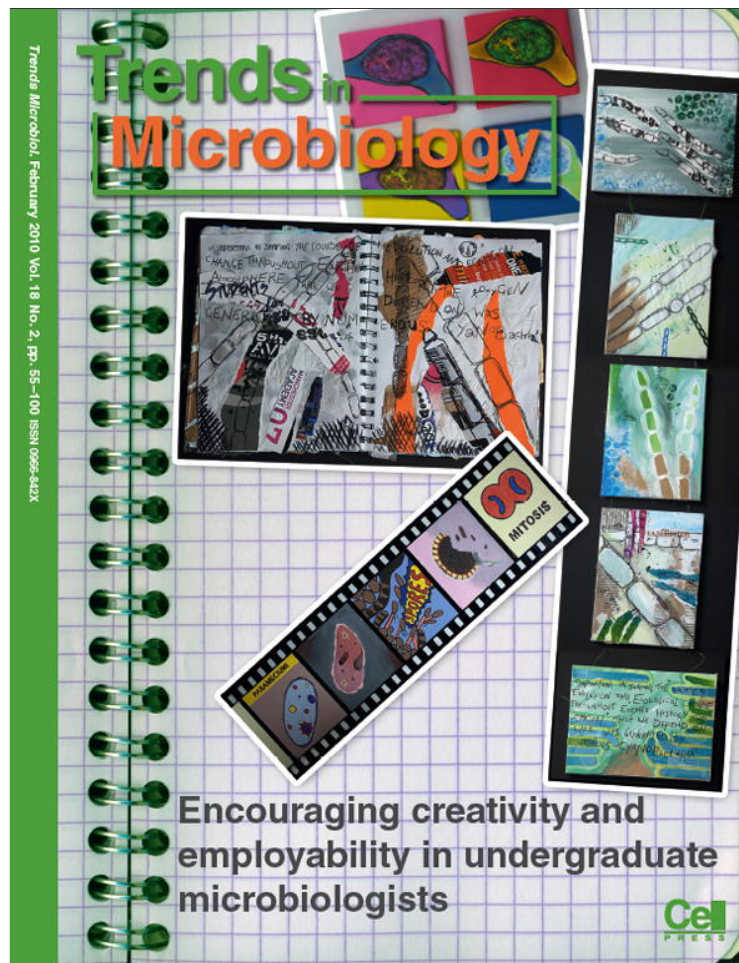


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Quorum sensing in natural environments: emerging views from microbial mats

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Much laboratory-based information exists on quorum sensing, a type of bacterial cell-to-cell communication that depends upon exchanges of molecular signals between neighboring cells. However, little is known about how this and other microbial sensing systems operate in nature. Geochemical and biological modifications of signals probably occur in extracellular environments, and these could disrupt intended communication if signals are no longer recognized. However, as we discuss here, signal alterations might result in other outcomes: if a modified signal is able to interact with a different receptor then further environmental information can be gained by the receiving cells. We also postulate that quorum sensing occurs within cell clusters, where signal dispersion might be significantly influenced by extracellular polymers. As a model system to discuss these points we use microbial mats – highly-structured biofilm communities living under sharply-defined, fluctuating geochemical gradients.

Introduction to quorum sensing

Quorum sensing (QS) is a density-dependent form of cell-to-cell chemical communication that uses small-molecule signals (Box 1). Over the past decades, QS has been examined in much detail under laboratory conditions and has generated an enormous literature base (reviewed in Refs [1–3]). However, comparatively little is understood regarding QS and other forms of microbial sensing within the complex milieu of natural environments [4]. In QS, cells produce molecular signals that are released outside the cell, traverse the extracellular environment, and are perceived by other cells in proximity, or by the producer cells themselves. Concentration-dependent responses to signals often result in changes in gene expression. Metabolic and behavioral changes associated with QS allow functional coordination that is generally favorable to bacteria when in high abundance (e.g. light production, swarming, virulence, extracellular enzyme production, biofilm formation) [5]. A growing list of signal classes (reviewed in Refs [1–3]) includes acylhomoserine lactones (AHL), autoinducing peptides (AIP), furanosyl diesters (also called autoinducer-2, AI-2), gamma-butyrolactones (GBL), *Pseudomonas* quinolone signals (PQS), and diffusible signaling factors (DSF) (Figure 1). There are likely to be many as yet undiscovered classes of signal molecules.

Outside the confines of laboratory cultures, and under ever-changing natural conditions, signal molecules are potentially susceptible to degradation or alteration once outside the cell. Fluctuations in the geochemical and photochemical conditions that characterize natural environments present substantial challenges to even the short-term persistence of intact signals and the efficient utilization of QS and other forms of chemical communication. Here we raise a set of questions regarding microbial signaling under natural conditions, using microbial mats as a model ecosystem. We propose that the fluctuations in physicochemical conditions that are typical of natural environments can alter signal molecules, sometimes in a predictable manner. As such, changes to signals could provide cells with important sensory information regarding their local environment.

Microbial mats

Microbial mats are diverse yet highly-structured biofilm communities living within the confines of sharply-defined geochemical gradients that predictably fluctuate over a diel (i.e. 24 h or diurnal) cycle (reviewed in Refs [6–8]). These laminated sedimentary biofilms provide an excellent dynamic system in which to examine environmental effects on QS for the following reasons. Mats have been shown to produce a wide range of extractable QS signals [9,10]. There are extensive small-scale (e.g. micrometer) horizontal gradients of physicochemical conditions (e.g. O₂, HS⁻, pH) [11,12] that fluctuate dramatically over a diel cycle [8]. Very high diversities of bacteria, comprising different functional groups, exist in microspatial proximity and are enclosed within a matrix of extracellular polymeric substances (EPS) [13,14]. Finally, confocal microscopy reveals dense clusters of bacteria that are a prerequisite for density-dependent QS. Given the high microbial diversities and abundances, it is likely that most known classes of signals can be found in mats. In the following sections the effects of natural conditions on signaling are categorized as physical, chemical or biological; however, it should be understood that most conditions are interdependent upon each other.

Physical environment

How do signals move once outside the cell?

The relative mobilities of different types of signals and the processes that might influence their movement are not well understood. The mobility of small molecules in aqueous

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Box 1. The LuxR/LuxI system

Gram-negative proteobacteria utilize acyl-homoserine lactones (AHLs) as signal molecules for a QS system that involves two key proteins: LuxI and LuxR. AHLs are synthesized from S-adenosyl methionine (SAM) and fatty-acid carrier proteins by LuxI-type AHL synthase enzymes [62]. The AHLs consist of a homoserine lactone and an acyl side chain of variable length that can be substituted at the C3 position (Figure 1a). AHLs cross the bacterial membranes largely through diffusion, and are detected and bound by LuxR-type regulatory proteins [2]. Binding occurs when the AHL acyl chain enters the hydrophobic acyl-binding pocket of the LuxR protein. The AHL–LuxR complex then binds to a DNA promoter and activates the transcription of several QS genes in the *lux* operon [43]. Expression of *lux*-like genes results in a wide range of activities in different bacteria, such as light production, virulence, EPS secretion, motility, and plasmid transfer [2]. Whereas the structures of AHLs are relatively conserved, the structures and amino-acid sequences of different LuxR homologs vary considerably.

solutions is mainly a function of their passive diffusion in water, that in turn is influenced by their spherical molecular diameter (i.e. approximate size) and surface reactivity with other molecules [15,16]. Small signal molecules (e.g. short-chain AHLs), therefore, should diffuse more rapidly than their larger counterparts (e.g. long-chain AHLs). The relative mobility of larger signal molecules will be further restricted by their solubility and their capacity to form weak interactions with other molecules. Short-chain AHLs are highly soluble in water, whereas AHLs having extended acyl-chain lengths are progressively less soluble. It can be predicted that large signal molecules will not easily move between cells in the diffusion-mediated manner that is thought to be usual for QS. Chemical alterations on an AHL (such as the addition of an oxo group at the C-3 position, or lysis of the lactone ring) will make the molecule slightly more hydrophilic and therefore more soluble in water; diffusivities, however,

should remain the same, all else being equal. Assuming that signals must be in a relatively water-soluble form in order to disperse, increased solubility would facilitate dispersion of a higher concentration of the signal, as opposed to being hydrophobically bound to a surface (e.g. sediment particle).

As mentioned above, the presence of functional groups makes signal molecules more susceptible to chemical alterations. Larger molecules can be bound to hydrophobic domains of other molecules that possess a more hydrophilic periphery, allowing them to disperse in the water phase – a hydrophilic shuttle, so to speak. For instance, cyclodextrins are ringed molecules with a hydrophobic core that could form a complex with the acyl chain of an AHL, and a hydrophilic periphery that can ‘solubilize’ large hydrophobic molecules. However, unless the signal is able to be released from the shuttle molecule after travel, the signal might not be able to enter the cell, and would then remain inactive. Additionally, some large signals can be packaged for travel in lipid vesicles or can be used for contact-only interactions [17].

How far do signals typically move or diffuse?

Chemical signaling operates over short distances (e.g. tens of micrometers) owing to diffusion constraints. Molecular diffusion over longer distances will simply take too long. However, Gantner and colleagues [18], using a reporter-engineered *Pseudomonas putida* strain on plant surfaces, showed that some signals were able to travel relatively long distances (up to 78 μm) and still effect autoinduction (i.e. induce gene expression), although most signaling occurred over relatively short distances (<10 μm). This was referred to as the cell-to-cell ‘calling distance.’

In the more complex geo- and physicochemical milieu of microbial mats the calling distance will be further compli-

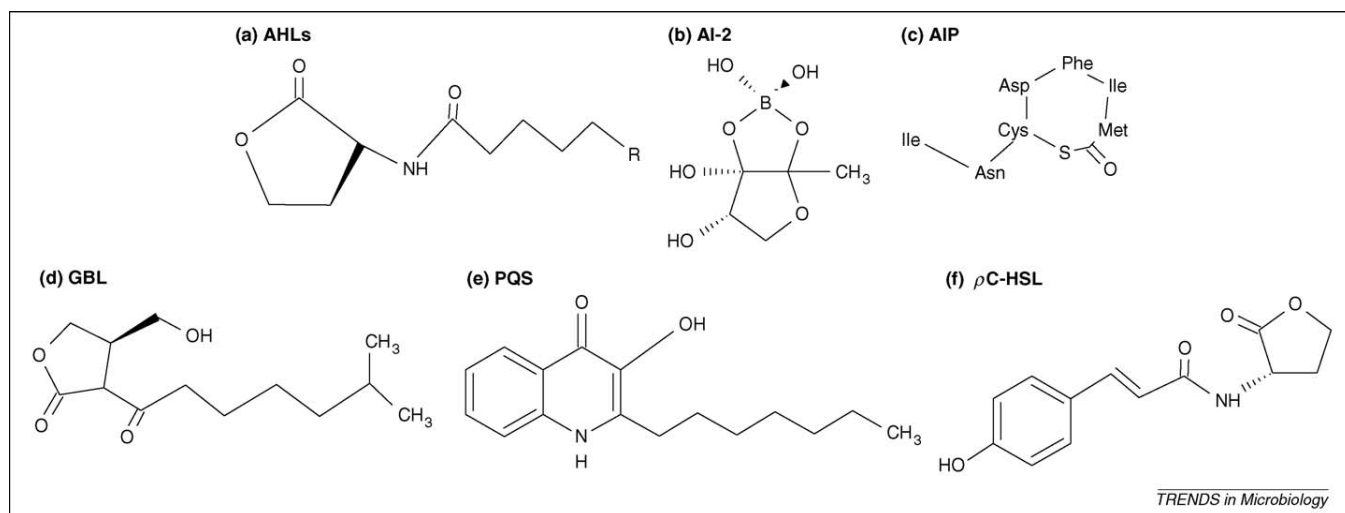


Figure 1. Major classes of signaling molecules in bacteria. (a) Acyl-homoserine lactones (AHLs) are produced primarily by Gram-negative proteobacteria such as *Vibrio fischeri* and *Pseudomonas aeruginosa*, and consist of a homoserine lactone (ring) and an acyl chain of varying length (C4 to C18) [2]. In the figure, R indicates the presence of additional carbon groups. (b) Furanosyl diesters, or autoinducer-2 (AI-2), are a collective term for signals produced from the 4,5-dihydroxy-2,3-pentanedione (DPD), a product of a homocysteine recycling pathway in bacteria. AI-2 compounds are detected by a wide range of Gram-positive and Gram-negative bacteria including *Vibrio harveyi*, *Salmonella typhimurium*, and *Escherichia coli*, and are thought to enable interspecific communication [2,63,64]. (c) Autoinducing oligopeptides (AIP) range from 5 to 17 amino acids in length and are utilized primarily by Gram-positive bacteria [65]. (d) γ -butyrolactones (GBLs) are structural analogs of AHL, but do not cross-react, and operate as separate signals in many bacteria including *Streptomyces* where they are used to regulate antibiotic production [66]. A wide range of signal molecules possess a γ -butyrolactone ring. (e) *Pseudomonas* quinolone signals (PQS) are produced by *P. aeruginosa* and are hydrophobic. They are transported between cells by enclosure in lipid vesicles [17]. (f) *p*-coumaric acid homoserine lactones (*p*C-HSL) have been found in the photosynthetic bacterium *Rhodospseudomonas palustris* [30]. This bacterium is unique in utilizing *p*-coumarate derived from exogenous sources to synthesize the signal, rather than fatty acids from cellular pools as in AHLs.

cated by the ability of the EPS matrix to constrain signal diffusion, by the alteration or degradation of the signal, and by the physicochemical properties of the signal itself. It is of interest, however, that some bacterial strains are known to secrete several different signals [5], and these will probably result in different mobilities and susceptibilities to modifications by the local environment. Thus, some signals could be efficient over relatively short distances, whereas other types of signals could have longer calling distances.

Efficiency sensing

Chemical signals can also be used as extracellular 'sensors' that provide cells with information regarding the properties of their proximal environment, such as local diffusivity [19]. This is a process that has been termed 'efficiency sensing' [20]. For example, gauging the relative diffusivity of molecules released by cells into the outside environment will be an important determinant in assessing the potential usefulness of trying to conduct extracellular processes (e.g. the production and release of extracellular enzymes or virulence factors, or biofilm construction). If a signal diffuses away too rapidly it might not be cost-effective for a cell to produce and release extracellular enzymes. Keller and Surette [21] calculated that energy expenditures involved in synthesizing signal-like molecules were comparatively less than those needed for producing enzymes. There seems to be an increasing realization that processes such as QS might be a subset of broader cell activities (e.g. efficiency sensing). Indeed, given that signals can travel relatively long distances, they could be utilized to sense collaborators or competitors [19].

Chemical environment

Can signals be altered by the environment?

Mats are characterized by geochemical fluctuations of parameters that can be easily measured, such as pH, concentrations of oxidants, O_2 , HS^- and other ions, UV irradiation, water availability and temperature (Figure 2). In mats, pH values can range from 6 to 11 over a diel cycle [11,22]. This is due to the extremely high rates of photosynthesis occurring during daytime within a thin subsurface layer (under 0.5 mm thick), whereas respiration predominates during the night [23]. During peak photosynthesis this very thin layer removes CO_2 more rapidly than it can be replenished by diffusion. As a result, the carbonate equilibrium will shift when bicarbonate ions dissociate into CO_2 and OH^- , the latter raising the pH [9]. During the nighttime the mats rapidly turn anoxic, and fermentation is one of the dominant microbial metabolisms. The formation of small organic acids during fermentation will lower the pH to values well below neutral. These pH changes are thought to influence the stability of AHLs [10].

How can signals be altered?

The alkaline pH (>9) that exists for half the daylight period [24] might contribute to AHL hydrolysis. The stability of an AHL against alkaline hydrolysis has been shown to vary depending on the acyl chain length [10,25], and AHLs with

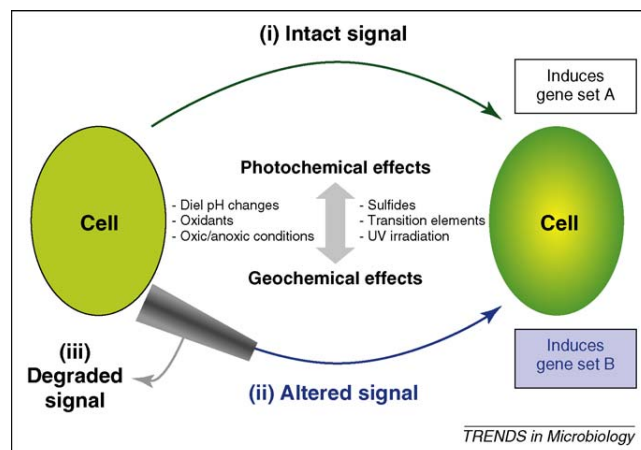


Figure 2. Interactions of signals with the natural environment. Because signal molecules traverse the extracellular environment to reach adjacent cells (or the cell of origin) their molecular structure might (i) remain intact allowing the compound to act as a signal, (ii) be altered in a predictable manner by environmental conditions (i.e. geochemical and/or photochemical) and elicit alternative effects on gene expression, or (iii) be degraded and not recognized as a signal or cue. It is proposed that if signals are altered in specific and predictable manner, they could be perceived by the cells and provide important sensory information.

longer acyl chains (>12 carbons) tend to be more resistant to hydrolysis than their shorter chain counterparts. If signals are degraded too rapidly by environmental conditions they might not be produced rapidly enough by cells to cause autoinduction in neighboring cells. This would imply that the activity of shorter-chain AHLs might be restricted to times when photosynthetic activity is low or absent. During daylight conditions and its associated alkaline pH only long chain AHLs would remain largely intact. However, both longer-chain and shorter-chain AHLs might remain active during the slightly acidic conditions at night. A recent study confirmed that the relative stability of AHLs in seawater increases with chain length [26].

Supersaturated O_2 concentrations during daylight facilitate the generation of oxidants through reactions involving carbonates, Fe(II) and certain types of organic matter, and photochemically-mediated Fenton reactions. As a result, oxidants (such as H_2O_2 or OH^*) might reach high concentrations within mats. Sulfides, especially abundant during nighttime anoxia [9], are highly reactive [27]. Whereas the periodic geochemical changes in microbial mats are well documented, the effects of these changes on signaling molecules and their efficacy on QS require further study.

Recent work conducted under laboratory conditions indicates that signal molecules could be altered to new, active forms by chemical reactions occurring in mats. For example, C6-AHLs (i.e. having six carbons on the acyl chain), when incubated in the presence of nitrates, are indirectly converted to several oxo forms, including the active signal 3-oxo-C6-AHL [28]. It is also possible that oxidants might open the lactone ring on an AHL.

Redox conditions, O_2 and sulfides could also alter signals. Sulfide, for example, is known to react rapidly with low-molecular-weight carbon compounds [27], especially during nighttime conditions. Therefore, the acyl side chains of AHLs (especially those containing oxo and

hydroxyl groups) could react in a similar fashion. The same study suggested that halides might also react with carbon compounds, especially under hypersaline conditions. Certain marine algae produce haloperoxidases that generate oxidized halogens and react specifically with the oxo group at the C3 position on an AHLs to negate its signaling activities [29]. The modified AHLs resulting from these reactions can be expected to have different effects on receptor sites than their native counterparts.

Additionally, bacteria might be able to synthesize modified signals depending on the availability of different substrates. Recently, it was shown that a new class of homoserine lactone signals was produced by a photosynthetic bacterium; the microbe used an exogenous molecule (*p*-coumaric acid) rather than the normal substrates (cellular fatty acids) to synthesize the structure [30] (Figure 1f).

Other possible alterations of signals include their degradation by enzymes. AHLs, generally produced by Gram-negative bacteria, can be degraded by many Gram-positive bacteria using two major groups of enzymes: AHL lactonases and AHL acylclases [31–33]. Certain Gram-negative bacteria also possess such enzymes, and these are thought to recycle AHLs so as to provide tighter control of auto-induction. Whereas most of these enzymes are intracellular, and therefore should not influence the extracellular movement of signals, their presence might decrease extracellular concentrations of signals, and furthermore might allow coexistence of Gram-negative and Gram-positive bacteria in proximity.

It is also notable that signals such as AHLs can be converted to forms exhibiting antimicrobial activities. Alterations of 3-oxo-AHLs, produced by Gram-negative bacteria, generate tetramic acid products that have antibiotic activity against Gram-positive bacteria [34]. Moreover, some antimicrobial agents exhibit signaling properties at low concentrations [35]. The multifunctional nature of signals has recently been addressed (e.g. the role of PQS in iron sequestration) [36].

The net effect of the above processes is that many permutations of microbial signal molecules can be generated through physical, chemical and biological processes in natural environments. Microbial cells must distinguish between molecules having useful information content for communication or sensing (i.e. signals or cues), and background organic compounds (i.e. chemical noise and nutrients). The molecular mechanisms that accomplish this, however, are not well understood, as discussed in the following subsection.

Can altered signals be perceived by cells?

If signals are modified by the local environment, two outcomes are possible: they could lose their ability to alter the expression of their respective target genes (i.e. signal degradation), or the modified signals might remain perceivable by cells. Many synthetic AHL derivatives have been tested for their ability to act as agonists or antagonists in QS assays [37–40]. Laboratory studies suggested [41] that responses to signal specificities often exist as a continuum, and the effect of a signal on gene expression could be dependent on growth phase. For example, certain bacteria

might respond best to a certain signal during stationary phase, but minimally respond to the same signal during the logarithmic phase of growth (or vice versa).

Biological environment

Complexity of natural communities

The biological and spatial heterogeneity of natural microbial communities results from, and contributes to, the diversity of chemical environments over microspatial (e.g. micrometer) scales. The highly localized diversities of species facilitate many interactions, either by chance or by self-organization [20]. These interactions will lead to exchange of signals between partners, non-intended sensing of cues, and chemical manipulation.

Physicochemical environmental conditions determine which microbes can thrive. For example, light is required for development of phototrophs, organic carbon for heterotrophs, temperatures between 45 and 80 °C for thermophiles. In turn, microbial metabolism can alter the physical and geochemical characteristics of the environment: for instance, the pH can increase through removal of acid substrates and/or production of alkaline metabolic products. If metabolic plasticity of the existing microbial community is inadequate to cope with these changes, a change in community composition will probably result. This feedback between environmental conditions and microbial populations is an ongoing process. In microbial mats, changes in environmental conditions display a diel cyclicity, although diel changes in diversity have not yet been documented. Remarkably, it appears that the community composition only changes over longer time spans (i.e. seasons) [13,14]. It is therefore of particular importance that the different types of microbes coordinate their metabolism, a process that could be enhanced by, although not necessarily being dependent upon, QS.

Sulfate-reducing bacteria (SRB), an important component of the mat, display maximum activity in mats during daytime [14] when oxygen levels achieve supersaturated concentrations. Although SRB are classically considered as anaerobes, it is conceivable that these microbes form close associations with other bacteria such as sulfur-oxidizing bacteria (SOB) (Figure 3). This synergy would allow both SRB and SOB to thrive under seemingly unfavorable environmental conditions (high O₂, and lack of sulfide, respectively) [42]. AHLs (or other types of signals) and metabolic products could be involved in coordinating metabolisms between SRB and SOB. However, data are currently lacking.

Complexities of natural environments?

QS has traditionally been considered to be a cell-density-dependent phenomenon wherein a single group of bacteria monitors its population and alters gene expression accordingly. In Gram-negative bacteria, two proteins, LuxI and LuxR, are essential for this process (Box 1). At low cell densities, the AHL synthase, LuxI, produces basal levels of AHLs. At high cell densities, the concentration (and sometimes production) of AHLs increase, resulting in binding and activation of the transcriptional regulator (LuxR) and subsequent activation of QS-regulated genes [43].

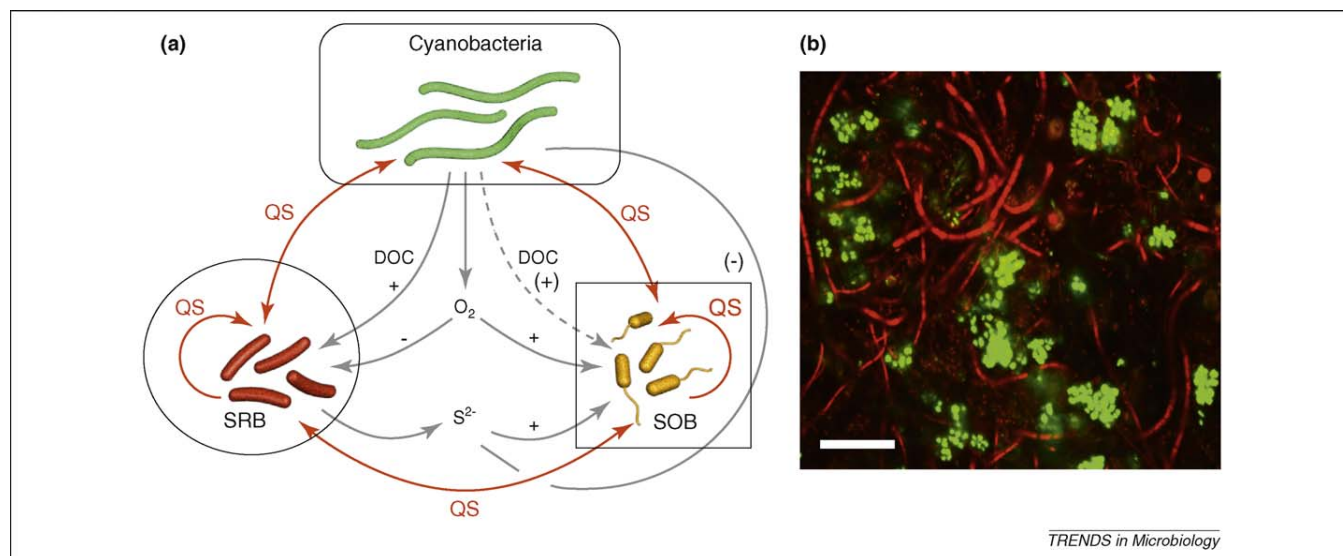


Figure 3. Signaling interactions between different groups of bacteria might facilitate the development of consortia in microbial mats. Initially, sensing molecules might be used to facilitate microspatial positions of cells in the mat. Diffusion of signals might increase awareness of nearby cell clusters and facilitate the development of consortia within or between clusters. **(a)** Very close and interdependent cycling of nutrients appears to occur among functional clades of microbial mats. Here, QS represents both quorum sensing and diffusion sensing. Cyanobacteria produce organic carbon during photosynthesis, that (in the form of dissolved organic carbon, DOC) stimulates the growth of heterotrophs such as sulfate-reducing bacteria (SRB), and to a lesser extent sulfur-oxidizing bacteria (SOB). However, the high O₂ level produced by cyanobacteria inhibits the growth of SRB, although it stimulates that of SOB. Finally, one of the metabolic products of SRB is S²⁻, that is toxic to cyanobacteria, and at higher concentrations also to SRB. However, S²⁻ is quickly removed by SOB. The resulting proposed consortium carefully balances the three metabolic modes. Many *in situ* rate measurements [7,14,22,24,27,67] clearly demonstrate that the three functional groups presented in this diagram operate at (near) maximum capacities, and this (in our opinion) requires sophisticated interspecific QS. Thus, signaling might occur within a cluster, or even between clusters, to facilitate (and periodically adjust) efficient recycling of nutrients in microbial mats. **(b)** A confocal microscopy image from a natural microbial mat showing clusters of heterotrophic bacteria (green cells) in proximity to each other, together with cyanobacteria (red string-like cells). Scale bar, 20 μm .

Many homologous systems have been revealed in proteobacterial species, where they regulate diverse phenotypes [2]. Each LuxR homolog demonstrates a level of specificity for its cognate AHL. Such specificity could establish an efficient signaling system for a single (or closely related group of) species to regulate gene expression within a mixed-species environment. Whereas all AHLs are composed of a conserved homoserine lactone, the fatty acid side chain can vary considerably in length (4–18 carbon atoms), level of saturation, and side chain (oxo or hydroxyl) substitutions. These differences affect binding efficiency in such a way that a particular LuxR homolog binds optimally to its cognate AHL [2].

Bacteria can contain multiple QS operons, and also single or multiple 'orphan' *luxR* homologs. Could harboring multiple QS systems (or *luxR* orphans) allow populations of bacteria to respond better to a vast array of environmental conditions? As pointed out earlier, most QS studies are performed within the confines of strictly controlled laboratory conditions where isolated cultures are thoroughly homogenized through intense shaking. Moreover, if the environment alters QS signals, could the modified signals bind to alternative receptors (e.g. different transcriptional regulators)? This might constitute an additional means of regulating gene expression in response to environmental changes. Also, it is important to note that some bacteria do not produce AHLs (i.e. they lack a *luxI* homolog) yet contain *luxR*-like genes and therefore might detect signals. Whereas researchers are starting to examine these possibilities, it is clear that QS-regulated gene expression is much more complex than originally thought.

Why do some bacteria harbor multiple QS systems? To address this question researchers have been examining the three QS systems of *Vibrio harveyi*: AI-1, CAI-1 and AI-2 [44]. Whereas it is not clearly understood why this bacterium utilizes three different autoinducers, this arrangement could allow community monitoring at three distinct levels: species (*V. harveyi*), genus (all vibrios), and bacteria of other genera, respectively [44]. Additionally, although not yet described, it is possible that certain environmental factors might differentially affect the stability of the three autoinducers, allowing *V. harveyi* to 'sense' a wide range of environmental changes. Furthermore, multiple QS systems might allow the formation of transcriptional regulator heterodimers (composed of two components, each one from a different QS system) that might bind to unique promoters, resulting in different gene expression profiles.

Can a single organism have several receptor proteins for a given signal that are active under different environmental conditions? Whereas QS has typically been examined in strict terms of LuxI–LuxR protein pairs, it is now evident that many proteobacteria can harbor a single *luxI*–*luxR* pair and many have single 'orphan' *luxR* homologs [45]. These orphans might allow bacteria to sense and respond to signals produced by other bacteria within a microbial community. Although not yet tested, an alternative hypothesis is that environmentally-modified autoinducers could bind to alternative orphan LuxR homologs instead of to its cognate protein, and in doing so provide a broader environmental sensor. Altered binding could allow access to a broader range of target genes and trigger global changes in gene expression,

allowing bacteria to sense surrounding environmental conditions.

EPS as a means to modify the signaling environment

In most natural environments, especially within microbial mats, bacteria are surrounded by a matrix of EPS that serves many functions [46]. How does this matrix affect signal transmission? EPS ranges in consistency from dense, firm hydro-gels to very loose arrays of soluble molecules. The diffusivities of signals, especially larger ones, might be slightly constrained by the EPS matrix. The mobility of a molecule within a gel depends on how fast it can diffuse through the nanopores between polymers. Within dense EPS gels, linkages between adjacent polymers will be frequent and pore sizes will be small, compared with loose-slime EPS where linkages are fewer and pore spaces quite large. Densities of EPS and the types of linkages between adjacent polymeric molecules vary over microspatial scales (e.g. nm to μm), called microdomains, and have the potential to impart specific chemical properties to the EPS [47,48]. Therefore, it is possible that bacterial colonies 'engineer' their surrounding EPS to better constrain the flow of signals and ions [49,50].

A final aspect of EPS and QS is that the polymer matrix provides a three-dimensional scaffold within which microbial cells can move and orient themselves. It is not surprising that microbial mats contain many clusters of bacteria of various sizes [10]. The proximity of cells within a cluster can be of major importance in facilitating signaling [20]. Interestingly, eukaryotic cells can transport large molecules very rapidly using a form of facilitated diffusion, and this occurs when normal three-dimensional diffusion is reduced to one or two dimensions. Here, filament tracks shuttle molecules in a particular direction, often faster than would be predicted if only passive diffusion was involved [51]. It is interesting to speculate that microbial cells in proximity to each other (e.g. within a biofilm cluster) might rapidly exchange large signals (or vesicles) using such extracellular tracks within the EPS.

Development of consortia facilitated by signaling

Given the plethora of environmental molecules and the complexity of signals encountered, how does a cell prevent information overload [52]? Bacteria in microbial mats are likely to encounter different combinations of signals. Therefore, integration of signals produced by other bacteria with signals from the home species could result in differential gene expression. This has been called a 'coincidence-detection system' in *V. harveyi* [53]. Such systems allow bacteria to differentially respond to the simultaneous presence of two signals versus either signal alone. This response requires the expression of different genes.

In nature, the integration of signals from diverse origins could be involved in the formation of groups of cells belonging to different species and having coordinated metabolic activities. Further, laboratory studies by Leadbetter and colleagues [54,55] showed that, through directed evolution (over many generations), bacteria can recognize other signals more efficiently. A signal might have multiple functions within a complex community, triggering expres-

sion of different gene sets in diverse taxa. It could act as a true signal, evolved to alter behavior and gene expression of another species. Alternatively, it could function as a sensing cue that is sensed by another organism but that did not evolve for this purpose (e.g. a metabolite that does not act as a signal for the producing species) [21]. Cells within a biofilm can participate in cross-talk where interactions of signals, cues and/or chemical manipulation molecules are exchanged with organisms different from the sender [56]. Nevertheless, how bacteria integrate the plethora of signals, cues and other molecules under natural conditions is largely unknown.

Signaling among bacteria within a complex community might enhance – but is not necessarily required for – the formation of stable, cooperative, and coordinated associations, even consortia. Recent sociobiological modeling studies of self-organization in biofilms predicted that organization can arise without active coordination (e.g. signaling), and that biofilms do not develop entirely as cooperative units [57]. Cooperative associations are not always beneficial to a system (e.g. biofilm) because cheaters (i.e. those who do not cooperate but gain from cooperation) will undermine the broader stability [58]. We suggest that local cooperation will occur intermittently at small spatial scales (e.g. within clusters), but not among all cells in a biofilm. Whereas QS *per se* might not be required for consortia development and other close associations, it provides a means for the modification of consortia. Hence, QS should be thought of as a network of signaling clusters occurring in small localized patches [59] that would coincide with the many clusters of cells that are observed within mats [10].

Conclusions and future directions

Many questions remain about how QS operates in natural environments (Box 2). The presence of signal molecules has been observed in a limited number of natural systems [10,26,60]. Given our present understanding of the physicochemical stability of signal molecules it appears that QS in natural biofilms will be confined to relatively small patches containing clusters of cells, and this raises the additional possibility that signals are altered by natural environments. Environmental alterations could be an asset to bacteria, and especially if chemical alterations of signals occur in a predictable manner that reflects the proximal geochemical environment. If signals are modified

Box 2. Outstanding questions

- What are the effects of shifting geochemical conditions on signaling (e.g. diel changes in pH, and oxic versus anoxic conditions)?
- How does the EPS matrix of biofilms influence diffusivity and signal concentration?
- How do different groups of bacteria genetically adapt (to each other) when they form consortia?
- How can bacteria adapt and respond to new signals?
- Do bacteria gain information from environmentally modified signals?
- How diverse are QS genes in natural systems?
- How do microenvironmental conditions influence gene expression *in situ*?

by the environment, and modifications are perceivable by receiver cells, then important environmental information can be gained that intact signals cannot provide.

Little is known about the genetic pathways involved in QS within natural systems. Currently, metagenomic approaches are being used to understand the overall diversity of QS in a variety of environments, including microbial mats and soils [61]. The determination of *in situ* environmental influences on gene regulation will be especially important in refining our understanding of how bacteria detect and respond to signals, and even change their gene expression patterns, under natural conditions. Finally, the inherent variability of natural environments and the presence of an EPS matrix over microspatial (*e.g.* micrometer) scales reinforce the likelihood of a broader role for QS in efficiency sensing.

Acknowledgements

This work was supported by National Science Foundation grants under the Environmental Genomics (En-Gen), Collaborative Research in Chemistry (CRC), and Biocomplexity (BE) Programs. We thank reviewers for their very helpful suggestions on many points mentioned in the manuscript.

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