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Review Article

Quorum sensing and Bacterial Pathogenicity: From Molecules to Disease

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ABSTRACT

Quorum sensing in prokaryotic biology refers to the ability of a bacterium to sense information from other cells in the population when they reach a critical concentration (i.e. a *Quorum*) and communicate with them. The "language" used for this intercellular communication is based on small, self-generated signal molecules called as autoinducers. *Quorum sensing* is thought to afford pathogenic bacteria a mechanism to minimize host immune responses by delaying the production of tissue-damaging virulence factors until sufficient bacteria have amassed and are prepared to overwhelm host defense mechanisms and establish infection. *Quorum sensing* systems are studied in a large number of gram-negative bacterial species belonging to α, β, and γ subclasses of proteobacteria. Among the pathogenic bacteria, *Pseudomonas aeruginosa* is perhaps the best understood in terms of the virulence factors regulated and the role the *Quorum sensing* plays in pathogenicity. Presently, *Quorum sensing* is considered as a potential novel target for antimicrobial therapy to control multi/all drug-resistant infections. This paper reviews *Quorum sensing* in gram positive and gram negative bacteria and its role in biofilm formation.

Keywords: Autoinducer, acyl homoserine lactone, biofilm, *Quorum sensing*

INTRODUCTION

Quorum sensing in prokaryotic biology refers to the ability of a bacterium to sense information from other cells in the population when they reach a critical concentration (i.e. a *Quorum*). The phenomenon of *Quorum sensing*, or *cell-to-cell communication*, relies on the principle that when a single bacterium releases autoinducers (AIs) into the environment, their concentration is too low to be detected. However, when sufficient bacteria are present, AI concentrations reach a threshold level that allows the bacteria to sense a critical cell mass and, in response, to activate or repress target genes.[1]

The discovery of intercellular communication among bacteria has led to the realization that bacteria are capable of coordinated activity that was once believed to be restricted to multicellular organisms. The capacity to behave collectively as a group has obvious advantages, for example the ability to migrate to a more suitable environment/better nutrient supply and to adapt new modes of growth, such as sporulation or biofilm formation, which may afford protection from deleterious agents. The "language" used for this intercellular communication is based on small, self-generated signal molecules called as autoinducers (AIs). Through the use of AIs, bacteria can regulate their behavior according to population density.[2]

It is now known that a significant portion of bacterial genome (4–10%) and proteome (≥20%) can be influenced by *Quorum sensing*. This only implies that *Quorum sensing* is a mechanism used by pathogenic bacteria not only to modulate virulence factor production but also to adapt to the metabolic demands of living in community.[3-7]

Quorum sensing is a generic regulatory mechanism used by many Gram negative bacteria and Gram positive bacteria to perceive and respond to factors as varied as changing microbial population density and the

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expression of specific genes. The concentration of a signal molecule reflects the density of bacterial cells in a defined environment, and the perception of a threshold level of that signal indicates that the population is "quorate" that is sufficiently dense to make a behavioral group-based decision. *Quorum sensing* is thought to afford pathogenic bacteria a mechanism to minimize host immune responses by delaying the production of tissue-damaging virulence factors until sufficient bacteria have amassed and are prepared to overwhelm host defense mechanisms and establish infection.[8]

HISTORICAL ASPECTS

Bacteria were for a long time believed to exist as individual cells that sought primarily to find nutrients and multiply. More than 30 years ago microbiologists found a way to induce luminescent bacteria to emit light prematurely. By adding cell-free culture fluid from a bright bioluminescent culture at high cell density to a non-luminous low cell density culture, they were able to eliminate the characteristic lag in the bioluminescence.[8-10] Nealson *et al*, proved that the "light switch" that controls the bioluminescence genes in the marine bacterium *Vibrio* (formerly *Photobacterium*) *fischeri* was cell density dependent, and they hypothesized that this light switch was controlled by AI signal produced by bacteria.[10]

In the above-mentioned example of bioluminescence, it was noted that bioluminescence could occur only after the AI accumulated in cultures to a threshold level that was attained at high cell density (>10^7CFU/ml).[11] Eberhard *et al*, went on to elucidate the structure of the AI molecule that was responsible for this effect, the first known acyl-homoserine lactone (AHL), 3-oxo-hexanoyl-homoserine lactone (3-oxo-C6-HSL).[12] 3-oxo-C6-HSL was found to freely diffuse from bacterial cells into the surrounding medium and vice-versa. This phenomenon of cell density dependent auto induction of specific bacterial genes involves two conserved regulatory gene products: (i) a Lux I-type acyl-HSL synthase and (ii) a Lux R-type transcriptional activator whose activity requires a particular acyl-HSL made by the cognate Lux I enzyme.[13] In addition to the Lux R/Lux I control, a second *Quorum sensing* system regulates the luminescence (*lux*) genes in *V. fischeri*. This second system consists of an acyl-HSL synthase, AinS, which directs the synthesis of octanoyl-HSL (C8-HSL). *V. fischeri ainS* mutants exhibited early luminescence, whereas the addition of C8-HSL delayed luminescence in cultures of wild type cells.[14] These examples illustrate how timing of a *Quorum-sensing-controlled (QSC)* process can be advanced merely by early exposure of the cells to a critical concentration of acyl-HSL. QSC phenomenon includes antibiotic production, virulence gene expression and other processes in many diverse bacteria.

Quorum sensing in Gram negative bacteria

AHL-mediated *Quorum sensing* systems are studied in a large number of Gram negative bacterial species belonging to the α, β, and γ subclasses of proteobacteria, including bacteria in the genera *Agrobacterium*, *Aeromonas*, *Burkholderia*, *Chromobacterium*, *Citrobacter*, *Enterobacter*, *Erwinia*, *Hafnia*, *Nitrosomonas*, *Obesumbacterium*, *Pantoea*, *Pseudomonas*, *Rahnella*, *Ralstonia*, *Rhodobacter*, *Rhizobium*, *Serratia*, *Vibrio*, *Xenorhabdus*, and *Yersinia*. Among the Gram negative bacteria, the most well-studied *Quorum sensing* system is the LuxR–LuxI homologous system and the cognate signal molecules: *N*-acyl-homoserine lactones (AHLs); similar to the system first studied in the marine bacteria *Vibrio fischeri*.[9]

The vast majority of gram negative *Quorum sensing* systems that have been studied thus far utilize N- AHLs as signaling molecules. When in high enough concentrations, these molecules can bind to and activate a transcriptional activator, or R protein, which in turn induces expression of target genes. The use of biosensors to screen spent culture supernatants has led to the discovery that AHLs are produced by a plethora of unrelated bacteria. Biosensors typically consist of a QSC promoter fused to a reporter such as *lac Z* or the *lux* operon. These biosensor strains contain a functional R protein but lack the AHL synthase enzyme; therefore, promoter activity depends on the presence of exogenous AHL. Despite the fact that R protein are exquisitely sensitive to their cognate AHLs, some infidelity does exist and this infidelity enables R proteins to be responsive to a range of AHL molecules, albeit higher concentrations of non-cognate AHL are usually required for activation.[2]

It is becoming apparent that in addition to AHLs, alternative gram negative signaling molecules do exist. For example, the plant pathogen *Ralstonia solanacearum* produces 3-hydroxy-palmitic acid methyl ester as a novel signaling molecule, which together with AHLs is used to regulate virulence.[15] *Xanthomonas campestris*, a cabbage pathogen, produces a diffusible extra cellular factor, which has yet to be chemically characterized but is not an AHL.[16] In *Pseudomonas aeruginosa*, a third AI, designated PQS (Pseudomonas Quinolone Signal), was identified that is different from the other two AHL AIs produced by this organism, in that it is a 2-heptyl-3hydroxy-4-quinolone.[3,17-19] The structural similarity between PQS and antimicrobial quinolones is quite intriguing, although

preliminary studies have showed no antimicrobial activity associated with PQS.[17] Butyrolactones have been isolated from *Pseudomonas aureofaciens* culture supernatants, and recently a novel family of signaling compounds, identified as diketopiperazines (DKP), were discovered in cell-free supernatants of *P. aeruginosa*, *P. fluorescens*, *P. alcaligenes*, *Enterobacter agglomerans* and *Citrobacter freundii*.[20]

Quorum sensing in *Pseudomonas aeruginosa*

With regard to bacteria that utilize *Quorum sensing* as part of their pathogenic life style, *P. aeruginosa* is perhaps the best understood in terms of the virulence factors regulated and the role the *Quorum sensing* plays in pathogenicity. Classified as an opportunistic pathogen, *P. aeruginosa* primarily infects individuals who are immunocompromised, such as patients with cancer or AIDS or those having breaches in normal barriers caused by burns, indwelling medical devices, or prolonged use of broad-spectrum antibiotics.[21-24] *P. aeruginosa* has an impressive armament of both cell-associated and extra cellular virulence factors. Expression of many of the extracellular factors is not constitutive but rather cell-density dependent with maximum protease production occurring during the late logarithmic and early stationary phases of growth. Two intertwined *Quorum sensing* systems have been shown to be involved in virulence, biofilm development, and many other processes in *P. aeruginosa*. The first system (Las) was discovered by Iglewski and colleagues and consists of a *lasI* -encoded acyl-HSL synthase and the *las*R-encoded transcriptional activator.[25,26] The second system (Rhl) was found by a number of investigators and consists of an *rhl I*-encoded acyl-HSL synthase and an *rhl*R-encoded transcriptional activator.[27-30] In the respective *Quorum sensing* systems each produce and respond to a specific acyl-HSL; LasI directs the synthesis of 3-oxo-dodecanoyl-HSL (3-oxo-C12-HSL) and RhlI directs the synthesis of butyryl -HSL (C4-HSL).[31]

Recently, Whiteley *et al*, using a *P. aeruginosa las I* and *rhlI* double mutant identified nearly 40 *qsc* genes that showed a five-fold or greater response to exogenously added acyl-HSL signals. The *qsc* genes were classified based on the temporal pattern of their responses to cells grown in presence of Las signal, 3-oxo-C12-HSL and/or the Rhl signal, CH-HSL. A number of "early" *qsc* genes were found that responded immediately to exogenously added signals, suggesting that these genes behave like the *lux* genes of *V. fischeri* and the carbapenem biosynthesis genes of *Erwinia*. Since these seminal observations were made, a number of other proteins have been found that support this hypothesis, including the stationary phase sigma factor RpoS, a third LuxR homolog (qscR), a secondary metabolite regulator, RsmA and the stringent response protein Rel A, all of which are involved in modulating expression of genes. qscR was found to negatively regulate expression of both rhlI and the 3-oxo-C12-HSL synthase gene, *LasI*. *P. aeruginosa qsc*R mutants showed early activation of a number of *qsc* genes and premature synthesis of both signals, C4-HSL and 3-oxo-C12-HSL.[25,32-36] Over expression of the *rsmA* gene product resulted in decreased production of *qsc* virulence factors and acyl-HSLs whereas deletion of *rsmA* led to early activation of *lasI* and thus early synthesis of 3-oxo-C12-HSL.[35] RsmA, RpoS, QocR all negatively regulate the Rhl or Las *Quorum sensing* systems, thus preventing early activation of these systems.

Quorum sensing in *P. aeruginosa* is involved in regulating expression of a number of virulence factors, and as such, this regulation is believed to play an important role in the pathogenicity of this organism. Using a number of different animal models, this presumption has been confirmed. In the neonatal mouse model of pneumonia, a *las*R deficient strain of *P. aeruginosa* was found to have significantly decreased virulence compared to that of parent.[37] Analysis of *lasI* mutant, a *rhlI* mutant and a *lasI, rhlI* double mutant in the same model revealed markedly decreased virulence, with the most notable reduction seen in double I mutant.[38] In a burned mouse model, strains deficient in *las*R, *lasI*, *rhlI* or both *lasI* and *rhlI* were found to be less virulent *in vivo* than in the parental strain. In addition, the total number of bacteria recovered from the spleens, livers, and skin of mice infected with the different mutants were significantly lower than those for the parental strain. These findings indicate that *Quorum sensing* plays an important role in the dissemination of *P. aeruginosa* throughout the body of burned mouse. In a study employing three different models of infection, namely *Caenorhabditis elegans* (nematode), *Arabidopsis thaliana* (plant) and a burned mouse model, a *las*R-deficient mutant generated through random mutagenesis exhibited greatly reduced virulence in all these models. These studies are extremely exciting because they suggest that the three aforementioned genes, which are all linked to *Quorum sensing*, contribute to trans-kingdom virulence of *P. aeruginosa*. Moreover, using the less costly and simpler plant or nematode model of infection enables identification of genes required for infection of other species. In the future, it will be intriguing to see if other bacteria that infect multiple species and employ *Quorum sensing* as part of their pathogenic lifestyles have genes that contribute to virulence in such diverse hosts.[39,40]

Quorum sensing in *Enterohemorrhagic Escherichia coli* O157:H7

The infection by Enterohemorrhagic *Escherichia coli* (EHEC)

O157:H7 can lead to potentially severe gastroenteritis and other extraintestinal manifestations, including fever, meningitis, and septicemia. The EHEC also express Shiga toxin (Stx) in the intestine which also has its receptors located in the kidneys and the CNS. Stx is a potent inhibitor of protein synthesis and can get absorbed systemically leading to hemolytic uremic syndrome (HUS), seizures, cerebral edema and coma. More so, in the management of EHEC infections, antimicrobial agents and antimotility agents are contraindicated as they promote the expression and release of Stx, thereby increasing the occurrence and severity of HUS and CNS involvement. For all these reasons, it becomes more imperative, the need for innovative and cost-effective treatment modalities.[41-43]

EHEC senses three signals to activate transcription of virulence genes: a bacterial aromatic AI (AI-3), produced by normal GI flora and two hormones (epinephrine/norepinephrine), produced by the host. Any of these signal molecules can trigger the QseC membrane bound sensor activity, ultimately leading to transcription of virulence genes. The QseC also activates the expression of second sensor QseE which also helps in fine tuning the signaling cascade. All these transcription events lead to formation of attachment and effacing lesions in the intestine and the production of Stx.[44,45]

Rasko *et al*, in their study identified a lead structure, LED 209 (*N*-phenyl-4-[[(phenylamino)thioxomenthyl]amino]-benzenesulfonamide), which selectively blocked binding of signals (AI-3/epinephrine and NE) to QseC, consequently inhibiting QseC-mediated activation of virulence gene expression. Significantly, LED 209 did not lead to killing of EHEC cell as that would have initiated Stx production. LED 209 was also not toxic to the host cell and only inhibited key virulence traits of EHEC.[46]

QseC homologs are present in at least 25 important human and plant pathogens, and AI-3/epinephrine/NE QseC receptor signaling system plays a central role in virulence of many important pathogens and *qseC* mutants of EHEC; *Salmonella typhimurium* and *Francisella tularensis* have been shown to be attenuated in animal models of infection.[46,47]

Quorum sensing in gram positive bacteria

A number of gram positive organisms are known to employ *Quorum sensing* systems. The nature of the signal molecules used in these systems varies from those of gram negative organisms, and thus far no gram positive bacteria have been shown to produce AHLs. Gram positive *Quorum sensing* systems typically make use of small post-translationally processed peptide signal molecules. These peptide signals interact with the sensor element of a histidine kinase two-component signal transduction system. *Quorum sensing* is used to regulate the development of bacterial competence in *Bacillus subtilis* and *Streptococcus pneumoniae*, conjugation in *Enterococcus faecalis* and virulence in *Staphylococcus aureus*.[48,49] The virulence in *S. aureus* is dependent on temporal expression of a diverse array of virulence factors, including both cell-associated products, such as protein A, collagen, and fibronectin-binding protein and secreted products including lipases, proteases, alpha-toxin, toxin-I, beta-hemolysin, and enterotoxin.[50] During the early stages of *S. aureus* infection, surface proteins involved in attachment (collagen and fibronectin binding protein) predominate. So is the case with protein involved in defense (protein A). However, once a high cell density is achieved at the site of infection, expression of *S. aureus* surface proteins is decreased and secreted proteins are preferentially expressed. Also, the *S. aureus* is known to exist in two phenotypes, an adhesive colonizer phenotype, which is tolerated by the host and the severe invasive infective phenotype that can damage the host tissues and is responsible for the disease manifestations.[41,51] The genetic basis for this temporal gene expression depends on two pleiotropic regulatory loci called *agr* (accessory gene regulator)[52-54] and *sar* (staphylococcal accessory gene regulator).[55] The *agr* locus consists of two divergently transcribed operons, RNAII and RNAIII.[56,57] The RNAII operon contains the *agrBDCA* genes that encode the signal transducer (AgrC) and response regulator (AgrA), and AgrB and AgrD, which are involved in generating the *Quorum sensing* signal molecule, the autoinducing cyclic thiolactone peptide (AIP).[52,58] The RNAIII operon encodes a hemolysin and is itself a regulatory RNA that plays a key role in *agr* response. During *S. aureus Quorum sensing*, the AgrC signal transducer is autophosphorylated in response to the octapeptide signal molecule, which in turn leads to phosphorylation of the AgrA response regulator.[59] Phosphorylated AgrA stimulates transcription of RNAIII and this, in turn, upregulates expression of numerous *S. aureus* exoproteins as well as the *agrBDCA* locus.[56,57] The latter leads to a rapid increase in the synthesis and export of the octapeptide signal molecule. At the second regulatory locus, the *sar* gene product (SarA) functions as a regulatory DNA-binding protein to induce expression of both the RNAII and RNAIII operons of *agr* locus.[60]

The survival of *S. aureus* is also dependent on genes regulated by a second QS system, RAP/TRAP. Here, the proposed AI, RNAIII activating protein (RAP) is believed to be secreted by an as yet unknown mechanism. This reenters the cell and activates the target of RAP (TRAP). The activated TRAP

upregulates *agr* expression and promotes cellular adherence, which is essential for biofilm formation.[61]

This system has the potential to be exploited as another alternative to antibiotics. This can be accomplished by using a heptapeptide, which inhibits the activity of TRAP. The heptapeptide RNAIII inhibiting peptide (RIP) has been shown to inhibit phosphorylation of TRAP and *agr* expression. It was shown that RIP is effective against some strains of MRSA and a non-peptide analog of RIP, hamamelitannin, was identified which has been shown to prevent device-associated MRSA infections in a concentration dependent manner.[61,62]

Quorum sensing and biofilm formation

Biofilms are now considered ubiquitous in the natural world.[14] In nature, bacteria are frequently found encased in polysaccharide matrix attached to a solid surface. This mode of growth, referred to as a biofilm, offers protection from environmental agents that would otherwise threaten their planktonic counterparts. Bacterial biofilms have been observed to be extremely heterogeneous, both structurally and with regard to the physiology of the bacterial cells within them. The prevailing conceptual model depicts bacterial biofilms as being made up of microcolonies, which serve as the basic unit of the greater biofilm structure. Microcolonies are hydrated structures consisting of bacterial cells enmeshed in a matrix of exopolymeric substances (EPSs). Bacteria may proliferate on the attachment surface, leading to microcolony expansion. Eventually, community growth becomes limited by substrate availability due to increased diffusion distances, and the biofilm reaches a steady state. Such mature biofilms often consist of "towers"and "mushrooms" of cells in an EPS matrix. Interstitial voids and channels separate the biofilm structures and facilitate a convective flow in order to transport nutrients to interior parts of the biofilm and remove waste products. Biofilms have become evident in many, if not most, environmental, industrial, and medical bacteria related problems. A recent public announcement from the NIH stated that more than 60% of all microbial infections involve biofilms.[22]

P. aeruginosa is an example of an organism frequently found growing in biofilms. Microscopic analysis of *P. aeruginosa* biofilm communities reveals that they are not just sugar-encased masses of cells, but rather distinct mushroom and stalk-like structures that contain intervening water channels to allow nutrients to flow in and waste products to flow out. In clinical setting, biofilms formed on medical devices and in bacterial infections can wreak havoc, largely because bacteria growing as biofilm are refractile to host defenses including phagocytes, antibodies, and complement.[63] Moreover, these organisms are highly resistant to antibodies making eradication by using conventional chemotherapy virtually ineffective. Thus, novel ways of preventing biofilm formation and eradicating those already established must be found. Recently, a link between biofilm formation and *Quorum sensing* was discovered in *P. aeruginosa*. Analysis of biofilms formed by a *P. aeruginosa* mutant deficient in the production of the las signal molecule, 3-oxo-C12-HSL, revealed a biofilm that was much thinner and lacked the three-dimensional architecture observed in that of the parent. Even more noteworthy was the fact that, while the parental biofilm was resistant to the detergent sodium dodecyl sulfate (SDS), the mutant biofilm rapidly dispersed from the underlying surface after SDS exposure. When grown in the presence of exogenous 3-oxo-C12-HSL, the mutant biofilm resembled that of the parent and was resistant to SDS. Thus, it appears that *Quorum sensing* plays a critical role in the formation of mature, differentiated biofilm structures. It is not known at this time whether other bacteria use *Quorum sensing* during biofilm formation. However, at least in case of *P. aeruginosa*, strategies designed to block *Quorum sensing* may be an effective means of preventing biofilm formation.[1]

Biofilm formation in *Candida albicans*

The process of biofilm formation by *Candida albicans* involves three main steps: the initial colonization of the substratum by the yeast cells, growth and hypha formation and the production of an extracellular matrix, primarily composed of β-1,3-glucan. The mature biofilm consists of yeasts, hyphae, and pseudohyphae; however, eventually, the yeast cells leave the biofilm. In *C. albicans* also the QS can modulate all stages of biofilm formation, i.e. attachment, maturation, and dispersal.[64] The best characterized QS molecule produced by *C. albicans* is "Farnesol,"[65] which regulates the inter-conversion between its yeast and filamentous form. In *in vitro* experiments, farnesol has been shown to reduce the size of biofilms. The other QS molecule that may also alter biofilm development in *C. albicans* is "tyrosol."[66] In experiments, farnesol has been shown to repress hyphal growth by inhibiting the Ras1-adenylate cyclase-protein kinase A signaling pathway. However, the role of farnesol in multicellular population can be better understood by discovering mutants with altered farnesol response and farnesol production.[67]

Future applications of bacterial *Quorum sensing*

Quorum sensing is a novel target for antimicrobial therapy.

The continuing emergence of multiple drug-resistant strains of bacteria has necessitated finding novel strategies for treating bacterial infections. The discovery that a wide spectrum of organisms use *Quorum sensing* to control virulence factor production makes it an attractive target for discovery of new anti-virulence therapeutics. The pathogenic organisms can be rendered avirulent if the *Quorum sensing* mechanisms that control virulence factors can be targeted. The scientific research has focused on the synthesis and characterization of AI analogs, mostly focusing on AHL-based QS systems. By virtue of the intrinsic cytotoxic activity of AHLs, they can aptly now be regarded as "small molecule toxins".[68]

The *Quorum sensing* pathways can be disrupted at various levels; for example at AIs and R protein levels, which have a unique specificity for one another. Non-cognate AIs typically only weakly activate or may inhibit R protein activation altogether. Therefore, analogs that bind to but do not activate R proteins could act as antagonists to prevent AI binding, which in turn would shut down the *Quorum sensing* cascade. The ability of AI analogs to inhibit activation of R proteins has already been demonstrated in a number of bacteria, including *V. fischeri*, *A. tumefaciens*, *Chromobacterium violaceum*, and *Aeromonas salmonicida*.[1,69-72]

Recently, enzyme from an isolate of *Bacillus* that is capable of degrading AHLs was discovered. This enzyme is encoded by the *aiiA* gene (AI inactivation) and contains two domains that are homologous to the active sites of the following metalloenzymes: glyoxalase II, metallo-B lactamase and arylsulfatase. Expression of *aiiA* in *E. carotovora* decreased generation of proteolytic enzymes and significantly reduced AI production.[73]

For finding another way of interfering with *Quorum sensing*, the biosynthetic pathways of some AHL molecules have been elucidated. Interrupting the AHL biosynthetic pathway and shutting down AHL synthesis, perhaps through the use of analogs of AHL precursors, would be a highly effective means of blocking the *Quorum sensing* cascade.[74-77]

Recently, an immunotherapeutic approach for QS quenching was pioneered by generation of the anti-AHL monoclonal Ab (mAb), RS2-1G9, elicited against a synthetic 3-oxo-C12-HSL analog. The RS2-1G9 was found to efficiently suppress QS signaling in *P. aeruginosa* and conferred protection upon mammalian cells via neutralization of 3-oxo-C12-HSL *in vitro*. The monoclonal antibodies against staphylococcal AIPs have been developed by using a hapten in which the hydrolytically labile thiolactone was replaced with a more stable lactone moiety. The mAbs thus developed were evaluated both *in vitro* and *in vivo* and were shown to possess potent *Quorum* quenching abilities, including the protection of mice from an otherwise lethal *S. aureus* infection.[68,78,79]

Recently, a novel Bacillus spp. strain, designated *Quorum sensing* Inhibitor-1 (QSI-1) by the researchers was isolated and identified from the intestine of the fish, *Carassius auratus gibelio*. The isolated QSI-1 is capable of utilizing AHL molecules as the sole source of energy and possesses at least one type of AHL degrading enzymes capable of degrading the AHLs of fish pathogen, *Aeromonas hydrophila*, thus significantly decreasing the production of its extracellular proteases, in turn increasing the survival rate of infected fish. Thus, perhaps this QSI-1 strain be used as a probiotic in aquaculture.[80]

CONCLUSION

The ability to coordinate behavior in a cell-density-dependent fashion has several obvious advantages. In the case of pathogenic microorganisms, the regulation of virulence determinants throughout the infection process is believed to play an important role in pathogenicity. Evading host defenses is a major goal of pathogens, and *Quorum sensing* equips these bacteria to appropriately time the expression of immune evading machinery. Using *Quorum sensing*, bacteria can amass a high cell density before virulence determinants are expressed, and in doing so, the bacteria are able to make a concerted attack and produce ample virulence factors to overwhelm the host defenses. As the list of bacteria that employ *Quorum sensing* systems continues to grow, so does the number of possibilities for exploiting these regulatory mechanisms. Because many important animal and plant pathogens use *Quorum sensing* to regulate virulence, strategies designed to interfere with these signaling systems will likely have broad applicability for biological control of disease causing organisms. In the future, it will be intriguing to see whether additional human pathogens utilize *Quorum sensing* as part of their pathogenic lifestyle and, if so, whether production of the signal molecules, AHL or otherwise, can be exploited to control infections.

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