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Impacts of Quorum Sensing on Microbial Metabolism and Human Health

Yang-Chun Yong and Jian-Jiang Zhong

Abstract Bacteria were considered to be lonely 'mutes' for hundreds of years. However, recently it was found that bacteria usually coordinate their behaviors at the population level by producing (speaking), sensing (listening), and responding to small signal molecules. This so-called quorum sensing (QS) regulation enables bacteria to live in a 'society' with cell–cell communication and controls many important bacterial behaviors. In this chapter, QS systems and their signal molecules for Gram-negative and Gram-positive bacteria are introduced. Most interestingly, QS regulates the important bacterial behaviors such as metabolism and pathogenesis. QS-regulated microbial metabolism includes antibiotic synthesis, pollutant biodegradation, and bioenergy production, which are very relevant to human health. QS is also well-known for its involvement in bacterial pathogenesis, such as iin nfections by *Pseudomonas aeruginosa* and *Staphylococcus aureus*. Novel disease diagnosis strategies and antimicrobial agents have also been developed based on QS regulation on bacterial infections. In addition, to meet the requirements for the detection/quantification of QS signaling molecules for research and application, different biosensors have been constructed, which will also be reviewed here. QS regulation is essential to bacterial survival and important to human health. A better understanding of QS could lead better control/manipulation of bacteria, thus making them more helpful to people.

Keywords Quorum Sensing · Metabolism · Pathogenesis · Biosensor · Bioenergy

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1 Introduction

Social and cooperative traits are believed to be the most important characteristics for human survival and development. Bacteria can easily adapt to various new environmental conditions, and they have thrived on earth ubiquitously with a much longer history and wider occupation than humans. However, bacteria were regarded as deaf mutes for at least 300 years since they were first observed by van Leeuwenhoek [1]. Scientists questioned how, as a simple single deaf germ, bacteria achieved such great success? Recently, researchers discovered that bacteria do not live as a non-communicating single cell, but they are organized like a society that shows unique social and cooperative traits [2]. They can use a so-called "quorum sensing" (QS) mechanism to 'talk to' and 'listen to' each other, and hence they can coordinate population behaviors like "multicellular" species [3]. In general, bacteria synthesize low molecular weight (MW) chemical molecules (serving as signaling molecules called 'autoinducers' [AIs]) and release them into the environment; the concentration of AIs increases with increasing cell densities; once the AIs (and cell density) reach a threshold concentration (the quorum), they bind to their cognate receptor and evoke a response (gene expression) in all individuals of the population [1, 3, 4]. Such a population-level response is cell-density dependent and was first termed as 'QS' by Fuqua in 1994 [5]. By the QS mechanism, bacteria can sense the population density and carry out some population behaviors that would be unproductive if done by individuals alone [2]. At present, scientists recognize that communication and cooperation are not exclusive characteristics for animals but rather are the norm in the bacterial world [1]. Since it was first described, QS has been identified in various bacterial species and involves in many important biological processes, including biofilm formation,

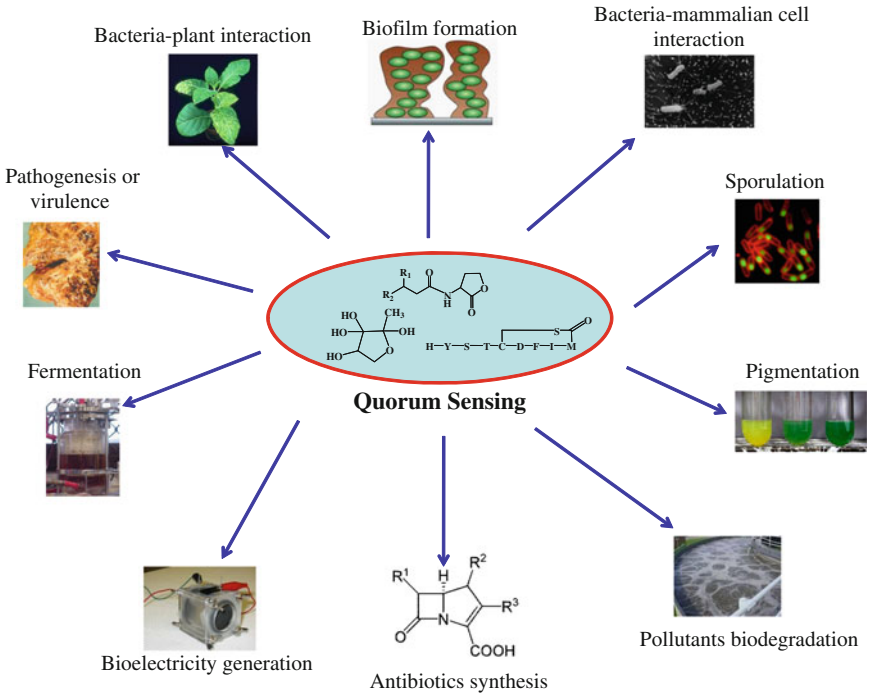

Fig. 1 Involvement of QS in various important biological processes

sporulation, virulence and pathogenesis, pollutant biodegradation, secondary metabolism, and bacteria-host interaction (Fig. 1).

To date, there are several QS systems, including QS in Gram-negative bacteria mediated by acylated homoserine lactone (AHL), hydroxy-palmitic acid methyl ester (PAME), diffusive signal factor (DSF), and *Pseudomonas* quinolone signal (PQS); QS in Gram-positive bacteria mediated by oligopeptide (known as auto-inducing peptides (AIP)), and A-factor; and interspecies QS mediated by autoinducer-2 (AI-2). Here, we selected three representative QS systems for a brief introduction: AHL-mediated QS in Gram-negative bacteria, oligopeptide (known as [AIP])-mediated QS in Gram-positive bacteria, and AI-2 mediated QS for interspecies communication (Fig. 2). AHL-mediated QS is widespread in Gram-negative bacteria. It is comprised of an AHL synthase (LuxI-type family protein) and an AHL receptor (LuxR-type family transcription regulator). AHL-mediated QS was first described in the bioluminescent bacterium *Vibrio fischeri*, in which the LuxI and LuxR proteins controlled the expression of *luxCDABE* operon (a luciferase) ([5], Fig. 2a). LuxI is responsible for the synthesis of the AHL signaling molecule 3OC6HSL (Fig. 3). LuxR is the receptor of 3OC6HSL and the transcription activator of Lux-controlled genes. Once the 3OC6HSL is produced, it can freely diffuse into the environment and accumulate with increasing cell densities. When the concentration of 3OC6HSL reaches its threshold, it will interact

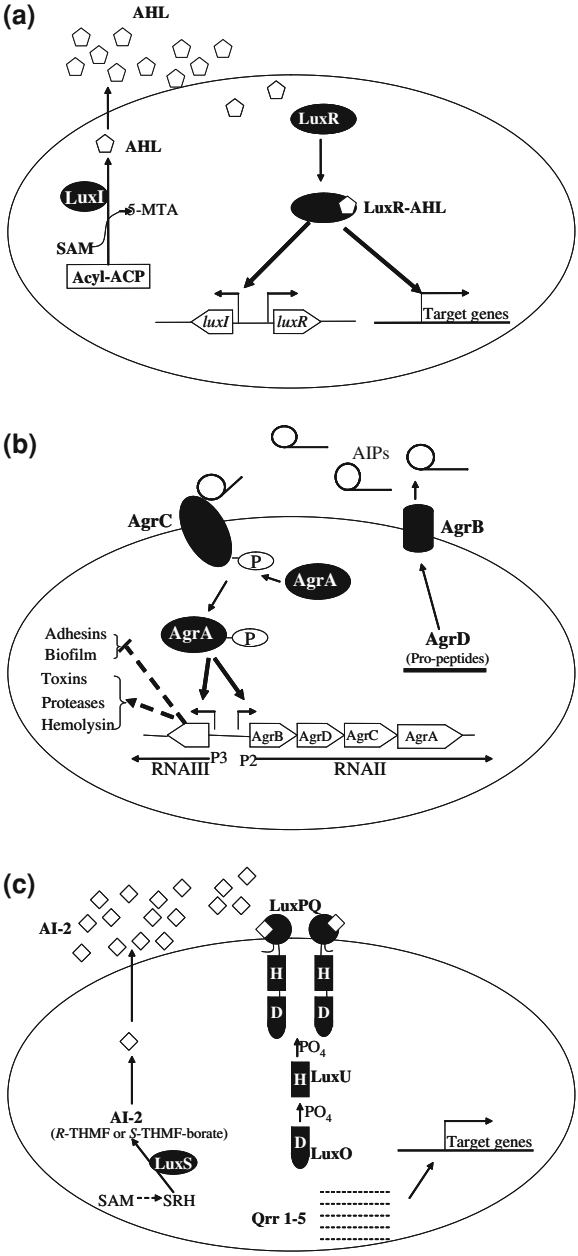

Fig. 2 Three typical QS systems in bacteria: **a** LuxI/R-type AHL-dependent QS system in Gram-negative bacteria (LuxI/R QS system in *V. fisheri*); **b** AIP-dependent QS system in Gram-positive bacteria (*agr* QS system in *S. aureus*); **c** AI-2-dependent interspecies QS system (LuxS QS system in *V. harveyi*) [1, 3, 4, 7, 9, 10, 16]

with the LuxR protein to form the LuxR-HSL complex and then activate the transcription of *luxCDABE* [3]. As this transcription activation and hence the luciferase expression could be activated simultaneously in all individual cells at the population level, *V. fischeri* could provide enough light for its host Hawaiian

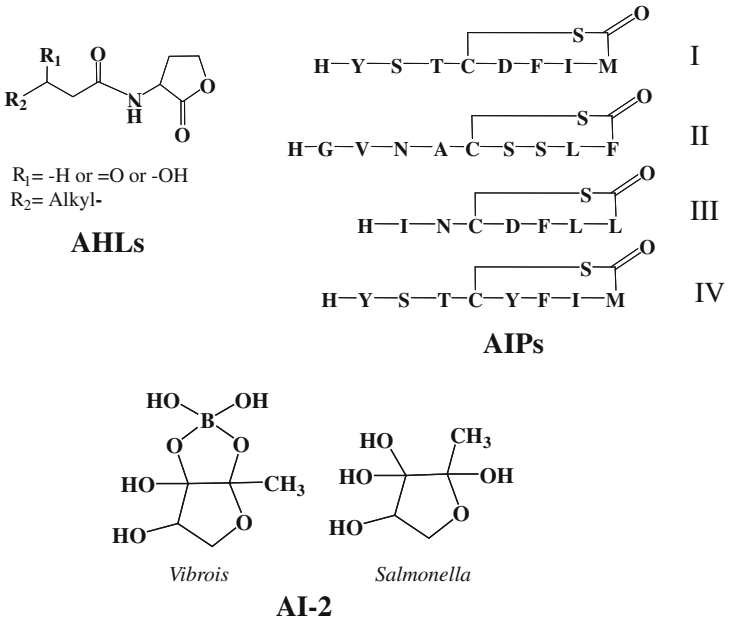

Fig. 3 Typical QS signaling molecules

squid *Euprymma scolopes* for counterillumination to mask its shadow and avoid predation [4, 6]. It is worth noting that the LuxI/R QS system usually has positive feedback for its AHL synthase LuxI [3]. Therefore if the quorum is reached, the AHL synthesis will be accelerated and the expression of QS controlled targets will also be rapidly enhanced.

In Gram-positive bacteria, the QS system is usually comprised of modified oligopeptides (AIP, Fig. 3) as signaling molecules and a two-component type of histidine kinase as signal sensing and transduction modules [7]. Besides using different types of signaling molecules, there are two main differences between AIP-mediated QS and AHL-mediated QS. First, AIP is a modified oligopeptide and cannot freely diffuse across the cell membrane; it should be exported with the aid of exporters. Second, the signaling process for AIP-mediated QS is relayed by two-component histidine kinase. The most typical example of AIP-mediated QS is the *agr* system in *Staphylococcus aureus* ([7, 8]). The *agr* QS system is encoded by *agrBDCA* operon. Gene *agrD* encodes the precursor of the signaling oligopeptide (AIP), and AgrB protein is responsible for the oligopeptide exporting and modification (add thiolactone ring). AgrC and AgrA consist of a two-component histidine kinase-response regulator. AgrC is a transmembrane protein that can bind with AIP. Once AgrC binds with AIP, the intracellular AgrA will be phosphorylated by a two-step phosphorelay. This phosphorylation can activate AgrA to induce the transcription of target genes. Similar to LuxI/R type QS, the phospho-AgrA can also

induce the expression of the *agrBDCA* operon to form a positive feed-back loop. (Fig. 2b).

Both of AHL- and AIP-mediated QS are involved in intraspecies cell–cell communication; the AIs and their cognate receptors are species specific and are only effective in intraspecies communication. In contrast, AI-2 can be synthesized in a remarkably wide variety of bacterial species and can be recognized by other species besides the producer [7, 9–11]. Hence, AI-2 mediated QS is an interspecies cell–cell communication system, and AI-2 is considered to be a universal language in bacterial world. AI-2 is a furanosyl borate diester (3A-methyl-5,6-dihydrofuro [2,3-D] [1,3,2] dioxa-borole-2,2,6,6A tetraol in *Vibrio harveyi*, Fig. 3) synthesized by LuxS from *S*-adenosylmethionine (SAM) [12, 13]. The signaling process relied on several phosphorylation-relay steps [7, 11, 14]. In *V. harveyi*, the signal-relay system is comprised of LuxPQ, LuxU, and LuxO (Fig. 2c). At low cell densities, in the absence of AI-2, LuxQ serves as a kinase and is autophosphorylated. The phosphate was transduced to LuxU and then transferred to LuxO. Phospho–LuxO (LuxO–P) then activates the transcription of Qrr1-5 (genes encoding five small regulatory RNAs (sRNAs)). Qrr sRNAs are master regulators controlling the transcription of a variety of genes [13, 14]. At high cell densities, once AI-2 reaches its critical concentration, it can be detected by the periplasmic proteins LuxP and LuxQ complex. Binding with AI-2 will switch the LuxQ from kinase to phosphatase and result in the dephosphorylation of LuxO–P. Therefore, the Qrr sRNAs are not transcribed and the response is the switch-off of the Qrr sRNAs regulation (Fig. 2c) [7].

It was found that some bacteria simultaneously have several QS systems. These systems may exist in parallel, cross-linked, or hierarchically organized in complex mode [7, 15]. Based on these QS systems, bacteria are not limited to normal cell–cell communication but are capable of signal interception or coercion [16], which allows them to evolve with more sophisticated social traits such as social cheating, exploitation, and kin-selection [17, 18]. Although some other new QS systems have been identified in bacteria and QS was found to be not just limited to the bacterial world, we take the above previously mentioned three typical QS systems as examples in this chapter to discuss the progress of in QS biosensors and the impact of QS on microbial metabolism and human health.

2 Impact of QS on Microbial Metabolism

From the beginning of history, humans have been continuously fighting against and benefiting from the little “bug” of bacteria. Bacterial metabolism is vital to human health. For example, the metabolic pathways of some virulence factor synthesis are crucial for pathogen infection in the human body, and they directly affect human health. In contrast, many other microbial metabolic pathways are related to biofuel production, antibiotic synthesis, biodegradation and bioelectricity production, which are beneficial and vital to the sustainable development of

human society and also closely related to human health. In this section, the QS regulation on microbial metabolism such as fermentation, biodegradation, and bioelectricity generation are discussed.

2.1 Butanediol Fermentation and Acetate Switch

With fermentable carbon sources, bacteria are able to produce a series of acids by using mixed-acid fermentation pathways or various neutral end products (e.g., butanediol or acetoin). The switch to neutral end products allows cells to prevent lethal acidification by limiting acidic end products production [19]. Recently, QS was identified to directly or indirectly regulate the 2,3-butanediol pathway in *Vibrio cholerae*, *Serratia plymuthica*, *Serratia marcescens*, and *Aeromonas hydrophila* [20–22]. In certain strains of *V. cholerae*, the QS systems repressed the *aphA* expression [23], while AphA showed strong repression on the acetoin and 2,3-butanediol synthesis pathway [20]. These studies indicated that QS systems positively regulated 2, 3-butanediol fermentation in *V. cholerae*. Recently, it was found that 2,3-butanediol fermentation in *S. plymuthica* RVH1 and *S. marcescens* MG1 is regulated by SplIR or SwrIR QS system [21]. This study was inspired by an incidental phenomenon in which *splI* mutant (*S. plymuthica* RVH1, deficient in AHL production) grows less well in LB medium compared to the wild-type strain; the *splI* mutant led to continued acidification of the medium and resulted in early growth arrest. Further analysis indicated that inactivation of QS systems in these two *Serratia* strains repressed the acetoin and 2,3-butanediol production and induced the acidic end products. Although the precise mechanism is still unclear, the evidence clearly indicated that the QS system controlled the switch to 2,3-butanediol fermentation in *S. plymuthica* RVH1 and *S. marcescens* MG1. Houdt et al. [21] also showed that the AhyIR QS system regulated the switch from mixed-acids fermentation to 2,3-butanediol fermentation in *A. hydrophila* AH-1 N. Hence, it effectively prevented the accumulation of acids that might lead to lethal acidification and allow further growth using the residual nutrient sources [21]. Butanediol fermentation is the first central metabolism identified to be controlled by QS system.

When grown on acetogenic carbon sources (e.g. D-glucose or L-serine), bacteria can transit from acetate dissimilation (production and secretion) to acetate assimilation (import and utilization) by using an acetate switch at certain conditions [24]. This central regulatory mechanism is crucial for bacteria survival by permitting bacteria growth rapidly when abundant nutrients are present and allowing cells to reclaim the secreted acetate in the absence of these nutrients [24]. However, this switch cannot occur unless the acetate assimilation machinery was activated. Induction of *acs* transcription and Acs activation are required to "flip" the acetate switch [25]. Studer et al. [26] described that wild-type *V. fischeri* showed an acetate switch during growth in a glycerol/tryptone-based medium. Interestingly, the C8HSL synthase (AinS) mutant strain cannot switch from acetate dissimilation to

assimilation, and the acetate accumulated to a lethal pH of about 4.6. Further analysis indicated that the *ainS* mutant showed about 20-fold lower *acs* transcription, which resulted in failure in acetate switch [26]. Meanwhile, the *acs* transcription and acetate switch could be restored by AHL complementation in *ainS* mutant. The evidence indicated that the AinS QS system regulates the *V. fischeri* acetate switch through the *acs* transcription control [26]. All the evidence indicates that QS not only plays important roles in non-essential bioprocesses (biofilm formation, motility), but also is involved in the essential metabolism process at certain conditions. It also implies the possibility to manipulate the of microbial metabolism by tuning QS system regulation to produce value-added products. However, the basic regulation mechanisms and ecological implication still need to be explored.

2.2 QS Regulation on Antibiotics Synthesis

2.2.1 Carbapenem in Gram-Negative Bacteria

The carbapenems are potent members of the most classic and important β-lactam antibiotics, which are widely used in chemotherapy, particularly for nosocomial, multidrug resistant infections [27]. Carbapenems are active against many important Gram-negative and Gram-positive bacteria, such as *S. aureus*, *Haemophilus* spp., and *Pseudomonas aeruginosa* [28]. Different kinds of carbapenems were identified in the metabolites of *Streptomyces*, *Erwinina*, and *Serratia* [28]. *Serratia* sp. ATCC 39006 and *E. carotovora* are able to produce a simple carbapenem antibiotic, 1-carbapen-2-em-3-carboxylic acid (Fig. 4a) [27, 29, 30]. A highly conserved gene cluster containing nine genes (*carRABCDEFGH*) was proven to be responsible for the production of this antibiotic in *Serratia* sp. ATCC 39006 and *Erwinia carotovora* [29, 30]. The *carA–E* genes are predicted as the structure genes for carbapenem synthesis, while *carF* and *carG* encode proteins are involved in the β-lactam resistance. The function of *carH* is unknown. CarR is a transcriptional activator responsible for the regulation of the remaining *car* genes [29, 30]. Interestingly, the expression of *car* operon and antibiotic production showed a cell density dependent manner that is characteristic of QS regulation [31]. Further analysis indicated that *E. carotovora* produced a QS signal molecule 3OC6HSL by an unlinked CarI. Purified protein CarR binds to its ligand 3OC6HSL with a stoichiometry of two molecules of the ligand per dimer of protein. In the presence of 3OC6HSL, the purified CarR could form a high MW complex with 3OC6HSL and bind to the promoter upstream of the first of the biosynthetic genes, *carA* [32, 33]. Therefore, when 3OC6HSL reaches its critical concentration at high cell density, CarR binds with the 3OC6HSL and multimerizes; the CarR-3OC6HSL then binds to the *lux* box of the *carA* promoter to activate the transcription. Thus, the carbapenem synthesis in *E. carotovora* is controlled by CarIR QS system at the transcriptional level [31–34]. In contrast to the LuxIR QS system, the AHL receptor

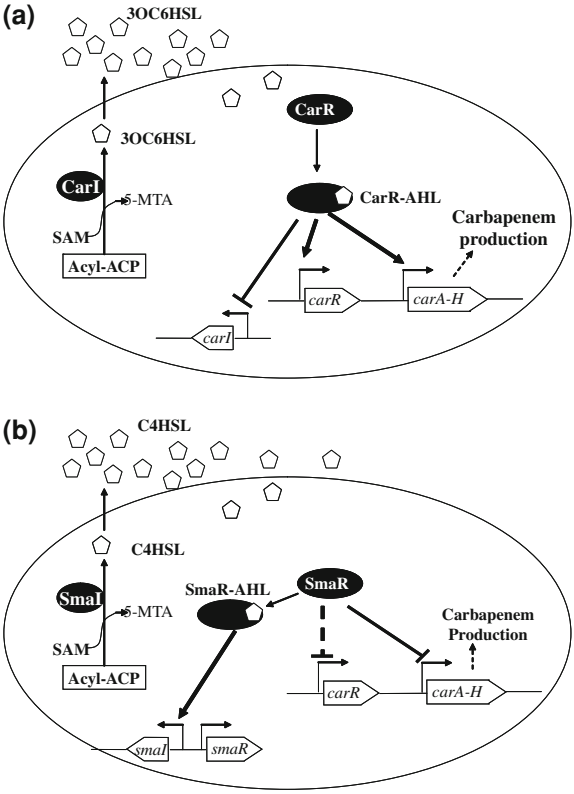

Fig. 4 Models for QS regulation in carbapenem synthesis in **a** *Erwinia carotovora* and **b** *Serratia* sp. ATCC 39006 [28, 199]

CarR shows positive autoregulation in the presence of 3OC6HSL, whereas AHL synthesis (CarI) is negative feedback repressed by the increasing concentration of 3OC6HSL [33] (Fig. 4a). Due to the sharing of a high identity of *car* operon to *E. carotovora* [30], the carbapenem production in *Serratia* sp. ATCC 39006 is expected to be controlled by the CarR/3OC6HSL QS system. Unexpectedly, the *lux* biosensor, which responded to 3OC6HSL, failed to detect AHL in *Serratia* sp. ATCC 39006. However, C4HSL and C6HSL were detected by using the *Chromobacterium violaceum* CV026 bioassay and TLC analysis [30]. More interestingly, although carbapenem production in *Serratia* was unsurprisingly proven to be C4HSL/C6HSL-dependent, the *Serratia* CarR protein showed AHL-independent regulatory activity and is functionally interchangeable with the *Erwinia* homologue [35]. The pheromone-independent nature of CarR inspired the researchers to identify a new SmaIR QS system in *Serratia* sp. ATCC 39006 [30]. SmaI, the homologue of LuxI, directed the synthesis of C4HSL and C6HSL. SmaR, which is the homologue of LuxR and the receptor of C4HSL/C6HSL, was a transcriptional repressor, an uncommon feature of LuxR-type transcriptional regulators [36]. In the absence of C4HSL/C6HSL, SmaR repressed the transcription of *car* gene cluster. In the presence of C4HSL/C6HSL, SmaR lost the repression

ability. Transcriptional analysis showed *Serratia carR* transcription (the AHL-independent transcriptional activator of *car* gene cluster, closely paralleled transcription of *carA*) is under the control of SmaIR QS system [36]. However, the QS regulation on *carR* did not occur directly as the SmaR protein did not bind the *carR* promoter as shown in in vitro gel shift assay [37]. Surprisingly, the purified SmaR directly bound with the *carA* promoter and C4HSL/C6HSL could abolish this binding [37]. It suggested that the SmaIR QS system controlled the carbapenem synthesis in *Serratia* through both an unclear indirect mode with *carR* and a direct control on transcription of structure genes (Fig. 4b). In addition, recent evidence implied that the AI-2 (produced by LuxS) QS system was also involved in the carbapenem synthesis in *Serratia* [38]. This evidence showed the complexity and diversity of QS regulation on carbapenem biosynthesis, which needs further investigation.

2.2.2 Phenazine Antibiotics Produced by *Pseudomonas*

During the past century, approximately 100 natural origin heterocyclic nitrogen-containing phenazines have been identified, and most of these showed antibiotic activity toward various plant bacteria and fungi [39–41]. Thus phenazines and their producers are widely used as biocontrol agents for plant protection. Although a lot of bacteria species could synthesize phenazine compounds, *Pseudomonas* spp. (including *P. fluorescens*, *P. chlororaphis*, and *P. aeruginosa*) were found to be the main producers [40, 42]. *P. fluorescens* only produced one phenazine compound of phenazine-1-carboxylic acid (PCA), while the other two genera simultaneously produced two or more phenazine compounds, which usually included PCA, 1-hydroxyphenazine (1-OH-PHZ), pyocyanin (5-N-methyl-1-hydroxyphenazine, PYO), and phenazine-1-carboxamide (PCN) [39, 40]. In most bacterial species, 1-OH-PHZ, PYO and PCN are derived from the common precursor PCA, while the PCA was synthesized from chorismate. The gene cluster responsible for PCA synthesis has been identified in several strains. Typically, *phzABCDEFG*, which is highly conserved among *P. aeruginosa*, *P. chlororaphis*, and *P. fluorescens*, was characterized and proven to be the biosynthetic operon that is responsible for the PCA synthesis from the chorismate precursor (Fig. 5). Then, PCA can be modified to diverse phenazine derivatives by various specific enzymes. In *P. aeruginosa*, PCA was transformed to PCN, PYO, and 1-OH-PHZ by PhzH, PhzM/PhzS, and PhzS, respectively (Fig. 5). Interestingly, the PCA production in Pseudomonas usually showed a population density-dependent manner; i.e., only a large amount of PCA was produced in the late exponential growth and early stationary phase, which suggested the regulation of QS. The QS system PhzI–PhzR was identified in *P. aureofaciens* 30–84, *P. fluorescens* 2–79, and *P. chlororaphis* PCL1391. Similar to other AHL-mediated QS systems, the *phzI* encodes an AHL synthase (side chain length differs from C4 to C14, with or without hydroxyl group substitution on C-3 position) and *phzR* encodes its cognate transcription regulator [40, 43]. The *phzI* and *phzR* genes are located directly upstream of the *phzABCDEFG* gene cluster, and

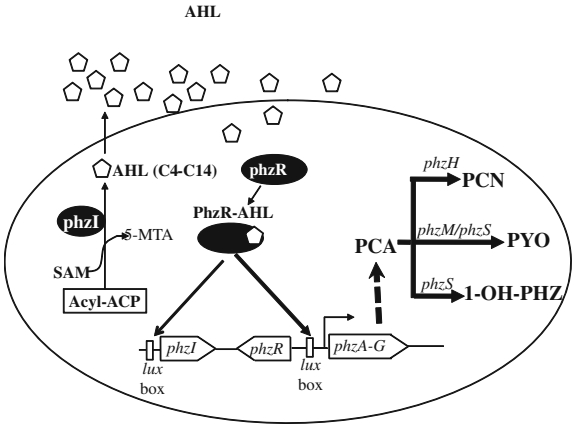

Fig. 5 Model for QS regulation in phenazine synthesis in *Pseudomonas*

positively regulated the transcription of core gene cluster for PCA synthesis through a well-defined *lux* box upstream of *phzA*. However, together with *P. aeruginosa* PAO1, another important biocontrol strain *Pseudomonas* sp. M18 possessed two complete sets of *phzABCDEFG* genes that are 99 % homolog, but without *phzI* and *phzR* [44, 45]. The PCA synthesis in *P. aeruginosa* PAO1 and *Pseudomonas* sp. strain M18 was regulated by another AHL-mediated QS system, i.e., RhlI–RhlR. In this regulatory context, the RhlI–RhlR QS system directly activated the transcription of *phzABCDEFG*, and thus promoted the PCA synthesis [44, 46, 47]. As the *las* and rhl QS systems are organized hierarchically and are interdependent in *P. aeruginosa* PAO1 and *Pseudomonas* sp. M18, the *las* QS system could regulate the PCA synthesis through modulation of the *rhl* QS system [44, 46, 48]. Besides, other environmental factors such as temperature or other global regulators such as GacA/S indirectly influenced the phenazine production through transcriptional or posttranscriptional regulation on the previously mentioned QS systems [47, 49, 50].

2.2.3 Lantibiotics in Gram-Positive Bacteria

Many Gram-positive bacteria produce small (<10 kDa) ribosomally synthesized peptide antibiotics (bacteriocins). These bacteriocins are usually categorized into two classes, i.e., class I (heat stable, post-translationally modified peptides) and class II (non-modified heat stable peptides) [51]. The great interest in these antimicrobial peptides biosynthesis is not just because they are potent antimicrobial agents, but also because they serve as QS pheromones, which in turn regulate the biosynthesis of themselves. Lantibiotics constitute a unique family of antibiotic peptides with a typical intracellular thioether bridges called (β-methyl) lanthionines and are the main members of class I bacteriocin [52]. Lantibiotics have been studied extensively because of their unusual structure properties, broad spectrum of antimicrobial activity, and increasing applications in the food industry [53]. Here we will take nisin and subtilin as examples to illustrate the QS regulation on lantibiotic synthesis (Fig. 6).

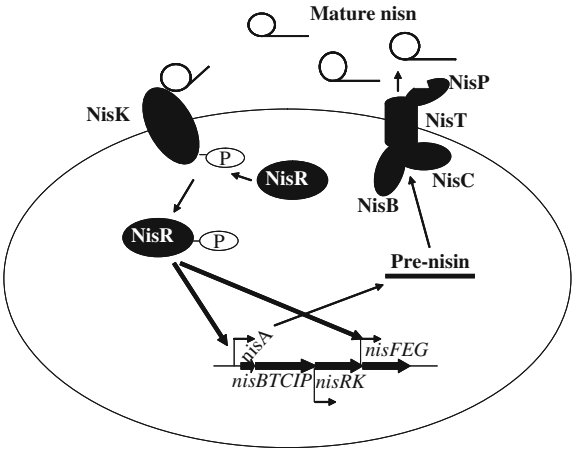

Fig. 6 Model for QS regulation on nisin biosynthesis [51, 52]

The genes for nisin and subtilin biosynthesis are organized in clusters, i.e., *nisABTCIPRKFEG* for nisin in *Lactococcus lactis* and *spaBTCSIFEGRK* in *Bacillus substilis* [54]. The gene clusters contain the structure gene encoding the lantibiotic precursors (*nisA* for nisin and *spaS* for subtilin), genes for post-translational modification (*nisB*, *nisC* and *spaB*, *spaC*, respectively), genes for secretion (*nisT* and *spaT*, respectively), genes for immunity (*nisIFEG* and *spaIFEG*, respectively), and a two-component regulation system (*nisR*, *nisK* and *spaR*, *spaK*, respectively) [54]. Gene *nisP* encodes an extracellular protease to remove the leader peptide of pre-nisin, and this cleavage is achieved by unspecific serine proteases for substilin [55]. Similar to other antimicrobial peptides, production of nisn and subtilin are inducible by the extracellularly accumulated peptides [52, 56, 57]. Production of these peptides usually only startes at the mid- to end-log growth phase, but rapidly reaches the maximum level at the beginning of stationary growth phase [52]. The rapid and massive production is achieved by positive feedback; i.e., the base-level antimicrobial peptide accumulates with increasing cell density When the concentration of peptide reaches its threshold, the peptide interactes with its cognate receptor, resulting in transmembrane signal transduction and leading to induction of the production of itself [52]. This typical QS regulation trait stimulated researchers to elucidate the QS regulation mechanism in nisin and substilin biosynthesis.

Introduction of 4-bp deletion in *nisA* gene of a nisin-producing *L. lactis* resulted in loss of nisin production and abolition of Δ*nisA* transcription [56]. However, the transcription of Δ*nisA* was restored by the exogenous addition of mature nisn or nisin analog into the culture medium. Also the induction of the transcription of Δ*nisA* was directly related to the amount of exogenously added nisin. This study indicates that nisin acts as a pheromone and induces the production of itself [56]. Further analysis showed that the two-component regulation system *nisRK* is indispensable for nisin autoregulation [58]. A QS regulatory circuit was also defined [52]. At the mid- to end-log growth phase, nisin biosynthesis started; the

extracellular nisin accumulated with increasing cell density. When the extracellular nisin reached its critical concentration, nisin interacted with the input domain of sensor kinase protein *nisK*, and the subsequent phosphortransfer from the sensor kinase transmitter domain to the receiver domain of the response regulator *nisR* led to its activation. The activated *nisR* would bind to specific *nis*-box within the promoter of *nisA*, which lead to transcription activation and induced nisin production. Studies also showed that substilin production is regulated by the *spaS–spaRK* QS system, which uses substilin (encoded by *spaS*) as the signaling molecule, and *spaRK* as a two-component signal transduction system [52, 57].

2.3 QS Regulation of Pollutants Biodegradation and Bioelectricity Generation

Although a variety of QS signaling molecules or gene circuits have been identified in bacteria that were isolated from pollutant treatment systems [59–63], only a few reports mentioned the involvement of QS in pollutant biodegradation [63–65]. Valle et al. isolated several AHL-producing bacteria from activated sludge and investigated the effect of AHL on its phenol biodegradation ability [59]. Without AHL addition, the phenol biodegradation by activated sludge was reduced at day 10 and completely lost at day 14. However, with the daily addition of 2 μM AHL mixture, phenol biodegradation ability of activated sludge remained stable at day 14 [59]. This is the first report on the effect of QS signals on pollutant treatment. They explained that the improvement on the performance of activated sludge was due to the change of the microbial community which was induced by AHL addition [59]. However, that work did not show any evidence about for the QS effect on bacterial biodegradation ability or the metabolism of pollutants. Although the mechanism is not mentioned, Kang and Park described an observation that QS signal elimination would lead to a reduction in hexadecane biodegradation by *Acinetobacter* sp. strain DR1 [64]. Recently, we isolated a strain of *P. aeruginosa* that could degrade broad-spectrum aromatics. This strain contained an active RhlIR QS system according to genetic analysis and biosensor detection [63]. AHL production was detected by *C. violaceum* CV026 during aromatics biodegradation. By using TLC and HPLC–MS/MS analysis, C4HSL and C6HSL were identified in its culture supernatant during phenol biodegradation. Interestingly, deletion of *rhlI* or *rhlIR* repressed the phenol biodegradation compared to the wild-type strain, and it could be reversed by complementation with wild-type *rhlI* or *rhlIR*, respectively. This repression by *rhlI* deletion could be relieved by exogenously adding AHL extracts or synthetic BHL. One report indicated that the RhlIR QS system is involved in the regulation of aromatics biodegradation by *P. aeruginosa* [63]. This report showed concrete evidence that the QS system is involved in the bacterial biodegradation ability. Further evidence on transcriptional and enzymatic levels indicated that the RhlIR QS system improved the catechol meta-cleavage

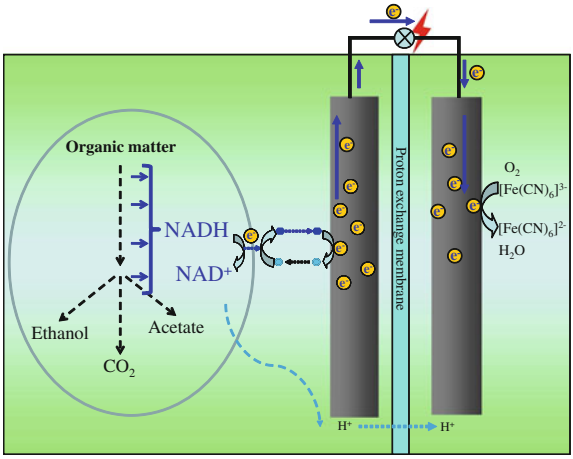

Fig. 7 Simple model for a two-chamber microbial fuel cell

pathway but with no effect on the first degradation step of phenol hydroxylation (unpublished data). It further suggested that the QS regulates metabolism of pollutants' biodegradation.

Besides environmental pollution, another serious problem that the human have encountered is the energy crisis. Microbial fuel cells (MFCs) are considered to be a promising "two birds, one stone" solution for simultaneous clean energy generation and pollutant treatment [66–69]. The bacteria in MFC could convert the chemical energy stored in the waste organic matters to bioelectricity by intracellular metabolism and extracellular electron transfer pathways (Fig. 7). Venkataraman et al. reported that *retS* knockout, which might indirectly activate the QS system, resulted in an increase in the current generation by *P. aeruginosa* (70). Recently, we proposed a novel mechanism in the reinforcement of the current output in the *P. aeruginosa* MFC, i.e., alteration of the electron transfer system to a more efficient electron shuttle by overexpressing the QS cassette [71] and Fig. 8). It was found that *P. aeruginosa* could use different electron shuttles in an MFC under different QS expression patterns, and the bioelectricity could be significantly improved by overexpression of the *rhlIR* QS system. In a dual-chamber MFC, the wild-type *P. aeruginosa* strain mainly used an electron shuttle (rather than phenazines) with a high mid-point potential of 0.20 V (vs. Ag/AgCl–KCl saturated electrode). This high mid-point potential is much closer to the cathodic potential ($\sim$0.3 V) and is believed to be unfavorable for electricity output (Fig. 8). As expected, the wild-type strain only delivered a low current output ($\sim$2.3 $\mu A/cm^2$). However, upon overexpression of the *rhl* QS system in this wild-type strain, the electron shuttle with high mid-point potential disappeared and more strikingly it was substituted by phenazines, which have a relatively low mid-point potential. According to HPLC analysis, the phenazine electron shuttles produced by the QS overexpression strain were pyocyanin and phenazine-1-carboxylate, which had a mid-point potential of -0.17 and -0.28 V, respectively. The substitution of high

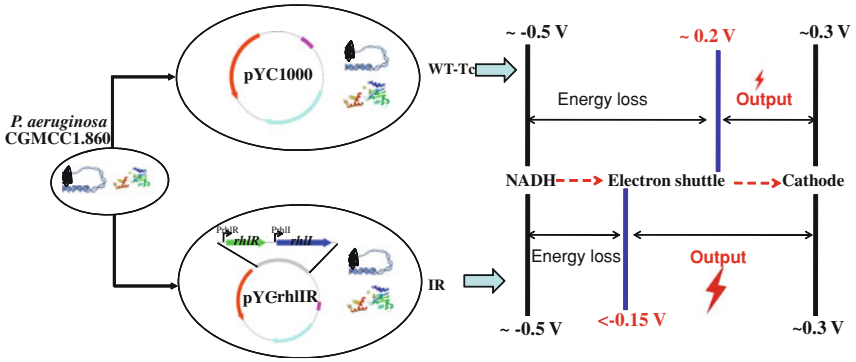

Fig. 8 Schematic illustration of bioelectricity improvement by QS overexpression in *P. aeruginosa*-inoculated MFC (modified from Ref. [71])

mid-potential electron shuttles with low ones directly led to an increase of about 1.6 times of the current output ($\sim$6.0 μA/cm^2) [71]. The results indicated that QS played important roles in bacterial electrochemical behavior.

Taken together, QS was proven to be involved in pollutant biodegradation and bioelectricity production. It may be expected to be an alternative to the solution for cleaner environment and cheaper bioenergy.

2.4 QS Regulatory Network for Synthetic Biology

Synthetic biology is an emerging and fast-moving field includes novel cellular behavior programming for a variety of applications [72–74]. Scientists believed that synthetic biology would be promising to provide solutions for many of the major global problems such as famine, disease, and energy crises [75]. The ultimate goal of synthetic biology is to build synthetic cells module by module—a bottom-up strategy, just like assembling a jumbo jet from a list of mechanical parts [75]. Although it is a long way from such a goal, a lot of genetic parts/circuits have been constructed, such as logic gates, bistable toggle switches, and oscillators. Because of the fancy molecule response characteristics of QS, it was widely used as the building block for genetic circuit construction. For example, an artificial genetic AND gate was constructed based on the hybrid promoter of $P_{luxI\text{–}lacO}$ and constitutively expressed LuxR and LacI [76]. The gene expression under this hybrid promoter can only be activated upon simultaneous addition of two kinds of activators (OHHL and IPTG). Thus, this minimal circuit showed logical AND response for induction with OHHL and IPTG. At the early stage, most constructed genetic parts mainly focused on single-cell behavior because stochastic gene expression caused cell–cell variation in a bacterial population. QS that realizes cooperative behavior at the population level could provide powerful tools for synthetic biology to coordinate microbial population dynamics and to engineer bacterial population behavior [74]. For example, a synthetic predator–prey ecosystem

consisting of two *Escherichia coli* populations was constructed by using a QS circuit to achieve bi-directional communication [77]. In this ecosystem, the predator cells can produce a QS signaling molecule that will activate the suicide gene to kill the prey cells. In contrast, the prey cells can also produce another QS signaling molecule to induce the expression of an antidote protein and then rescue the predator cells. The QS communication system ensured these bacterial behaviors could be coordinated at the population level. More interestingly, this synthetic ecosystem displayed defined eco-behaviors, such as competition or predation depending on the specific experimental conditions [76]. In all, QS is a vital building block in synthetic biology and should be crucial to future synthetic living systems, which are expected to substantially change our life-styles.

3 QS Biosensors

During the past decades, QS was proven to play important roles in many essential bioprocesses, which including microbial metabolism (primary and secondary metabolism), pathogenesis (host colonization, invasion, antibiotic resistance, etc.), plasmid conjugation, motility, and biofilm formation [1, 78]. The importance of QS led to a burst of research on QS mechanism and development of tools for QS signaling molecule detection/quantification. Because AIs are difficult to detect by conventional physical and chemical methods, biosensors that provide rapid and cost-effective alternatives have attracted much attention [2, 79]. During the past decades, there have been a series of QS signaling molecules identified in different bacterial systems (Fig. 3). Meanwhile, a large number of AI-specific receptors have been identified, which provided the possibility to construct various biosensors to meet research and application needs.

3.1 Biosensors for AHL, AIP, and AI-2

The AHL-mediated QS system is the most intensively studied cell–cell communication system, and various bacterial biosensors were constructed for the detection/quantification of AHL in samples ([80], Table 1). LuxR-type proteins usually specifically recognize AHLs produced by its cognate LuxI-type synthase. Therefore, various LuxR-type proteins have been used as detectors for AHL biosensors. For detection of short-chain AHL (C4–C8), LuxR (*V. fischeri*), TraR (*Agrobacterium tumefaciens*), CviR (*Chromobacterium violaceum*), AhyR (*A. hydrophyla*), RhlR (*P. aeruginsoa*), or SmaR (*Serratia* sp.) have been used as the sensing module [2, 81–83]. For long-chain AHL detection (C10–C16), LasR (*P. aeruginosa*) and SinR (*Sinorhizobim meliloti*) have been used [84, 85]. The output modules, which usually are bioluminescent, β-galactosidase, or fluorescence genes, are reassembled downstream of the LuxR-type regulator controlled promoters [80]. In the presence

Table 1 Typical biosensors for detection of QS signaling molecules

Strain/Plasmid	Host	QS system used	Most sensitive AI detected	Ref
pYC-rhlR	*P. aeruginosa* (*rhlI*$^-$*rhlR*$^-$)	RhlI/R	C4HSL	[2]
C. violaceum CV026	*C. violaceum* (*cvi*$^-$)	CviI/R	C6HSL	[82]
pSB401	*E. coli*	LuxI/R	3OC6HSL	[179]
pSF105 + pSF107	*P. fluorescens*	PhzI/R	3OHC6HSL	[180]
pAS-C8	Broad host range	CepI/R	C8HSL	[181]
PJZ384 + PJZ410 + PJZ372	*A. tumefaciens*	TraI/R	3OC8HSL	[105]
PSB1075	*E. coli*	LasI/R	3OC12HSL	[177]
BB170	*V. harveyi* (*luxN*::tn5Kan)	LuxS	AI-2	[94]
MM32	*V. harveyi* (*luxN*::cm, *luxS*::tn5Kan)	LuxS	AI-2	[95]
pRN7035	*Staphylococcus aureus* (*agrD*$^-$)	*agr*	*S. aureus* AIPs (Based on host)	[91]
pRN7062	*S. aureus* (agr-null)	*agr*	Group I AIPs (*S. aureus*)	[91]
pRN7105	*S. aureus* (agr-null)	*agr*	Group II AIPs (*S. aureus*)	[91]
pRN7131	*S. aureus* (agr-null)	*agr*	Group III AIPs (*S. aureus*)	[182]
pRN7107	*S. aureus* (agr-null)	*agr*	Group IV AIPs (*S. aureus*)	[91]
BD2876	*B. subtilis* (*comQ*$^-$)	*com* (*B. substilis* 168)	Group I ComX	[183]
BD3020	*B. subtilis* (*comQ*$^-$)	*com* (*B. subtilis* RO-E-2)	Group II ComX	[92]
BD2962	*B. subtilis* (*comQ*$^-$)	*com* (*B. mojavensis* RO-H-1)	Group III ComX	[183]
BD2877	*B. subtilis* (*comQ*$^-$)	*com* (*B. substilis* natto NAF4)	Group IV ComX	[183]

of its cognate AHL, the LuxR-AHL complex could activate the transcription of the output module and hence result in luminescence, enzyme activity, or fluorescence (reviewed in [80]). In addition, natural pigments are used as the output module. The pigments chosen usually are natural AHL controlled ones. Therefore, the simple design idea is to change the pigment production control from self-synthesized AHL to exogenously added AHL. By mutation on AHL synthase, the self-synthesized AHL is abolished and the QS-regulated pigment production can respond to the exogenous AHL [2, 82, 83]. *C. violaceum* CV026, which was constructed based on the CviI/R QS system, is a commonly used biosensor for C6HSL [82]. *C. violaceum* CV026 is a *cviI* mutant without AHL production, and its violacin (purple pigment) production responded to the exogenously added C6HSL. However, strain CV026

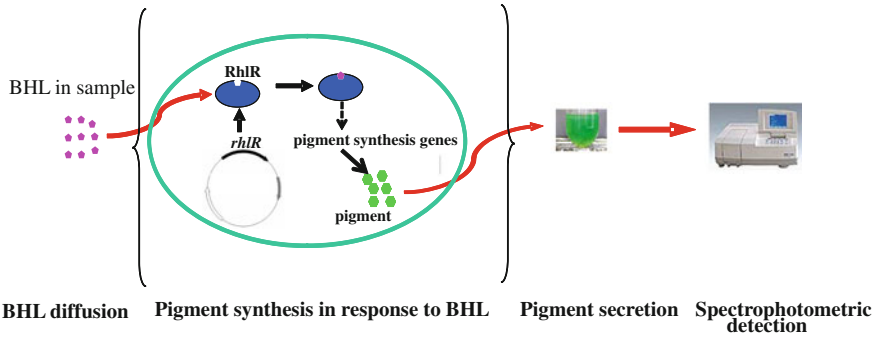

Fig. 9 The schematic illustration of the pigment-based whole-cell BHL biosensor (modified from Ref. [2])

can respond to other AHLs (C63OHSL, C8HSL, and C4HSL) although with less activity than the natural *C. violaceum* AHL (C6HSL). More interestingly, the violacin induced by short chain AHL (C4–C8) can be inhibited by long-chain AHLs (C10–C12) [82]. By quantification of extraction of the induced violacin by spectrophotometer, Blosser and Gray developed a quantification method for C6HSL by using another *C. violaceum* mutant CVblu [86]. Recently, we constructed a C4HSL biosensor by using an RhlI/R QS system [2] (Fig. 9). Similarly, a C4HSL-negative mutant was constructed and an overexpressed RhlR was used as the AHL sensing module. The engineered *P. aeruginosa* can produce a blue–green pigment in a specific response to the exogenous C4HSL. By optimization of the sensing conditions, a high sensitivity (10^4-fold more than CV026, and 300-fold more than LC–MS) for C4HSL quantification was achieved (Fig. 10). When applied in pollutant samples, the selected host strain *P. aeruginosa* CGMCC 1.860 is an aromatic resistant strain. Remarkably, this biosensor was successfully applied for C4HSL quantification in environment samples with toxic aromatic pollutants [2].

Regarding the genetic polymorphism of the AIP QS system, the AIP sensing/response system in Gram-positive bacteria is highly specific [87, 88]. For example, *S. aureus* has been divided into four groups based on the specific interactions between AIP and ArgC [89, 90]. Each group produced the structurally distinct AIP (different amino acid sequences and lengths), and only activated the *agr* response in itself and in the same group members, but showed intra-group inhibition on *agr* response [88, 90]. Therefore, a series of AIP biosensors have been constructed for application in different species and groups (Table 1). The most common AIP biosensors are constructed in the AIP gene mutant (e.g., *agrD* mutant of *S. aureus*, *comQ* mutant of *Bacillus* species) of itself or the same group members. The reporter genes (*gfp*, *yfp*, *lacZ*, *lux*, etc., optimized for expression in Gram-positive bacteria) are usually harbored on plasmids and transcriptional fused reporter genes with the AIP QS controlled promoters (e.g., P3 promoter in *agr* system in *S. aureus*, *srfA* promoter in com system in *Bacillus*) (Table 1). These biosensors use the host-expressed AIP receptors (e.g., AgrC in *S. aureus*) to detect the

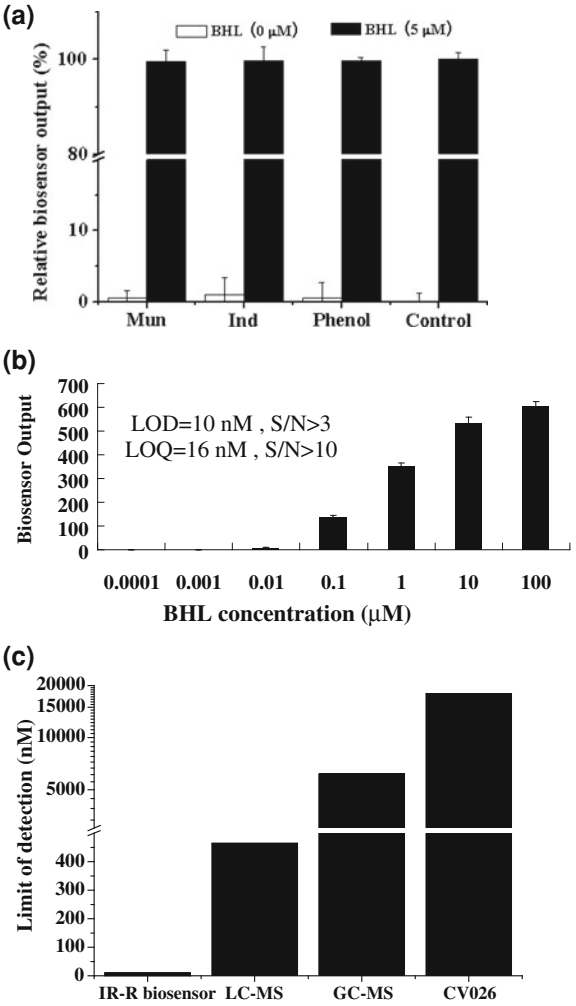

Fig. 10 Characterization of a genetically engineered BHL biosensor (*P. aeruginosa* IR-R). **a** The effect of different interferences on the biosensor output. Mun, municipal wastewater; Ind, industrial wastewater; PHE, phenanthrene; NAP, naphthalene. **b** Dose-dependent response to synthetic BHL. **c** Comparison of limit of detection (LOD) with previous reported BHL quantification methods. CV026, *C. violaceum* CV026 biosensor. (modified from Ref. [2])

exogenous AIP, process the signaling based on the AIP QS system in the host, and then activate the reporters by the inducible promoters (Fig. 3). This kind of biosensor can only specifically respond to the AIP of itself or the same group. However, by heterogeneously expressing another group AgrC in *agrC* and *agrD* double mutant, the strain of *S. aureus* could be converted to detect other groups of AIP [91]. Similarly, the ComX pheromones in *Bacillus* are defined as four different groups [92]. Biosensors for different groups of ComX were constructed in a *Bacillus substilis* mutant (*comQ*::Kan, ComQ is responsible for the maturation of

ComX precursor) by replacing the original *comXP* genes with another *comXP* that belongs to other groups [93].

For AI-2 detection, a well-known QS reporter kit (designed for detection of QS molecule AI-2 in *Vibrio*, ATCC # BAA 1116–1121) was developed by Bassler et al. (*V. harveyi* BB170 (*luxN*::tn5Kan) and *V. harveyi* MM32 (*luxN*::cm, *luxS*::tn5Kan)) is commonly used [94, 95]. Because the AI-2 QS system regulates the luciferase operon in *V. harveyi*, *V. harveyi* should be a suitable host for biosensor construction by rewiring the bioluminescence with exogenous AI-2. In *V. harveyi*, there is another QS system that used AI-1 (N-(3-hydroxybutanoyl)-L-homoserine lactone) as a signaling molecule interfering with the AI-2 signaling process [7]. Therefore, both of the AI-2 biosensors, BB170 and MM32, had an insertion mutation on the LuxN receptor needed for AI-1 detection [94]. They only responded to exogenous AI-2 by producing induced luminescence [94]. Because its original LuxS is intact in BB170, BB170 can synthesize AI-2 and produce low base-level bioluminescence without exogenous AI-2. It is also widely used as a simple tool for a qualitative test for the production of AI-2 in various bacteria [96–98]. However, for BB170 detection, supernatants from *V. harveyi* BB152 (*luxLM*::Tn5; AI-1^{-}; AI-2^{+}) and MM77 (*luxLM*::Tn5, *luxS*::Tn5; AI-1^{-}; AI-2^{-}) are usually required to serve as positive and negative controls [99]. By mutation on AI-2 synthase LuxS and AI-1 receptor LuxN, the strain MM32 thus produced no endogenous AI-2 and only responded to exogenous AI-2; it showed better performance than BB170 with lower base-level luminescence [95]. However, it should be noted that these biosensors were sensitive to growth conditions (such as pH and glucose) and bioluminescence could be inhibited by a high concentration of AI-2 [100–103].

3.2 Improvement on QS Biosensors

Even though a number of QS biosensors have been constructed so far, there are only a few reports on QS biosensor improvement. By using genetic and protein engineering strategies, one can expect to enhance the biosensor sensitivity, as well as the wide or narrow substrate spectrum [104]. It is reasonable to expect to enhance the biosensor sensitivity by engineering the sensor module (higher concentration of sensor module or higher bounding activity with target). Overexpression of the LuxR-type protein is confirmed as a useful strategy to synthesze more AHL detectors intracellularly and hence enhance the sensitivity of AHL biosensors [2, 105]. By overexpression of RhlR using a multi-copy plasmid, the sensitivity of a C4HSL biosensor was significantly enhanced [2]. Also, by using T7 expression system to overproduce TraR, the limit of detection (3 pM for 3OC8HSL) for this biosensor was reduced by at least 100-fold [105]. Moreover, Ling et al. constructed a *P. aeruginosa* biosensor for detection of a broad spectrum of AHLs by using a reporter plasmid containing *tacp-lasR*, *tacp-rhlR*, and *lasB*'-*lacZ* [106]. This reporter plasmid confers to non-signaling *P. aeruginosa* the ability to respond to a

wide variety of exogenous AHLs (C4HSL, C6HSL, 3OC6HSL, C7HSL, C12HSL, and 3OC12HSL) by induction of β-galactosidase [107]. Another versatile plasmid that tandemly assembles 8 *luxI*-family gene promoters (*luxI*, *cviI*, *ahlI*, *rhlI*, *cepI*, *phzI*, *traI*, and *ppuI*) upstream of a promoterless *lacZ* gene was constructed by Steindler et al. [108]. This plasmid biosensor was demonstrated to be useful to identify QS LuxR-family orphans in bacterial strains not producing AHLs [108]. If it is co-transformed with plasmid harboring different LuxR-type receptors, a super biosensor that could detect much a very broad spectrum of AHLs would be expected.

By using site-directed mutagenesis or direct evolution, a series of autoinducer receptor variants that respond to different AHLs at different levels and specificities were obtained [109–112]. By two-generation screening of the variants exhibiting increased response to C8HSL (only showing a weak interaction with wild-type LuxR) in LuxR mutant libraries (constructed by error-prone PCR and DNA shuffling), a LuxR-G2E variant that maintained a wild-type response to 3OC6HSL (the native AHL) and showed 100-fold increase in sensitivity to C8HSL was obtained [109]. As a result, LuxR-G2E could detect a much broader substrate spectrum (C5HSL, C6HSL, 3OC6HSL, C8HSL, C10HSL, C12HSL, 3OC12HSL, C14HSL) than the wild type [109, 111]. By an additional two rounds of directed evolution based on LuxR-G2E, the substrate spectrum was further broadened; LuxR-G4E obtained showed a strong response to C4HSL, which could not be detected by G2E [110]. In contrast, a specificity-enhancing variant of LuxR-G2E, which only responded to straight-chain AHL but no longer responded to 3OC6HSL and 3OC12HSL, was obtained by using a novel dual positive–negative selection system [111]. The alternation in autoinducer specificity of TraR or AgrC was also achieved by site-directed mutagenesis [109, 112]. Three residue changes in the AgrC-IV receptor were identified to be sufficient to convert the AIP recognizing specificity to AgrC-I [112]. Although most of these studies did not focus on biosensors, these AI receptor variants with different specificities and sensitivities could be used for biosensor construction, and improvement in specificity or sensitivity would be expected. The results of these studies imply that the biosensor specificity and sensitivity could be efficiently manipulated by engineering the AI receptors with various molecular strategies such as directed evolution, site-directed mutagenesis, and promoter engineering. Moreover, in the near future, with adequately detailed structure–function information on autoinducer receptor, we can expect computational design of new receptors with desired specificity and sensitivity [113, 114] to have customized QS biosensors.

Most of the reported QS biosensors were constructed for pure laboratory research use (for detection of samples with several extraction/purification steps and in well-defined culture condition), while a few efforts have been made toward their application in real environmental samples. Intended for application in samples containing toxic pollutants, we previously isolated aromatics resistant *P. aeruginosa* from rubber rubbish and used it as a host strain to construct a whole-cell biosensor for quantification of C4HSL in wastewater samples [2]. As expected, the biosensor response was not affected by toxic aromatics, and it was

demonstrated to be successfully applied in synthetic and real wastewater samples (Fig. 10). Two in situ AHL biosensors were also constructed recently [115, 116]. By using an indigenous soil bacterium as the host strain, a biosensor containing a P_{luxI} controlled *gfp*-reporter plasmid was constructed [115]. This biosensor was introduced into compost soil microcosms; after incubation, cells were harvested and analyzed by flow cytometry. By using this in situ technology, the AHL-producing microcosms were successfully discriminated [115]. Another approach for in situ AHL monitoring used a self-transmissible biosensor plasmid. Lumjiaktase et al. [116] constructed a RP4 replicon based AHL biosensor plasmid. This plasmid could self-spread within mixed biofilm, and it was applied for in situ identification of AHL-producing bacteria in lake sediment [116].

4 Effect of QS on Pathogen Infection and its Application in Diagnosis and Drug Discovery

4.1 QS Regulation on Pathogen Infection

The pathogen infection process is like a war between pathogens and human defense systems (immune defense and antimicrobial agents' treatment). Surpassing and overtaking the human defenses is the main goal for pathogens in achieving successful infections. The QS systems provide a sophisticated mechanism for pathogens to escape from the host immune defense at the early colonization stage, and to overwhelm and destroy the host defenses by rapid production of massive virulence factors once the population expands to a critical level (reviewed in [117–119]). In addition, QS systems also render antibiotic resistant strategies for pathogens to fight against the antimicrobial treatment [120–123]. Therefore, there is great interest in elucidating the QS effect on pathogenesis, which will provide insights on pathogen infection and the opportunity to develop new diagnostic or treatment tools. Here, we take two significant pathogens as examples to briefly introduce QS regulation on Gram-negative and Gram-positive bacterial infections.

4.1.1 *Pseudomonas aeruginosa*

P. aeruginosa is an important opportunistic pathogen that is commonly involved in nosocomial infections and burn wound infection; it is a leading cause of death in cystic fibrosis (CF) lung disease [124, 125]. So far, at least three QS systems have been identified in *P. aeruginosa* and are proven to be involved in its pathogenesis [15] (Fig. 11). Two of these are LasIR and RhlIR QS systems, which use 3OC12HSL and C4HSL as signaling molecules, respectively [126–129]. The third QS system is an AHL-independent system that consists of a LysR-type regulator PqsR and the

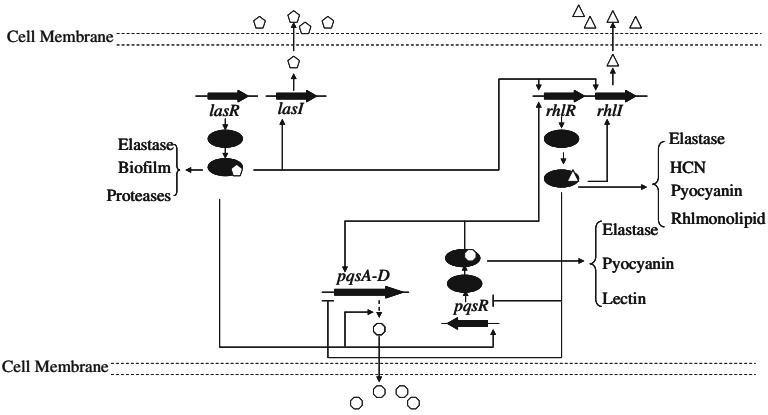

Fig. 11 Model for QS networks in *P. aeruginosa* and its regulation on virulences [3, 15, 16]. *Solid arrows* indicate positive regulation; *solid T-bars* indicate, negative regulation

pseudomonas quinolone signal (PQS, 2-heptyl-3-hydroxy-4-quinolone) called PQS system [130, 131].

P. aeruginosa usually secretes a variety of extracellular virulence determinants to kill or change signal transduction in mammalian cells. However, expression of these toxins is metabolically expensive [132]; early secretion of toxins will trigger the innate immunity system, which is a disaster for pathogens. Therefore, toxin expression should be exquisitely regulated so that it is secreted at the right time and location [132]. It has been determined that most of the virulence factors are regulated by the fine-tuned QS systems in *P. aeruginosa* [133]. According to transcriptome analysis, the *las* and *rhl* QS systems controlled the transcription of over 300 genes, representing about 6 % of the *P. aeruginosa* genome [134]. These include a large number of virulent genes such as *lasAB*, *rhlAB*, *hcnABC*, and *phzCDE* [134]. By comparative analysis between site-specific QS gene deletion mutants and wild-type strains, *las* and *rhl* QS systems were found to regulate the production of multiple virulence factors including elastase [135], alkaline protease [136], hydrogen cyanide [137], exotoxin A [138], rhamnolipid [139], pyocyanin [140], and lectins [141] (Fig. 6). More interestingly, an additional timing regulation mechanism (suggested at the LasR and RhlR receptor level) besides AHL accumulation may exist to ensure that the virulence factors are produced at the right time [134]. The PQS system has been shown to control several virulence factors, such as pyocyanin, elastase, and lectin [118, 142] (Fig. 6). Because the three QS systems are cross-linked, one specific virulence factor might be simultaneously regulated by two or three systems; this regulation may occur in parallel, crossover, or hierarchically [15] (Fig. 6). Besides various virulent weapons, *P. aeruginosa* evolved some defense helmets such as the formation of biofilm and resistance to multiple antibiotics, which were also regulated by QS systems [143, 144]. In addition, in vivo and clinical studies also confirmed the importance of QS regulation on *P. aeruginosa* infection. Both C4HSL and 3OC12HSL have

Table 2 Typical antibiotics biosynthesis controlled by QS systems

Antibiotics	Strain	QS system involved	Ref
Carbapenem	*Serratia* sp. ATTCC 39006	SmaI/R	[30]
	E. carotovora	CarI/R	[33]
	Serratia sp. ATTCC 39006	LuxS	[38]
Prodigiosin	*Serratia* sp. ATTCC 39006	SmaI/R	[30]
	S. marcescens ATCC 274	LuxS	[38]
Thailandamide	*Burkholderia thailandensis*	ThaA/?	[184]
Phenazines	*P. chlororaphis* 30–84	PhzI/R	[185]
	P. fluorescens 2–79	PhzI/R	[180]
	P. chlororaphis PCL1391	PhzI/R	[186]
	P. aeruginosa PAO1	RhlI/R, PQS	[15, 140]
	Pseudomonas sp. M18	RhlI/R	[44, 46]
Pimaricin	*Streptomyces natalensis*	PimM/PI	[187, 188]
Mupirocin	*P. fluorescens* NCIMB 10586	MupI/R	[189]
Pyoluteorin	*Pseudomonas* sp. M18	RhlI/VqsR	[190, 191]
		PQS	[192]
Pyrrolnitrin	*B. cepacia*	CepI/R	[193]
	Serratia plymuthica	SplI/R	[194]
Bactobolin	*B. thailandensis* E264	BtaI2/R2	[195]
Fosfomycin	*Streptomyces fradiae*	FomR/?	[196]
Nisin	*L. lactis*	Nisin/NisRK	[56]
Subtilin	*B. subtilis*	Subtilin/SpaRK	[57]
Blp Bacteriocins	*Streptococcus thermophilus*	Blp/BlpRH	[197]
Plantaricin	*Lactobacillus plantarum*	Plantarcin/PlnB/PlnC/D	[198]

been detected in sputum from *P. aeruginosa* infected patients with CF [145, 146]. Studies on various animal infection models showed that mutation on QS regulon leads to less severe tissue destruction, reduced pneumonia, reduced infections dissemination, and reduced mortality compared to wild-type *P. aeruginosa* [147–152]. These results indicate that QS systems are active during *P. aeruginosa* infection and functional QS systems play essential roles in acute or chronic infections (Table 2).

4.1.2 *Staphylococcus aureus*

S. aureus is a typical Gram-positive pathogen that uses QS to control its pathogenesis [118]. *S. aureus* is considered to be another versatile and dangerous human pathogen. It causes multiple high mortality infections such as bacteremia, endocarditis, sepsis, and toxic shock syndrome (reviewed in [153, 8]). *S. aureus* is very dangerous to humans not just because of its capability to produce a diverse arsenal of virulence factors and its increasing resistance to multi-antibiotics [8], but also because it is commensally colonized in 30 to 50 % of healthy adults [153]. *Agr* The QS system is responsible for its switch from a colonizing commensal bacterium to an aggressive pathogen [118, 154]. The *agr* system in *S. aureus* has been

studied as a model QS system in Gram-positive bacteria as introduced in Sect. 1, and its effect on *S. aureus* infections will be discussed here.

According to transcriptomic and proteomic analyses, over 20 virulent genes are regulated by the *agr* QS system [155, 156]. As described in Sect. 1 and Fig. 2b, *agr* regulation is mainly relies on the P3 promoter controlled RNAIII. RNAIII is a 514-nt bifunctional RNA, i.e., mRNA encoding δ-hemolysin and a regulatory RNA [156, 157]. The regulatory RNA RNAIII is the QS effector that regulates a variety of virulence factors. The *agr* response virulence factors are comprised of two groups, i.e., cell surface factors (group I) responsible for early-stage attachment to host cells and evasion of host innate immune systems, and extracellular virulence factors (group II) responsible for the invasion and toxin production [118, 156]. Murphy et al. [156] showed that cell surface associated factors are predominately expressed during early-log-phase and repressed by the *agr* system at high cell densities. The extracellular virulence factors, including exoenzymes and toxins, are produced at the basal level during early-log-phase and upregulated by the *agr* system at high cell densities [156]. This *agr* regulation mechanism facilitated the initial *S. aureus* colonization on host cells and strictly repressed the expression of exoenzymes and toxins to evade the host immune system during the early stage. The upregulation of exoenzymes and toxins facilitates the bacteria to rapidly invade the host cells and causes disease when high cell density is reached. In contrast to *P. aeruginosa*, the QS system activates the detachment of biofilm in *S. aureus* by repressing the group I factors that are responsible for biofilm formation at high cell densities [158]. The detached cells can colonize other sites to cause a spreading invasion or infect other persons to cause person-to-person transmission [8, 153]. Although the mechanism is still controversial, it is in line with its metastatic infections and high frequency observations of acquired nosocomial infections [153].

4.2 QS as a Potential Biomarker for Pathogen Detection and Disease Diagnosis

As described previously, a considerable amount of in vitro and in vivo evidences shows that QS plays an important role in pathogen infections. Therefore, researchers hypothesized that QS may serve as a biomarker for diagnostic purposes in the management of bacteria-related diseases [159, 160].

Some PCR-based methods have been developed to use QS as a biomarker to specifically detect various pathogens [161–163]. By designing a set of primers targeting the QS genes of pathogens, some pathogens were specifically detected and the presence of pathogens in various samples was rapidly discriminated [161–163]. For example, by using a set of primers targeting *sdiA* QS genes in *Salmonella* spp., a total of 101 different serotypes of *Salmonella* (155 strains) could be specifically detected [81 non-*Salmonella* strains (24 different species) showed negative results]. Moreover, this PCR-based method was successfully applied to specifically detect

Salmonella in contaminated human fecal samples with a detection limit of 10^2 CFU/g [161]. Although the PCR-based diagnostic method could specifically check for the presence of the targeting pathogen, it is hard to provide information about the QS activity in the infected samples. However, the activity of QS is much more important than its existence for determining the infection stage. For example, the activation of QS is the key step to switch *S. aureus* from a commensal to an aggressive pathogen (Sect. 4.1.2). Specific quantification of QS signaling molecules or effectors, which was the advantage of biosensor, could give us insight into the activity of QS.

Various biosensor systems for the detection/quantification of QS signaling molecules have been developed (introduced in Sect. 4). Kumari et al. attempted to detect AHLs in clinical samples and test whether AHL could be employed as a biomarker for bacteria-related disorders by using the *gfp*-based biosensing system [160]. They successfully detected the presence of AHLs in saliva and stool samples. More interestingly, the levels of AHLs were significantly different between samples from healthy volunteers and samples from patients with Crohn's disease [159, 160]. The difference was further confirmed by using HPLC–MS/MS analysis [159]. Although it is difficult to conclude the precise relationship between AHLs (type and concentration) and the bacteria-related disorder due to the limited number of samples analyzed, they suggested that QS signaling molecules may be potential biomarkers for the diagnosis of bacteria-related diseases [159, 160]. However, special attention should be paid to the following aspects: A significant number of clinical samples should be carefully analyzed to test whether QS signaling molecules could be considered as biomarkers for each specific disease. Before testing, rigorous studies should be conducted to assess analytical performance when applied to such clinical samples. Notably, the co-existence of different QS signaling molecules may cause cross-inhibition on biosensor detection (e.g., long-chain AHL would inhibit violacin of *C. violaceum* CV026, which responded to short-chain AHL). The concentration of QS signaling molecules is much lower at the early infection stage, which might result in negative results by biosensor diagnosis for early diagnosis. Therefore, a combination of PCR and the QS biosensor method could provide comprehensive information about QS during pathogen infection. However, a combination of QS diagnosis with another conventional strategy has been strongly suggested for pathogen infection diagnosis, and the sensitivity of QS biosensor should be improved for early diagnosis.

4.3 QS as an Antimicrobial Target for Drug Discovery

Considering the increasing antibiotic resistance and threat of postantibiotic era [164, 165], new strategies to combat microbial infections has attracted much attention. The selective pressure/motivation for bacterial resistance evolution is the stress imposed by conventional antibiotics that are aimed at bacterial viability [165, 166]. New strategies targeting the functions essential for infections rather

than bacterial viability have been investigated over the past decades [164]. Anti-virulence, which did not impose any growth stress and thus no risk of resistance, was considered to be a promising strategy [164, 165].

As described in Sect. 3.1, a QS system acting as a global regulator controlled a variety of virulence factors in many clinically important pathogens. Moreover, many QS-deficient pathogens lost the ability to infect or at least caused less severe infections. Therefore, interference with the QS system was considered to be a compelling approach to fight against microbial infections [165]. Theoretically, interruption in any step of the QS regulatory circuit (autoinducer synthesis, autoinducer-receptor interaction, signal transduction, etc.) would abolish its regulation on microbial infection. Based on the different targets, the identified inhibitors or antagonists could be divided into at least four groups. The group I inhibitors target autoinducer synthesis and accumulation. SAM (S-adenosylmethionine) analogs (interfering with AHL synthesis) [167] and SRH (S-ribosyl-L-homocysteine) analogs (interfering with AI-2 synthesis) [168, 169] are the main representatives of this group. In addition, enzymes with autoinducer degradation abilities (review in [170]) and antibodies that sequestrate the autoinducers [171] could abolish pheromone accumulation and disrupt the QS. Autoinducer receptors are the target of Group II inhibitors/antagonists. Analogs of autoinducers are a large family of this group. The various analogs with QS inhibition activity could bind to the autoinducer receptors to prevent interaction with its actual autoinducer, resulting in disruption of QS [172]. The signal transduction process and the downstream QS effectors are the targets of groups III and IV, respectively. These two groups are mainly composed of the inhibitors of peptide-dependent and AI-2 QS systems.

By using QS bioreporter strains for in vitro screening, researchers identified a large number of potential QS inhibitors or antagonists (reviewed in [172]) from synthetic or natural compound libraries. Most of the inhibitors and antagonists showed strong inhibition on the production of virulence factors by important pathogens [172]. For example, two inhibitors that resembled the acyl-homoserine lactone molecule were screened out from a library of 200,000 compounds by using an ultra-high throughput in vitro screening based on PAO-MW1 bioreporter strains [173]. More importantly, one of these two inhibitors (12-carbon aliphatic tail attached to a phenyl ring) inhibited 129 QS-controlled genes in *P. aeruginosa* PAO1 at a concentration of 100 μM. With the addition of this inhibitor, the pyocyanin production was inhibited by about 90 % and elastase production was reduced by 60 % [173]. Remarkably, the in vivo anti-virulence/anti-infection activity of several inhibitors or antagonists has been confirmed in different animal infection models [171, 172, 174]. Interestingly, some inhibitors/antagonists disrupted QS with several different mechanisms. For example, the well-known halogenated furanone, (5Z)-4-bromo-5-(bromomethylene)-3-butyl-2(5H)-furanone, showed broad-spectrum QS inhibition activity by interfering with AHL receptors [175, 176] or inhibiting AI-2 synthase LuxS [177]. To date, a large series of inhibitors and antagonists of the bacterial QS system have been identified (reviewed in [172]), and most of these showed strong inhibition on production of virulence factors. Although many potential inhibitors/antagonists have been

obtained, more in vivo research should be conducted to address their efficiency on infection control. Because QS is a widespread global regulatory mechanism, the ecological effect of QS disruption/interference on other commensal beneficial microbial flora should be taken into account. More importantly, although no risk of microbial resistance was claimed, attention should be directed to concrete long-term and rigorous evidence to address whether there is the possibility for bacteria to evolve resistance to QS disruption [178].

5 Concluding Remarks and Perspectives

This chapter reviewed the recent progress in QS regulation on microbial metabolism and bacterial pathogenesis. It introduced recent endeavors in novel antimicrobial agents development based on quorum quenching, disease diagnosis based on QS signaling molecule detection, and sensitive QS biosensors developed for various applications.

The findings of QS regulation on microbial metabolism have provided us with a possibility to manipulate the target metabolic pathway at the population level. Population- level control could coordinate the cell populations to maximize production ability, and it is substantially different from previous regulation patterns that only focused on the individual cells. We envision that QS regulatory circuits can also serve as a model system to be incorporated into the traditional fermentation/biodegradation process, and hence control the fermentation or pollutant degradation processes at the population level. To date, knowledge of QS regulation on pathogenesis is still limited to several model bacteria. Development of novel antimicrobial agents or diagnosis strategies based on QS mechanisms is heavily dependent on our knowledge in its role on pathogenesis. Systematic research on the role of QS in disease-related bacteria should be addressed because QS quenching is expected to be the next generation of antimicrobial strategy.

QS may be one of the most important bacterial regulatory systems for human life. It is not only a key regulon in death (mortal infections), but it also provides hope for life because it is related to the production of fuels and antibiotics, as well as diagnosis. QS expected to be the target of the next generation of antimicrobial agents, and it may be is the sole hope for the postantibiotic era. Bacteria are surely a double-edged sword for human beings, but the QS regulatory system links these two sides. QS can to reduce the danger of bacteria and fully use their advantages for the benefit of human life.

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