Quorum Sensing and Microbial Biofilms

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Abstract Some bacterial species engage in two well-documented social behaviors: the formation of surface-associated communities known as biofilms, and intercellular signaling, or quorum sensing. Recent studies have begun to reveal how these two social behaviors are related in different species. This chapter will review the role quorum sensing plays in biofilm formation for different species. In addition, different aspects of quorum sensing in the context of multispecies biofilms will be discussed.

1 Introduction

Microbiology has traditionally followed a guideline of isolating and studying pure cultures of bacterial species. This has accelerated our understanding of bacterial physiology and molecular biology, particularly in the context of pathogenesis as

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dictated by Koch's postulates (Kaufmann and Schaible 2005). However, conventional pure culture microbiology fails to inform us about aspects of bacterial interactions and group behavior important in the environment and in disease.

Many bacteria and simple eukaryotes are often found growing as surface-associated aggregates, commonly referred to as biofilms. Biofilms have been recognized as a common form of microbial growth on aquatic surfaces in natural, clinical, and industrial environments.

Although natural and laboratory biofilms formed by different species have been shown to exhibit a wide variety of structural characteristics, most appear to be encased in a self-produced extracellular matrix. The contents of the matrix can vary from organism to organism, but are usually abundant in polysaccharides, nucleic acids, and proteins (Sutherland 2001). Biofilm formation has been suggested to result from a developmental programme of gene expression. Indeed, biofilm development and maintenance have been shown to require a wide range of genetic determinants and to involve bacterial subpopulations carrying out different functions. One of the regulatory mechanisms suggested to play a significant role coordinating biofilm formation for many species is intercellular signaling, or quorum sensing (QS) (Parsek and Greenberg 2005).

Microorganisms secrete a wide variety of small molecules that can be self-recognized in a concentration-dependent manner and subsequently induce or repress expression of QS-controlled genes. These QS signals are often referred to as auto-inducers (AIs) and can be classified based upon their structures (Camilli and Bassler 2006). In this review, we will discuss some of the primary classes of QS systems and how (or if) they contribute to biofilm development in different bacterial systems. Additionally, the potential role of QS in multispecies biofilms will be discussed.

2 Different Systems for Sensing a Quorum

The term quorum sensing was coined to describe microbial signaling mediated by self-recognized, secreted molecules, also known as auto-inducers (Fuqua et al. 1994). There are several molecular mechanisms for intercellular signaling in microbes. We will briefly describe a few different, commonly studied quorum sensing systems.

2.1 Acyl Homoserine Lactones

The first described acyl homoserine lactone (AHL) QS system was in *Vibrio fischeri* (Nealson 1977). *V. fischeri* is a marine species that can bioluminesce in the light organs of various marine animals, such as the Hawaiian bobtail squid

Euprymna scolopes (Ruby 1996). *V. fischeri* was found to bioluminesce at high cell densities in liquid batch culture (Nealson 1977). Due to an accumulation of the AHL signal, 3-oxo-hexanoyl homoserine lactone (Eberhard et al. 1981; Kaplan and Greenberg 1985).

AHL signaling has subsequently been described in a wide number of Gram-negative α-, β-, and γ-proteobacteria species (McDougald et al. 2006). AHL signals consist of a homoserine lactone moiety that is linked by an amide bond to an acyl side chain. As shown in Fig. 1, the acyl side-chain lengths and degree of substitution can vary from one QS system to another. AHLs can exhibit a wide range of diffusion characteristics. Short side-chain AHLs diffuse freely across cell membranes, as long side-chain AHLs may partition to the membrane, requiring active efflux for signal export (Pearson et al. 1999). AHL synthesis is primarily catalyzed by a single enzyme belonging to the LuxI family. While signals are perceived by cytoplasmic DNA-binding regulatory proteins belonging to the LuxR family (Parsek and Greenberg 2000).

2.2 Peptide Auto-inducers

While AHL signaling has been found exclusively in Gram-negative bacterial species, many Gram-positive species have been shown to utilize peptides for QS (Fig. 1). Competence signal peptides (CSP) are examples of QS molecules frequently used by streptococcal species. An accumulation of CSP induces autolysis, releasing chromosomal DNA into the environment (Steinmoen et al. 2002). Subsequent uptake of DNA by neighboring cells is thought to promote horizontal gene transfer (Thomas and Nielsen 2005). Natural competence is therefore thought to be advantageous as a form of group behavior, but CSPs and other peptide-based QS molecules are also implicated to regulate other group-associated behaviors in different Gram-positive species, such as biofilm formation (Li et al. 2001) and bacteriocin production (van der Ploeg 2005).

There is a basic molecular scheme for peptide-based signaling (Kleerebezem et al. 1997). A pro-peptide signal is translated in the cytoplasm. This signal can be processed both before and during transport across the cell membrane. Signals usually vary in size from 5 to 87 amino acids depending on the system. Additionally, signals often have unusual, modified amino acids and in certain cases are cyclized via lactone and thiolactone linkages. These modifications/lactonizations are thought to promote the stability of the signal in the extracellular environment (Horswill et al. 2007). There can be several differences and variations upon this scheme. For instance, *Streptococcus pneumoniae* CSP has been shown to be unmodified (Håvarstein et al. 1995). Signal molecule export is mainly carried out by an ABC transporter. Unlike AHLs, signal detection usually occurs at the surface of the cell by a membrane-associated sensor kinase, which then transduces a signal to a DNA-binding response regulator.

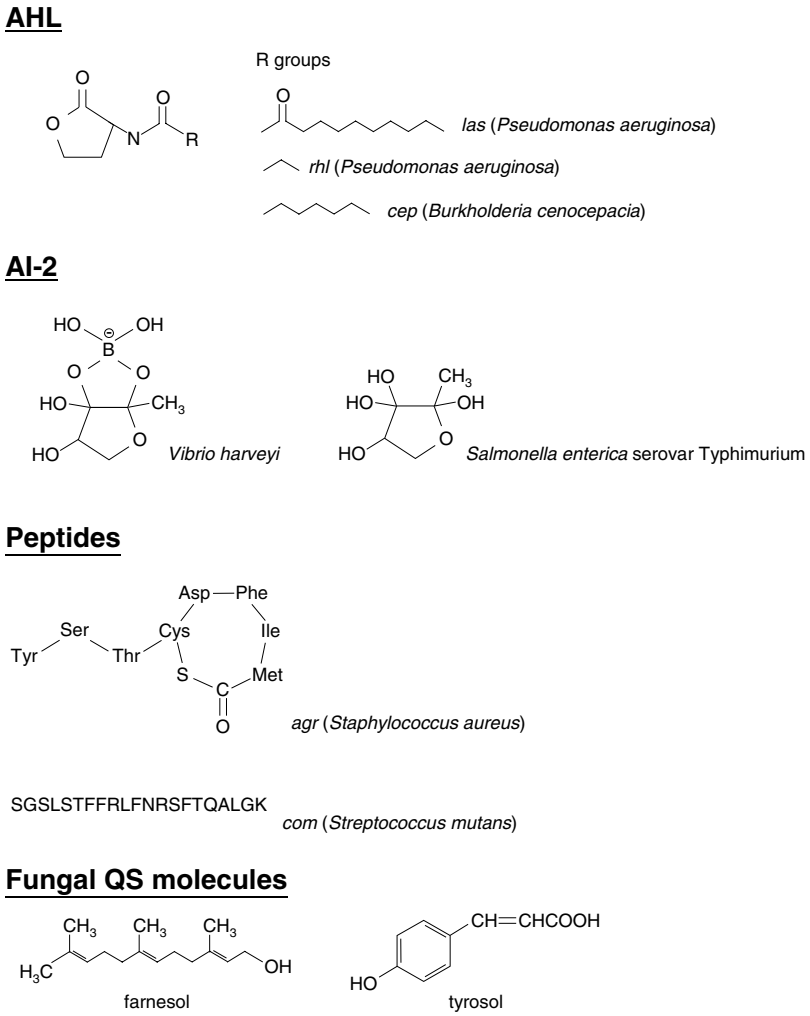

Fig. 1 Representative chemical structures of QS molecules

2.3 Auto-inducer 2

Auto-inducer 2(AI-2) is a QS signal produced by some Gram-positive and Gram-negative bacterial species. To date, only two AI-2 structures have been solved: those of *Vibrio harveyi* (Chen et al. 2002) and *Salmonella enterica* serovar Typhimurium (Miller et al. 2004) (Fig. 1). While the two structures are distinct, possibly allowing for species specificities, cross-species signaling appears to be prevalent (Camilli and Bassler 2006).

A key step in AI-2 synthesis is catalyzed by a highly conserved enzyme LuxS (Pei and Zhu 2004). The *luxS* gene is found in a vast number of bacterial species, implicating its importance for basic biological function (Xavier and Bassler 2003; Vendeville et al. 2005). AI-2 is derived from the precursor compound, 4,5-dihydroxy-2,3-pentanedione (DPD). LuxS converts *S*-ribosyl homocysteine to homocysteine and DPD. Once outside the cell, DPD can undergo a number of spontaneous chemical rearrangements to form different furans, including the two known AI-2 structures. AI-2 is perceived by the cell in different manners depending on the system. In the *V. harveyi* system, AI-2 is recognized at the cell surface by cell membrane-associated receptor LuxP (Bassler et al. 1994), while in the *S. enterica* sv. Typhimurium system AI-2 is first transported inside the cell prior to initiating a signaling cascade (Taga et al. 2001).

Various species appear to use the AI-2 QS system to regulate different functions, and furthermore, LuxS catalysis has an alternate role in cell metabolism as an integral component of the activated methyl cycle (Vendeville et al. 2005).

2.4 Fungal QS Systems

Eukaryotic microbial QS systems have recently been discovered and characterized in the dimorphic fungal species *Candida albicans* and *Saccharomyces cerevisiae*. Several QS molecules have been found, including farnesol (Hornby et al. 2001), tyrosol (Chen et al. 2004), phenylethanol, and tryptophol (Chen and Fink 2006). Farnesol is the best studied and appears to block yeast-to-mycelium conversion of *C. albicans* (Hornby et al. 2001). The timing of response to farnesol also regulates dimorphism by determining the commitment to morphotype conversion, but the molecular mechanisms are largely unknown (Nickerson et al. 2006). Interestingly, QS response kinetics of *C. albicans* to farnesol and tyrosol were different (Alem et al. 2006) and may represent a check-point for regulation during development.

3 Rationale for Using Quorum Sensing to Control Biofilm Formation

One can envisage different ways in which QS might influence biofilm formation. For example, QS-regulated functions might serve to initiate biofilm formation. Inducing concentrations of QS signals might precede starvation and other types of stress associated with crowded planktonic bacterial populations. To protect themselves from such types of stress, bacteria may form biofilms, a lifestyle that is characteristically more stress-resistant. Most biofilm systems have demonstrated enhanced resistance to external insults such as antibiotics, shear force, and the host immune system (Lewis 2001; Davies 2003; Jesaitis et al. 2003).

QS may also function to control the population size in a biofilm. Biofilm formation has been described as a developmental cycle (Fig. 2), and QS may serve as

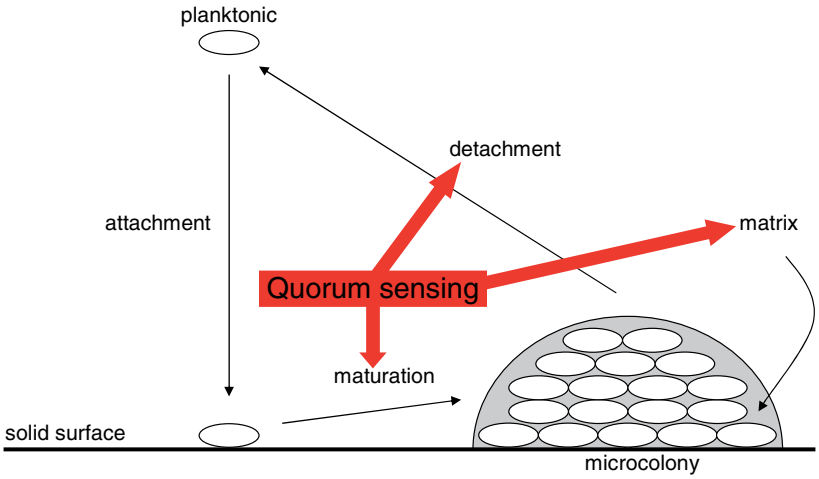

Fig. 2 Developmental biology of biofilms. Unattached free-swimming bacteria (planktonic) initially attach to a solid surface, eventually maturing into structured aggregates called microcolonies. Biofilms are composed of these microcolonies, often encased in extracellular polymeric substances known as the matrix. Some biofilm bacteria detach from microcolonies and become planktonic, presumably to colonize a new surface. QS is believed to be involved in regulating different steps of biofilm development, depending on the organisms and growth conditions

the checkpoint for reinitiating the cycle by promoting dispersion or dissolution of a subpopulation of cells. In this case, dispersing cells might escape the nutritional stress that accompanies or follows inducing concentrations of QS signal. For nonmotile species, QS might serve to regulate population density in a biofilm using a different mechanism. Some Gram-positive bacteria initiate autolysis in response to reaching a quorum (Steinmoen et al. 2002).

Finally, QS may induce behaviors in biofilm cells (as they transition from a QS-uninduced state to a QS-induced state) that alter the course of biofilm development, such as the production of secreted factors, such as exopolysaccharides or other adhesins. Alternatively, QS might induce or repress group activities such as surface motility, which in turn could have a profound impact on biofilm structure.

4 Examples of QS-Regulated Biofilm Phenotypes

The above points all represent plausible scenarios of how and when QS may impact biofilms. Thus, perhaps it is not surprising that a survey of the current literature reveals that QS can influence biofilm development in a myriad of ways for different species (Table 1). These studies are not necessarily definitive, they were generally conducted for biofilms grown under a single culturing condition. Even identical

strains of the same organism can form biofilms with profoundly different structural characteristics when grown under different nutritional conditions, and the effect of QS on biofilms can be nutritionally conditional (Yarwood et al. 2004; Shrout et al. 2006).

Another potential complication in interpreting the results in Table 1 is that many species have multiple, integrated quorum-sensing systems. Identifying the primary quorum-sensing system responsible for biofilm-related phenotypes can be difficult. For instance, in *Pseudomonas aeruginosa*, there are at least three primary quorum-sensing signals: two AHLs (butyryl homserine lactone and 3-oxo dodecanoyl homserine lactone) and a quinoline-like signal, PQS. The *las* QS system controls expression of the *rhl* QS system and both AHL systems can regulate expression of the PQS system (Latifi et al. 1996; Pesci et al. 1997).

As shown in Table 1, QS has been shown to be involved at multiple stages of biofilm formation in different species. Some QS systems appear to promote biofilm

Table 1 Effects of disabled QS system on biofilm formation in representative organisms

Organism	Disabled QS system	Effect on biofilm	References
Aeromonas hydrophila	*ahy* (AHL)	Defective maturation of biofilm	Lynch et al. 2002
Burkholderia cenocepacia	*cep* (AHL)	More susceptible to ciprofloxacin (double mutant)	Huber et al. 2001
	cci (AHL)	More susceptible to SDS (single mutants) Impaired maturation (*cep*)	Tomlin et al. 2005
Candida albicans	farnesol	Deficient in biofilm dispersal	Kruppa et al. 2004
Klebsiella pneumoniae	AI-2	Delayed biofilm development	Balestrino et al. 2005
Listeria monocytogenes	AI-2	*luxS* Mutant 58% more biofilm than wild type	Belval et al. 2006
Pseudomonas aeruginosa	*las* (AHL)	Flat, unstructured biofilm, more sensitive to SDS (*las*)	Davies et al. 1998
	rhl (AHL)	More susceptible to tobramycin and H_2O_2 (double mutant) Varies with growth conditions	Bjarnsholt et al. 2005a
Pseudomonas putida	*ppu* (AHL)	Formation of more structured biofilm with distinct microcolonies and water channels	Steidle et al. 2002
Serratia liquefaciens	*swr* (AHL)	Thinner biofilm, lacking cell aggregates and cell chains	Labbate et al. 2004
Serratia marcescens	*swr* (AHL)	No biofilm dispersal	Rice et al. 2005
Staphylococcus aureus	*agr* (peptide)	Varies with growth conditions	Yarwood et al. 2004

formation, while others appear to be involved in biofilm dispersion. In *Vibrio cholerae*, QS negatively regulates production of the *vps* exopolysaccharide (Hammer and Bassler 2003; Zhu and Mekalanos 2003). Expression of *vps* has been shown to promote biofilm formation (Yildiz and Schoolnik 1999). Thus, at high cell densities, QS induction would dissuade biofilm formation. QS mutations result in upregulated matrix production, and therefore enhanced biofilm production (Hammer and Bassler 2003; Zhu and Mekalanos 2003). A completely opposite phenomenon has been recently observed in *P. aeruginosa*, where QS positively regulates *pel*, a major biofilm-related exopolysaccharide operon (Sakuragi and Kolter 2007). In *Serratia*, the conserved AHL system (*swr*) works differently between two species of the same genus. *Serratia liquefaciens swr* appears to promote biofilm formation (Labbate et al. 2004), while in *Serratia marcescens swr* promotes biofilm dispersal (Rice et al. 2005).

QS has also been shown to regulate biofilm formation in simple eukaryotes such as *C. albicans*. Alem et al. reported that early in biofilm development, the QS signal tyrosol is responsible for promoting hyphal formation, while during later stages, another QS signal, farnesol, promotes dispersal of yeast cells from the biofilm (Alem et al. 2006). This suggests that the syntheses of tyrosol and farnesol are differential throughout biofilm development, and cells respond according to which QS signal accumulates at particular developmental stages.

Although QS has been shown to be important during biofilm formation for a wide variety of species, in many cases the QS-regulated functions responsible for observed biofilm phenotypes have not been identified. This is an emerging challenge for researchers, particularly since this question may be complicated. In the case of *P. aeruginosa*, several QS-regulated functions have been shown to influence biofilm development (Kirisits and Parsek 2006).

5 Relationships Between QS, Biofilms, and Infection

It has been suggested that a majority of chronic bacterial infections are characterized by biofilm formation (Parsek and Singh 2003; Hall-Stoodley et al. 2004). One of the paradigm biofilm diseases are the chronic airway infections that afflict people who suffer from cystic fibrosis (CF). *P. aeruginosa* is one of the major CF pathogens and exhibits many characteristics of biofilms in the airways, including the formation of large cellular aggregates and distinct AHL production patterns (Singh et al. 2000).

Virulence factors required for acute infection are often repressed during chronic infections for species capable of causing both types of infection. Acute virulence factors can stimulate the host immune system and cause damage to host tissues, while establishing chronic infection necessitates avoiding the host immune response and maintaining a stalemate with the host, where invasive tissue damage is minimized. Biofilm formation may be an important mechanism for host immune modulation and/or virulence factor downregulation (Irie et al. 2004; Kuchma et al. 2005).

V. cholerae is an interesting example of integrating QS and biofilm formation during its pathogenic life cycle. *V. cholerae* does not cause a chronic infection, but causes acute gastroenteritis, which leads to severe diarrheal symptoms. *V. cholerae* has an aquatic environmental niche, and infection is thought to be only transient in its lifestyle, promoting its proliferation and dissemination. *V. cholerae* most likely resides in biofilm communities while in the environment (Watnick and Kolter 1999; Watnick et al. 2001). Upon ingestion, bacteria first encounter stomach acid, and it has been shown that biofilm cells are more resistant to acid than planktonic cells (Zhu and Mekalanos 2003). Subsequently, QS downregulates extracellular matrix production (Hammer and Bassler 2003; Zhu and Mekalanos 2003) and dislodges the bacteria from biofilms once inside the intestinal lumen, leading to more widespread colonization. Bacterial proliferation in the intestines is thought to induce QS once again, which downregulates adhesins required for biofilm formation (Miller et al. 2002; Zhu et al. 2002) as well as the matrix production, allowing the bacteria to detach from the intestinal epithelia and exit the host via diarrheal flow.

Studying biofilm infections in the laboratory has been problematic. Very few biofilm models of animal infections have been established, and even fewer have tested virulence of QS mutants in such models. Deletion of *luxS* in *Staphylococcus epidermidis* resulted in enhanced biofilm formation in vitro, and enhanced virulence in the catheter-associated infection model (Xu et al. 2006). Similar observations were made with *agr* QS mutants of *S. epidermidis* (Vuong et al. 2004). On the other hand, decreased chronic virulence has been observed for QS mutants in other species, including *S. marcescens* (Coulthurst et al. 2004), *Vibrio vulnificus* (Kim et al. 2003), and *Neisseria meningitidis* (Winzer et al. 2002),

Although it is unknown whether QS-regulated biofilm is important for enteropathogenic *Escherichia coli* (EPEC) in vivo during infection, there is evidence that QS mutants are deficient in biofilm formation in vitro (Moreira et al. 2006). The primary QS system in EPEC that appears to control important adhesins for in vitro biofilm formation is AI-3, an alternative class of QS molecule distinct from previously reported AHLs and AI-2 (Sperandio et al. 2003). AI-3 also controls other virulence factors such as the LEE (locus of enterocyte effacement) pathogenicity island, which encodes for type III secretion system (T3SS) and toxins secreted by T3SS. Furthermore, EPEC AI-3 receptor QseC also binds to epinephrine and norepinephrine (Clarke et al. 2006), which are secreted into the intestinal lumen by the host, providing evidence for interkingdom signaling pathway. It is intriguing to speculate whether the AI-3/epinephrine/norepinephrine QS signaling pathway is inducing in vivo biofilm formation in the intestinal lumen during EPEC infection of the host.

6 QS in Multispecies Biofilm Communities

Biofilms found in many clinical, industrial, and natural environments are frequently mixed species. In these communities, the high cell densities might result in high QS signal concentrations. This is valuable information, both for the species producing the

signals and other species occupying the same habitat. There is growing interest in the importance of interspecies QS in shaping multispecies communities. In this section we will discuss some important considerations for QS in mixed-species systems.

6.1 Synergistic Interactions

Studies of oral bacterial communities have been the most progressive in terms of mixed-species biofilms. There are as many as 500 species found in dental biofilms, and colonization by different species appear to be spatiotemporally dynamic (Kolenbrander et al. 2002). AI-2 QS has been suggested to be important for promoting mixed-species interactions in the oral cavity. For example, the AI-2 QS system is required for the oral commensal species, *Streptococcus gordonii,* to form a mixed biofilm with the oral pathogen *Porphyromonas gingivalis* (McNab et al. 2003). Interestingly, the bacteria are capable of forming dual-species biofilm even if the other species is unable to produce AI-2, suggesting a cooperative usage of the AI-2 signal. Only when both AI-2 signal are inactivated are co-culture biofilms disrupted.

There are examples of interspecies QS communication among AHL-producing organisms important in CF lung infections. Interspecies AHL QS has been demonstrated in *P. aeruginosa–Burkholderia cenocepacia* dual-species biofilms (Riedel et al. 2001). In this system, *B. cenocepacia* could perceive *P. aeruginosa* signal, but *P. aeruginosa* did not respond to *B. cenocepacia* AHL. This study suggests that AHLs produced by *P. aeruginosa* may promote AHL-regulated virulence factor production by *B. cenocepacia*.

There are other mixed-species biofilm systems that have enhanced phenotypes only when multiple species are present, such as biofilms formed by bacteria isolated from marine algae (Burmølle et al. 2006). These data are suggestive of cooperative or synergistic QS interactions, but the specific molecular mechanisms of interspecies communications are still undetermined.

6.2 Antagonistic Interactions

Microorganisms occupying the same niche are constantly competing for common resources. Competition within mixed-species biofilms may be fierce, with high cell numbers of competing species spatially fixed within close proximity of one another. Bacteriocin production (Tait and Sutherland 2002) and lowering of pH (Burne and Marquis 2000) are two of the mechanisms within biofilm communities that may help some species to compete. Bacteriocin production can be regulated by QS (Kleerebezem 2004) and may represent one of the mechanisms through which QS is important for competition. *P. aeruginosa* appears to utilize QS to help achieve population dominance over *Agrobacterium tumefaciens* in biofilm growth

(An et al. 2006). Yeast species appear to take a direct approach at dealing with competitors, using their QS molecule farnesol. Farnesol-exposed *Staphylococcus aureus* displays enhanced antibiotic susceptibility, as well as decreased biofilm formation (Jabra-Rizk et al. 2006).

QS might also provide established bacterial biofilms a means to prevent predation by simple eukaryotes. In addition to promoting biofilm dispersion in *S. marcescens*, *swr* QS system mediates resistance against protozoan grazing (Queck et al. 2006). It is unclear, however, whether QS promotes active resistance against grazing. It also remains to be determined whether a similar observation can be made for biofilms under attack by the predatory bacterial species *Bdellovibrio bacteriovorus* (Kadouri and O'Toole 2005).

Another case of microbial warfare in the context of biofilms has been studied for the soil microorganisms *P. aeruginosa* and *C. albicans* interactions. *P. aeruginosa* can degrade farnesol, the QS signal required for *C. albicans* filamentation and mature biofilm formation (Cantwell et al. 1978). *P. aeruginosa* can also use QS-regulated virulence factors to kill *C. albicans*. *C. albicans* exposed to the *las* QS AHL signal does not filament, remaining in the yeast morphotype (Hogan et al. 2004). Yeast-only *C. albicans* has been demonstrated to be impaired for biofilm formation (Lewis et al. 2002; Ramage et al. 2002). The yeast form of *C. albicans* is, however, more resistant to killing by *P. aeruginosa* (Hogan and Kolter 2002), enhancing their survival in this environment.

6.3 Listening but Not Talking

Some microbes have been shown to specifically respond to QS signals that they do not produce. This may constitute an effective means of gearing one's physiology to neighboring competitors. For example, despite the inability to produce AI-2, *P. aeruginosa* upregulates virulence factors such as phenazine, elastase, and rhamnolipid in its presence. This may be significant in mixed-species environments such as CF airway infections (Duan et al. 2003). AI-2 molecules have indeed been found in CF sputum where *P. aeruginosa* is thought to produce biofilms. *P. aeruginosa* may produce virulence factors to eliminate competing microbial species colonizing the respiratory tract. Similarly, *S. enterica*, despite their inability to produce AHL QS signal, encodes a probable AHL-responsive transcriptional regulator (SdiA). This allows *S. enterica* to detect and respond to AHL molecules produced by other bacteria (Ahmer 2004; Henke and Bassler 2004).

Bacteria are not the only organisms that are eavesdropping. In the ocean, zoospores of the green seaweed *Ulva* are attracted to AHL molecules produced by bacteria (Tait et al. 2005). Biofouling is often initiated by bacterial biofilms colonizing a surface, followed by eukaryotes such as algae (Beech et al. 2005). It is unclear why algae are specifically attracted to bacterial biofilms, but it has been reported that several green algae have developmental defects in the absence of periphytic bacteria (Provasoli and Pintner 1980; Stratmann et al. 1996).

6.4 Interfering with QS Signaling

Recent work has demonstrated an abundance of organisms capable of degrading QS signals. In spatially structured microbial communities, QS signal degradation may prevent signal propagation from one region of a biofilm to another. This may result in signaling being spatially confined to different regions of the community. One example of QS signal degradation is the ability of *P. aeruginosa* to degrade *C. albicans* signal, farnesol (Cantwell et al. 1978).

Two of the most common quorum quenchers of AHL systems are AHL-lactonases and AHL-acylases (Dong and Zhang 2005). AHL-lactonases can hydrolyze the lactone ring while AHL-acylases hydrolyze the amide linkages. An AHL-lactonase was initially isolated from *Bacillus* (Dong et al. 2000) and has subsequently been found in several other bacterial species. AHL-acylases were initially discovered in *Variovorax paradoxus*, which can utilize AHLs as a carbon source (Leadbetter and Greenberg 2000). *P. aeruginosa* (Huang et al. 2003) and *Ralstonia* species (Lin et al. 2003) have also been discovered to produce AHL-acylases.

Interference of QS signaling can also be achieved through the use of QS signal mimics (McDougald et al. 2006). For example, the *P. aeruginosa las* QS molecule can inhibit the *Chromobacterium violaceum* AHL QS system (McClean et al. 1997). Another example is *Xanthomonas campestris* pathovar campestris, which produces cis-11-methyl-2-dodecenoic acid (DSF) (Barber et al. 1997), which is a structural analog of farnesol (Wang et al. 2004). DSF can inhibit germ tube formation of *C. albicans*, but farnesol does not interfere with *X. campestris* (Oh et al. 2001). Finally, another classic example is the truncated peptides of some *S. aureus agr* QS systems, which can inhibit QS in other *S. aureus* strains (Lyon et al. 2000).

Eukaryotes also possess the ability to interfere with bacterial QS. Human airway epithelia can degrade *P. aeruginosa las* QS molecules through the activity of secreted paraoxygenases (Chun et al. 2004). Interestingly, the *rhl* QS molecule was not inactivated by the epithelia. Since the *P. aeruginosa las* QS system controls the *rhl* QS system (Latifi et al. 1996), inactivation of *las* may be more effective for the host to reduce the virulence of the pathogen.

Another example of eukaryotic inactivation of bacterial QS was discovered in the Australian marine macroalga, *Delisea pulchra*, which uses QS signal mimics called furanones to interfere with AHL signaling in Gram-negative bacterial species, such as *P. aeruginosa* (Manefield et al. 1999; Hentzer et al. 2002). This is thought to inhibit microbial biofilm formation on the surface of its leaves.

7 Summary

Studies of social behavior in bacteria have begun to clearly shape how microbiologists perceive microbial communities. The relationship between QS and biofilm formation has the potential to shape these communities. Closely related species seemingly employ QS for very different purposes during biofilm development.

Many environments are colonized by biofilms consisting of multiple species. In this context, the likelihood of interspecies interactions in the form of QS signaling may be high. Cooperative QS signaling has been demonstrated in several multispecies biofilm systems (McNab et al. 2003; Burmølle et al. 2006). In some cases, QS appears to be utilized by microbes to compete with one another. Biofilms probably represent a common relevant context for these types of interactions.

Microbial biofilms have strong relevance to chronic bacterial infections (Parsek and Singh 2003; Hall-Stoodley et al. 2004). Since biofilm formation for many organisms are QS-mediated, therapeutic strategies targeting QS systems are attracting attention in the drug development fields. Microbes have long utilized various anti-QS strategies for competition against other species (Zhang and Dong 2004). Understanding these molecular mechanisms may be fruitful in developing therapeutic strategies against pathogenic species. Already, some quorum-quenching chemical compounds have demonstrated success in inhibiting microbial biofilms (Dong and Zhang 2005). Unlike modern antibiotics, QS-directed therapies are not designed to cause bactericidal or bacteriostatic effects, and thus, emergence of resistance may be less problematic. Anti-QS measures have been demonstrated to be effective for plant infections. Heterologously expressed AHL-lactonase rendered tobacco and potato plants significantly more resistant to *Erwinia carotovora* (Dong et al. 2001).

As the studies of microbial communities continues, understanding how different species percieve and respond to one another will be crucial to understanding community composition and function. The most relevant context for such studies may be microbial biofilm.

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