to benefit from the public good. Soon after, the QS signal of *S. pneumoniae* (commonly known as a pheromone) was found to be a small peptide and *Staphylococcus aureus* was discovered to use a small cyclic peptide pheromone to activate extracellular toxin-producing genes. Thus, quorum sensing has been shown to occur in Gram-positive and Gram-negative bacteria through different chemical signals (Fuqua *et al.,* 1994). A first study shows that a bioluminescent marine bacterium called *Vibrio harveyi* can detect a self-generated signal as well as a signal or multiple signals produced by other bacterial species to produce light. This phenomenon is considered a type of QS, in which cells of many species in a mixed microbial community sense the density of the general bacterial population through a molecule called Autoinducer-2 (AI-2). The strengths and weaknesses of this concept will be discussed later in this review. Other studies have also described QS-like systems in eukaryotes (Candida and Histoplasma) and more recently in viruses, thus providing clear examples of convergent evolution. Some QS signals are unstable, such as DSF and PAME signals, and there is evidence that signal instability can occur in the local environment.

Quorum Sensing and Social microbiology

An evolutionary perspective on quorum perception early research suggested that SQ is a social trait (a trait that affects both the individual performing the behavior and the recipient), but this concept has not been tested experimentally. In principle, QS can be asocial, e.g. allowing individual bacteria to sense their physical environment (this is called diffusion sensing). However, there is now ample evidence that in some settings QS is social and that at the population level, QS regulates extracellular production of public goods. It is important to note that while public goods directly benefit the producer cell, they also indirectly benefit the surrounding cells. Because the production of public goods is expensive, these behaviours are likely to be exploited by non-productive "fraudsters," creating bacterial social dilemmas. QS has now been shown to be exploitable by cheaters in laboratory cultures. One well-studied example is the pathogenic

bacterium *Pseudomonas aeruginosa* (Vadakkan *et al.,* 2018b). This bacterium requires QS to stimulate the production of extracellular proteases required for growth on proteins. QS Mutants cannot grow on their own with milk proteins, but when co-cultured with competent QS protease-producing cells, mutants have the advantage of fitness in relatively low amounts. In fact, mixed rogue and cooperative cell infections have been shown to be less virulent than cooperative monoclonal infections. This led to the idea of using bacterial trickery to aid in the treatment of infections by attenuating virulence or introducing the 'Trojan horse' gene into virulent populations (Rumbaugh *et al.,* 2012)

Autoinducers: Script of bacterial language

Autoinducers play a significant character in quorum sensing and subsequently considered as alphabets of bacterial language (Vadakkan *et al.,* 2018a). Autoinducers are categorized into three different classes based on their structure and specific function; such as AHLs (Acyl Homoserine Lactones), AIP (Autoinducing Peptides) and Autoinducer-2 (AI-2) (Hense and Schuster, 2015). AHLs are small diffusible particles with a core lactone ring and acyl side chain which is vital for assisting signaling in case of gram negative bacteria (Manefield and Turner, 2002). In general, Quorum sensing in gram positive bacteria are mediated by AIPs. AIP lack free transportation across the cell membrane hence requires specialized membrane transport proteins (Kolibachuk and Greenberg, 1993). AI-2 are furonone derived signaling molecules found functioning in both gram negative and gram positive bacteria [20] also exhibit features of both AHLs and AIPs (Xavier and Bassler, 2003).

Classical QS in Bacterial Communication

QS is defined as the bacterial cell-cell communication process that controls bioluminescence, capacity, antibiotic production, spores, and membrane formation and secretion of virulence factors in bacteria. Recent work has shown that the scope of QS extends to inter-kingdom communication, mediated by a number of newly identified extracellular signaling molecules called autotransformers (AIs). Among these AIs, five major signaling molecules are involved in the classical QS system. Acyl-homoserine lactone (AHL) QS system in Gram-negative bacteria Gram-positive and Gram-negative bacteria use QS to communicate with each other. Although the types of QS pathways differ in Gram + and Gram - bacteria, they all have fundamental biological roles. The Gram-QS system has several features in common (Papenfort and Bassler, 2016). First, AIs are molecules synthesized from S-adenosylmethionine (SAM) as a substrate. Acyl-homoserine lactones (AHL) are the most common type of AI. They have an N-acylated homoserine-lactone ring as their core and a 4-18 carbon acyl chain containing the transformations. The length of the acyl chains affects their stability. The LuxI-type enzyme is the main, but not the only, producer of AHL. A LuxM fusion found in *Vibrio harveyi*, which is not a homolog of LuxI, can induce AHLs to communicate within their species (Bassler *et al.,* 1993). SAM can be converted into specific signals that can be detected by different bacterial species. Diffusion signaling factor (DSF)-like molecules is synthesized by RpfF proteins in *Pseudomonas aeruginosa* and *Burkholderia cenocepacia*. Cholera autoinducer 1 (CAI-1) is produced by CAI-1 AI synthase (CqsA) in *Vibrio cholerae*. Due to the ubiquity of CqsA homologues in *Vibrio* sp., many different CAI-1s are produced by this bacterial species. In addition, *Vibrio* sp. may have different affinities for non-self-induced CAI-1, suggesting that CAI-1 is a Vibrio sex-communicating molecule (Miller *et al.,* 2002; Rutherford and Bassler, 2012; Papenfort and Bassler, 2016).

Second, AI binds to specific membrane receptors or cytoplasmic proteins (Papenfort and Bassler, 2016). LuxR-like receptors, cytoplasmic transcription factors, detect AHLs that can diffuse freely in the cytoplasm and bind to co-derived AHLs. Stable LuxR-AHL complexes can bind DNA while unbound LuxR proteins are rapidly degraded (Rutherford and Bassler, 2012). These LuxR/LuxI

analog systems, including LasR/LasI and RhlR/RhlI in *Pseudomonas aeruginosa*, are involved in cell-to-cell communication (Papenfort and Bassler, 2016). Several annotated LuxR proteins belong to LuxR-solo receptor or orphan LuxR class. In the absence of LuxI synthase, they detect different AHL molecules produced by other bacterial species, thus ensuring interspecies communication. Examples of these are QscR in *P. aeruginosa* (Papenfort and Bassler, 2016), SdiA (LuxR peer) in *Escherichia coli* (Hughes *et al.,* 2010). Third, coupled receptors act as transcription factors to regulate tens to hundreds of genes affecting biofilm formation, virulence, and other biological processes in bacteria. The receptors of the QS molecule establish a relay loop during the regulation of gene expression, known as auto-induction. This mechanism increases the synthesis of auto-inducers, thereby promoting the expression of synchronous genes in the population (Papenfort and Bassler, 2016).

QS in Natural Habitat

Most of the studies that provide our current understanding of the molecular biology and evolution of QS have used mixed culture and laboratory media. The experiment is not intended to mimic the natural environment. These *in vitro* systems provide reproducible conditions for biochemical and evolutionary studies, as well as the ability to grow large culture volumes often required for signal purification and identification. These systems have also provided a wealth of solid background knowledge that we can build on to investigate the role of QS in regulating the composition and function of natural microbial communities. Working in microbial natural habitats is challenging and requires QS scientists to grasp the complexity of these environments while leveraging cutting-edge multidisciplinary approaches. An elegant system that has been developed to initiate QS studies in a natural bacterial community in the host animal is a mutual symbiosis between the squid *Euprymna scolopes* and its symbiotic organ *V. fischeri*. The squid lives in the coastal waters of the Hawaiian Islands, being born with sterile light organs. The luminous organ was particularly concentrated by *V. fischeri*, present in small quantities in the surrounding seawater. *V. fischeri* uses its QS system to activate luminescence genes in the environment of high-light-dense organs. Mutualism is simple in that there are only two partners, and it is easy to manipulate in the lab. This model system provides insights not only into the role of QS in host -microbe interactions, but also on how a microbiome, although simple, can influence the host development. Researchers are now beginning to study many habitats with a more complex microbiome than the squid symbiosis. Here, we focus on three emerging areas of interest with important and relatively unknown functions in natural ecosystems: (1) LuxR orphan homology; (2) association between QS and microbial biogeography and (3) extinction representative.

Orphan LuxR or single homolog and interspecies interactions LuxR-like transcription factors consist of two domains, an N-terminal signal-binding domain and a C-terminal DNA-binding domain, and similarity searches can be used simple copper to identify LuxR homologues in genome sequencing. Such a search of the *Salmonella typhimurium* genome revealed the first example of what we now call the orphan55 or solo56 LuxR homolog. The *S. typhimurium* genome has a luxR homolog called sdiA but it lacks a luxI-like gene and *S. typhimurium* does not produce AHL. In fact, many Gram-negative bacteria have luxR-like genes and no luxI-like genes, and many Gram-negative bacteria have more luxR-like genes than luxI-like genes. In the case of *S. typhimurium*, we learned that SdiA reacts with AHLs produced by other bacteria, leading to the activation of specific genes. There is also an sdiA gene in *E. coli*. SdiA has been reported to react with AHL and small molecules produced by the mammalian host.

QS System of Self-Inducible Peptide (AIP) in Gram-Positive Bacteria

Although the QS circuit shares some common features, there is a clear distinction between Gram+

and Gram– bacteria. The AI of many Gram+ bacteria is an oligopeptide (AIP). There are two types of canonical AIP-QS circuits. An AIP class is encoded as a precursor to the QS operon, which is then processed and secreted extracellularly by specialized transporters. AIPs with 5 to 17 amino acids can be linear or cyclic (Thoendel *et al.,* 2011; Rutherford and Bassler, 2012). Membrane-binding two-component sensing kinases, such as the Agr system in Streptococcus aureus and the Fsr system in *Enterococcus faecalis*, act as AIP receptors. Sensor kinase autophosphorylates after binding to AIP and the phosphoryl group is transferred to an involved cytoplasmic response regulatory protein that controls the expression of QS-related genes (Rutherford and Bassler, 2012). In *S. aureus*, AIPs can change and co-evolve with their receptors. Unrelated AIPs inhibit QS in other strains, allowing a strain to establish its specific niche (Lyon *et al.,* 2002). In other canonical AIP-QS circuits, pre-AIP is secreted by the secretory system and processed by extracellular proteases such as the neutrally secreted protease B (NprB). AIP is imported and then binds to transcription factors to regulate DNA expression through (Opp) (Gominet *et al.,* 2001). In addition, there is transcriptionally-derived autophagic induction of the QS operon encoding AIP pro-AIPs, transporters, receptors, regulators and proteases, leading to synchronization of the QS response (Gominet *et al.,* 2001).

Representative sensing in biofilm communities

Bacteria adhere to surfaces and together form biofilm communities (Kolter *et al.,* 2006). We now understand that biofilms are a predominant microbial life form on Earth and that these stalkless communities are involved in the environment (Donlan *et al.,* 2002), medicine, and industry. Biofilm cells are enclosed in an extracellular matrix consisting of polysaccharides, proteins, and extracellular DNA (Branda *et al.,* 2005). Unlike bacterial cultures that are well mixed in liquids, biofilms are heterogeneous and can reorganize over time, raising questions about nutrient acquisition and diffusion. Furthermore, understanding how quorum sensing occurs within the architectural constraints of biofilms is an important question facing the field.

Effect of fluid flow and surface topography on the Quorum Sensor Signal

Bacteria form biofilms on a variety of surfaces, including soil, river beds, sewage, deep-sea vents, and plant and animal tissues. The natural environment differs from the media traditionally used in laboratories for biofilm studies by two main features: the presence of irregular surfaces (e.g. on rocks, corrugated tubes, velvet, cilia, leaves, teeth, etc.) and the presence of fluid flow (Drescher *et al.,* 2013). Recent studies aimed at mimicking natural scenarios have taken advantage of advances in microfluidic technology that allow for precise control of surface topography and fluid flows.

Bacteria exhibit biofilm-forming behaviours distinct from their quorum sensing state

For example, *Pseudomonas aeruginosa* forms high cell density (HCD) biofilms in response to the accumulation and detection of autoinducers, while *Vibrio cholerae and Staphylococcus aureus* form biofilms have low cell density (LCD), and accumulation and detection of inducers automatically suppress biofilm formation. Regardless of whether the quorum sensing regulation of biofilm formation is positive or negative, a common theme that has emerged is the amount of bacterial biomass required to initiate quorum sensing in a microbial population. Specific bacteria increased with increasing fluid flow. In particular, the fluid flow removes the vectors of self-conductors and thus higher cell densities are required to achieve under-flow quorum than in well-mixed liquid cultures. One counterintuitive finding from new studies in the field is that in bacterial species such as *V. cholerae* and *S. aureus* in which the quorum sensor prevented biofilm formation, the increase in biofilm formation occurred in the flow condition compared with the no flow condition. The removal of the self-conductor by flow reduced inhibition, promoting increased biofilm formation

compared with biofilms formed on non-fluid surfaces. However, once a thick biofilm is established, quorum sensing is activated in cells residing at the bottom and inside the biofilm, possibly because these cells are shielded from progression. Self-inductive by neighbouring cells and extracellular matrix. Because outer-resident cells undergo a different flow regime than internally-resident cells, cells in distinct regions of the biofilm apply distinct gene expression programs that are regulated & controlled by the delegate sensor.

Quorum Sensing in Eukaryotic Hosts

Within a host, bacteria often exist in mixed species communities and, therefore, quorum sensing of a species can influence and be affected by sensors. Quorum variables or other operations performed by the species involved. In addition, host processes such as the immune response can also influence bacterial quorum sensing and vice versa. Here, we review some recent advances related to quorum sensing function in mixed bacterial communities and how host processes affect quorum sensing signal transduction in infection process.

In summary, there has been an explosion of activity on QS bacteria and the field continues to grow rapidly. An area ripe for progress involves complex adaptive microbial communities. QS has the potential to control behaviours that are important for the development and success of these communities in different environments such as the human gut microbiome and chronic human infections. Unravelling the role and function of QS in natural ecosystems requires a continued willingness to accept the complexity of these microbial communities and incorporate ecological principles at the system level.. We will continue to see the underlying biological incorporation of bacteria's social dynamics into thinking about how to intervene in some infectious diseases, and will continue to use bacteria to understand the biology of communication and disease. We already understand that there are many different small molecule-dependent interactions between bacteria and between bacteria and their hosts. There is certainly much more to discover and the opportunity to sort out the fundamental differences between these different systems. Understanding these issues will be critical as we move towards translating basic QS studies to meet future needs, including functional studies of the human microbiome. Environmental features ranging from fluid flows and surface topography to host immune responses and the presence or absence of other bacterial species influence bacterial communication. It is imperative to study quorum sensing under complex conditions, such as in biofilms and in the context of the microbiome in the eukaryotic host for the field to understand how this works. Cell-to-cell communication in real-life scenarios and to understand how the behaviours controlled by the quorum sensor are deployed outside the laboratory. Exciting studies are underway in this direction, and in addition to providing background information, they hold promise to advance the field in efforts to prevent quorum sensing in harmful bacteria and promote sensing representative of beneficial bacteria.

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