CHAPTER 9

Pseudomonas aeruginosa *Quorum Sensing and Biofilm Inhibition*

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1 Introduction

Antibiotics have been used against infections widely since their discovery. However, misuse of these drugs resulted in the adaptation of microorganisms by gaining antibiotic-resistance. There are major concerns that antibiotics will become dysfunctional within a short period of time, prevention and treatment of infections will become impossible, and classical infections will be resurrected as one of the major causes of mortality (Van Hecke et al., 2017). The World Health Organization reports that we are in a shortage of treatment options against antibiotic-resistant bacteria, and an international coordinated effort is essential to overcome this situation (WHO, 2017). Laxminarayan et al. (2016) estimates that an average of 214,000 neonatal sepsis deaths are caused by antibiotic-resistant pathogens. It is also reported that approximately 23,000 people die every year in the United States due to antibiotic-resistant infections (CDC, 2017). Hospitalized patients suffer the consequences of antibiotic-resistant bacteria directly, and among these bacteria, *Pseudomonas aeruginosa* can be difficult to treat due to multidrug resistance.

P. aeruginosa is a Gram negative opportunistic pathogen that is responsible for approximately 10% of all nosocomial infections (Diekema et al., 1999). *P. aeruginosa* can cause infections in lungs, bloodstream, urinary tract, and surgical sites. Although it mostly causes infections in immunocompromised patients, healthy people can also get infected. Multidrug resistant (MDR) *P. aeruginosa* is presented as the main cause of high mortality rates in cystic fibrosis (CF) patients. CF is a disease that is encountered frequently and affects approximately 70,000 people in the world, known to degrade lung functions by affecting the respiratory system (Rivas Caldas and Boisrame, 2015). With concentrated treatments, CF patients can have a life

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expectancy of 35–50 years. Left untreated, many CF patients can die at a young age. MDR biofilm forms make the disease very hard to treat with antibiotics.

P. aeruginosa colonies are known to produce a polysaccharide matrix and attach to surfaces when they reach a certain population density, in a macroscopically seen and hard to remove biofilm form. It is reported that this biofilm form is up to 1000–3000 times more resistant to antibiotics compared with the same species in planktonic form (Olson et al., 2002). Biofilm form provides many advantages, such as amassing nutrition and protecting the microorganisms against disinfectants, antibiotics, UV light, pH, moisture, and heat fluctuations, organisms that feed on bacteria and viruses (Hall-Stoodley et al., 2004).

The evident response against *P. aeruginosa* infections is to develop new treatment methods in addition to the efforts of preventing infections and reorganizing antibiotic usages. The antivirulence approach is the leading progression toward this goal. Antivirulence does not aim to exterminate pathogens directly but to alternately inhibit virulence (Fuqua and Greenberg, 2002). Accordingly, specific biological information is needed for the targets of antivirulence methods. There are many recognized virulence factors, such as bacterial toxins, surface proteins, immunoevasion factors, and adhesins. It is now understood that most of these factors in addition to numerous behaviors are controlled by quorum sensing (QS) systems, including bioluminescence, swarming, conjugation, protease activity, and also biofilm formation.

2 Quorum Sensing and Quorum Quenching

It has been established that planktonic cells cannot exist freely in environments because they have to compete with other coexisting organisms and survive in extreme conditions. Therefore, they communicate via QS. Higher levels of bacterial population density trigger QS system for intraspecies, interspecies or interkingdom interactions. Small and diffusible chemical signaling molecules called autoinducers (AI) are secreted into bacterial local milieu. Three types of main AIs were identified. AI-1, called also AHLs (*N*-acylated L-homoserine lactones), are utilized by Gram negative bacteria. Autoinducing peptides (AIP) are used by Gram positive bacteria and autoinducer-2 (AI-2) by both Gram positive and negative bacteria for interspecies interaction (Rutherford and Bassler, 2012). The QS system consists of five elements that are responsible for QS regulation: AI molecules, signal synthase enzymes, receptors, regulators, and genes. Most pathogenic bacteria orchestrate their virulence factors, biofilm formation, and antibiotic resistance by QS systems (Li and Tian, 2012).

The term *quorum sensing* was firstly introduced in 1970 in *Vibrio fischeri* and *Vibrio harveyi*, which are known marine bacteria with a characteristic luminescent feature. Through the study of the bioluminescence of these bacteria, the details of QS systems were revealed. QS has been defined as a sensory system of bacteria to detect the environmental changes with regard to population density in their surrounding milieu. The luminescent characteristics of the aforementioned bacteria can be easily monitored after the lag phase of bacterial growth. To

reach the maximum luminescence in early log-phase cultures as in the stationary phase, cell-free supernatant from the stationary phase can be added to the medium. AI molecules are small diffusible compounds that are released to the surrounding environment of the bacteria. The concentration of these signaling molecules remains at lesser levels in low cell densities, whereas it accumulates up to a threshold of concentration at higher cell densities.

The first identified AI of *V. fischeri* (VAI) was *N*-3-(oxohexanoyl)-homoserine lactone (3OC6HSL) (Eberhard et al., 1981). It has been well established that the biosynthesis of VAI is encoded by *luxI* gene in a positive feedback mechanism. The increase in bacterial population density leads to the accumulation of VAI molecules at higher concentrations in the surrounding milieu. The interaction between these signal molecules and LuxR gene leads to transcription of the *luxICDABE* operon. Then, the luciferase is encoded by the *luxCDABE* operon, and QS activation becomes visible through luminescence. On the other hand, lower concentrations of VAI due to low cell densities are insufficient to activate the *luxR* and *luxI* genes, and luciferase is not encoded. This information proves convenient for several studies focused on the QS mechanism.

It is understood that AHL molecules only function as QS signals when the bacterial cell population density increases and certain threshold levels of AHL are obtained. For the regulation of the QS system, the synthesis of AHLs via AHL synthase and the accumulation of these signals at higher concentrations due to population density are needed. Therefore, QS systems can be evaluated by AHL monitorization. AHL accumulation depends on physical and chemical factors in bacterial communities. For example, AHL molecules can mostly diffuse across the membranes.

Several pathogenic bacteria such as *P. aeruginosa* cause high mortality and morbidity rates despite high doses of antibiotics, especially in immunocompromised patients (Borges et al., 2016). These pathogenic bacteria develop various ways to avoid bactericidal and bacteriostatic effects of antibiotics, such as horizontal gene transfer and spontaneous mutations (Kalia, 2013). Also, the failure of antibiotic treatments occurs due to group transfer, redox mechanism, or enzymatic hydrolysis (Hentzer and Givskov, 2003). Therefore, recent studies have focused on developing alternative strategies to prevent bacterial resistance against antibiotics by the disruption of bacterial QS. These anti-QS approaches are called *quorum quenching* (QQ; Hentzer and Givskov, 2003). Several natural or synthetic QS inhibitor (QSI) compounds from plants, animals, fungi, bacteria, and algae have been discovered and examined for their QSI potentials. Moreover, technological advances in computational sciences and in silico methods allow a rapid screening of these compounds. Their biological and therapeutic effects have been reported by many studies, as explained in this chapter.

2.1 *Quorum Sensing Systems and Biofilm in* P. aeruginosa

P. aeruginosa QS systems regulate crucial functions such as virulence, motility, biofilm formation, and secondary metabolites production. Like other Gram negative bacteria,

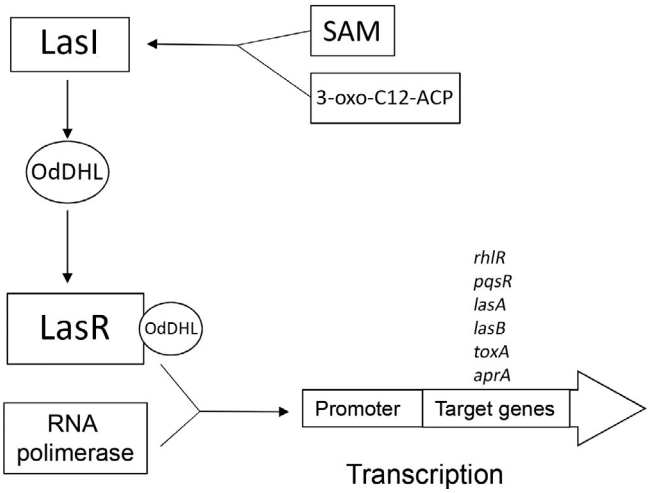

Fig. 1

A basic diagram of *las* signaling network in *P. aeruginosa*. The *las* system detects 3-oxo-C12-HSL produced by signal synthase LasI, via the transcriptional regulator LasR, and regulates virulence factors via two-component signal transduction system.

P. aeruginosa also utilizes AHLs for its main QS systems. It is known that *P. aeruginosa* has four hierarchically connected QS systems for interspecies communication: *las, rhl, pqs,* and *iqs* (Daniels et al., 2004; Lee and Zhang, 2015).

The *las* system consists of the transcriptional regulator LasR, signal synthase LasI, and the autoinducer *N*-3-oxododecanoyl-homoserine lactone (3-oxo-C12-HSL, OdDHL), as shown in Fig. 1. The *rhl* system similarly has RhlR, RhlI, and the autoinducer *N*-butanoyl-homoserine lactone (C4-HSL, BHL). The *pqs* system has PqsR as its regulator, *pqsABCDE-phnAB* operon, and PqsH for signal synthesis, and 2-alkyl-4-quinolones (AQs) as its signal molecules, including 2-heptyl-4- hydroxyquinolone (HHQ) and the 2-heptyl-3-hydroxy-4(1H)-quinolone named pseudomonas quinolone signal (PQS). Finally, the recently discovered *iqs* system utilizes 2-(2-hydroxyphenyl)-thiazole-4-carbaldehyde as its signal and is related to environmental stress.

The main QS systems *las, rhl,* and *pqs* regulate the production of many virulence factors, such as elastase, exotoxin A, rhamnolipids, pyocyanin, lipase, pyoverdine, lectins, and so on. It is also thought that the efflux pump system of MDR bacteria is related to a *pqs* system. Among these three systems, *las* governs other systems hierarchically, while *pqs* seems to mediate between *las* and *rhl* systems while regulating some virulence factors (Lee and Zhang, 2015). However, the hierarchy of QS systems in *P. aeruginosa* may change and adapt according to the environmental stress. For example, an *iqs* system can take over *las* functions in the case of severe phosphate depletion stress, or the *pqs* system can activate without the *las* system. Therefore, the virulence pathways can change according to environmental conditions and stress. Other regulators such as QscR and RsaL can inhibit signal production, maintaining a balance for this complex overall signal mechanisms.

QS is known to be involved in the biofilm formation of *P. aeruginosa*. QS mutant strains form flat and weak biofilms compared with wild type strains, although it is important to consider the culturing conditions. It is accepted that QS plays a role in biofilm formation, but some of the proposed mechanisms remain debatable.

The first step in biofilm formation is the attachment of bacteria to a surface. Flagellar motility (type IV pili) and adhesins are important factors in this stage. After irreversible attachment, microcolonies are formed, increasing QS communication. The maturation of biofilm starts accordingly. The 3D structure of mature biofilm varies according to environmental conditions, as well as the amount of factors produced by the bacteria. These factors are exopolysaccharides (EPS) like alginate, structural DNA, iron chelator pyoverdine, and surfactants like rhamnolipid, most of which are directly controlled by QS metabolism. The right amount of rhamnolipid secretion is crucial for a mature biofilm and the next step: dispersion. Overexpression of rhamnolipid causes the biofilm to disperse, thus allowing the bacteria to colonize other surfaces (Boyle et al., 2013).

The role of *P. aeruginosa* QS systems on biofilm formation during these stages seems obvious. However, there have been contradictory and diverse results and opinions. QS-biofilm relation is usually studied by using flow-cell systems. These systems have small channels through which media is circulated constantly. Bacteria strains form biofilm structures in these channels, and they are monitored by confocal laser microscopes. The strains are QS-deficient mutants that are compared with their wild-type counterparts. The varying results of these studies concerning QS relation to biofilm formation lead to the common opinion that culturing and environmental conditions have significant impacts on biofilm structures. However, it is known that QS systems have important effects on biofilm formation, as previously explained, and their inhibition seems a reasonable approach from a therapeutic point of view (Joo and Otto, 2012).

2.2 Screening for Quorum Sensing Inhibitors

QSI molecules have to be efficient, stable, and practicable compounds with low-molecular weight and high specificity for signal regulators. It is important not to cause adverse effects for bacteria and the host. Furthermore, these compounds must not be affected by hydrolytic enzymes of the host. On the other hand, some compounds bind to receptors and activate them acting as agonists and cause an upregulation in QS-related genes. QSIs are preferred to display antagonistic effects on their inhibition targets.

Quorum quenching (QQ) is a general term which is used for all processes targeting to inhibit QS system. The purpose of QQ approaches is to disrupt bacterial communication without killing bacteria or preventing their growth. There are several targets for QS inhibition. A summary of QQ approaches for *P. aeruginosa* are shown in Fig. 2.

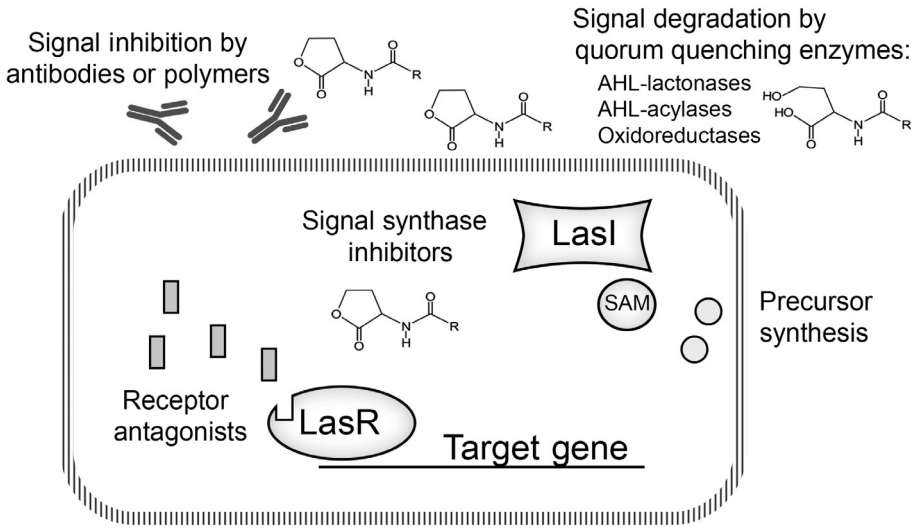

Fig. 2

QQ approaches and QSIs for *P. aeruginosa las* system. These approaches mainly focus on autoinducer signal synthesis inhibition including the synthesis of their precursors, inhibiting or degrading signal molecules and blocking the detection of signal molecules.

2.2.1 Inhibition of Quorum Sensing Signal Biosynthesis

Inhibition of QS signal biosynthesis is one of the QQ approaches used against *P. aeruginosa*. In Gram negative bacteria, enoyl-acyl-carrier-protein (ACP) reductase (ENR) and *S*-adenoysl methionine (SAM) may be targeted for *N*-Acyl homoserine lactone (AHL) synthesis (Dong et al., 2007). In AI-2 QS inhibition, the synthesis of 4,5-dihydroxy 2,3 pentanedione (DPD), which is formed from cleavage of *S*-ribosyl-L-homocysteine SHR by LuxS enzyme, can also be a QQ target (Galloway et al., 2011).

Since bacterial antibiotic-resistance is a major global health care problem, *P. aeruginosa* QS systems have been studied in detail. Most pathogen bacteria such *as P. aeruginosa* coordinate its pathogenesis via QS system. The genes responsible for AHL synthesis and accumulation have been targeted as an alternative approach, and the potential of AHL synthase as an antimicrobial target has been revealed. For this purpose, repressor genes have been used to decrease the transcription of *luxI* homologues. *dksA,* a suppressor gene for *rhlI* from *P. aeruginosa*, has been isolated by Branny et al. (2001). The *rhlI* gene is responsible for the transcription of C4-HSL synthase. On the other hand, the repressor gene *qscR* targets the *lasI* gene and modulates the synthesis of QS signals, along with the virulence factors in *P. aeruginosa*. It was reported that the mutant *qscR* gene results in premature signals and also premature transcriptions in the following steps. Other QS signal synthesis repressor genes are known, like *rsaL* in *P. aeruginosa*.

NADH-dependent ENR (FabI) is responsible for the synthesis of acyl-ACP and also the formation of acyl chains for AHLs. Despite the fact that triclosan, an antibacterial agent, can inhibit Fabl enzyme production and C_4HSL, it was reported that the resistance of *P. aeruginosa* cannot be prevented by triclosan due to efflux pump system (LaSarre and Federle, 2013). The relationship between efflux pump systems and QS is a compelling topic in this regard, and further investigations may reveal intriguing prospects.

2.2.2 QS Signal Degradation and Inactivation

Degradation of QS signals may be achieved chemically, metabolically, or enzymatically. In chemical degradation, alkaline pH values cause the lactone ring to open, whereas acidic pH values lead to recyclization (Rasmussen and Givskov, 2006). However, QS signal degradation is mostly handled by enzymes, and inactivation can occur using antibodies.

2.2.3 Inhibition of Signal Detection

The inhibition of signal molecule detection may be achieved by competing antagonist molecules by binding to the receptor before the signal molecules. Inactivation of the signal receptors provides an inhibition in virulence factor expression. Most natural QSIs are known to show activity against *P. aeruginosa* through the inhibition of LasR, RhlR, and PqsR.

2.3 Natural Quorum Sensing Inhibitors

Natural QSIs are mostly compounds, extracts, enzymes, and antibodies obtained from natural sources.

2.3.1 Quorum Quenching Enzymes

AHL-lactonases, acylases, oxidoreductases, paraxonases, and 2,4-dioxygenase (Hod) are reported to be QQ enzymes.

AHL-Lactonases

AHL lactonases are involved in the group of metalloproteins, and they form acyl homoserine by hydrolyzing ester bond of the HSL ring. This group of enzymes shows significant specificity for AHL molecules due to a highly conserved HSL ring (LaSarre and Federle, 2013). The autoinducer inactivation gene (AiiA), firstly described lactonase enzyme, has been discovered in *Bacillus genus—B. anthracis, B. cereus, B. mycoides, B. subtilis, B. thuringiensis, Arthrobacter* spp., *Acidobacteria, Agrobacterium* spp., and *Klebsiella* spp., and demonstrated to degrade AHL (Kalia and Purohit, 2011).

It was also reported that AiiA alleles in *P. aeruginosa* and *Bacillus thailandensis* inhibited the aggregation of AHLs. AttM, found in the plant pathogen *Agrobacterium tumefaciens*, exhibits low levels of similarity to *AiiA,* despite the same conserved HXDH motif. Also, AiiB, AhlD,

AhlK, AidC, QlcA, BipB01, BipB04, BipB05, BipB07, QsdA, AiiM, AidH, and QsdH have been reported to act as lactonase enzymes. They differ in their DNA sequence and dependence for metal ions (LaSarre and Federle, 2013).

Mammalian enzymes, paraoxonases 1, 2, and 3 (PON1, PON2, PON3), exhibit lactonase activity. Hraiech et al. (2014) discovered a new variant molecule for *Sso*Pox (hyperthermostable lactonase). They demonstrated that *Sso*Pox-I (phosphotriesterase-like lactonase) had a QQ potential in a rat model of acute pneumonia, and the survival rates were increased by the intratracheal application of *Sso*Pox-I. Also, *lasB* activity, pyocianin synthesis, proteolytic activity, biofilm formation, and lung tissue damage were reduced.

Recently, Tang et al. (2015) reported that protease and pyocyanin production by *P. aeruginosa* was inhibited by MomL, newly identified AHL lactonase from *Muricauda olearia*. The researchers evaluated the effects of MomL on *P. aeruginosa* virulence in a *Caenorhabditis elegans* model, and virulence inhibitions were observed.

Acylases

The amide bond between HSL and acyl side chain are cleaved by this group of enzymes. After this cleavage, a fatty acid chain and an HSL moiety are formed. The specificity of these enzymes are reported to be the length of acyl chain and substitution on the third position of the chain (LaSarre and Federle, 2013).

Pseudomonas have an acylase activity for its own AHL (Grandclement et al., 2016). The amidase encoding genes called *pvdQ* (PA2385), *quiP* (PA1032), and *hacB* (PA0305) were revealed in *P. aeruginosa* (Huang et al., 2006; Wahjudi et al., 2011; Sio et al., 2006).

The acylase enzyme from *Aspergillus melleus* was immobilized into polyurethane coatings by Grover et al. (2016). This immobilized enzyme inhibited the biofilm formation and also pyocyanin production of *P. aeruginosa* ATCC 10145 and PAO1 strains. Sunder et al. (2017) investigated the effects of penicillin V acylases (PVAs) against *Pectobacterium atrosepticum* and *A. tumefaciens*. Researchers transferred these enzymes to *P. aeruginosa*, and reported an inhibition in virulence factors and biofilm formation, and also an increase in survival rate in an insect model of acute infection.

Paraxonases

Paraoxonases are mammalian enzymes acting as QQ enzymes. There are three types of paraoxonases (PON1, PON2, and PON3). PONs have been described as lactonase-like enzymes regarding the disruption of QS system (Grandclement et al., 2016). Stoltz et al. (2007) reported that overexpressed PON2 caused $3OC_{12}$-HSL degradation in murine tracheal epithelial cells.

Recently, SsoPox-W263I was tested in *P. aeruginosa* strains obtained from patients with diabetic foot ulcers. An inhibition was reported in virulence factors (proteases and pyocyanin

production). The researchers indicated that SsoPox-W263I was more efficient when compared with 5-fluorouracil and C-30 (Guendouze et al., 2017). Moreover, human serum paraoxonase 1 (hPON1) displayed a reduction in pyocyanin, rhamnolipid, elastase, staphylolytic LasA protease, and alkaline protease activities (Aybey and Demirkan, 2016).

Oxidoreductases

Another group of enzymes, the oxidoreductases, oxidize or reduce the acyl side chain of AHL without degradation. These enzymes function as QSIs by modifying the C_3 keto group of the fatty acid side of AHL molecules. The inactivation AHL-mediated biofilm formation of *P. aeruginosa* by BpiB09, NADP-dependent oxidoreductase from a metagenome-derived clone is also reported (Weiland-Brauer et al., 2016).

2,4-Dioxygenase (Hod)

Hod, another QQ enzyme, causes noctanoylanthranilic acid and carbon monoxide to be formed from PQS. Pustelny et al. (2009) reported that exogenous addition of heterocyclic-ring-cleaving enzyme Hod from *Arthrobacter* sp. Rue61a into *P. aeruginosa* cultures inhibits key virulence factors and tissue damage in a plant leaf infection model.

2.3.2 Antibodies

QQ antibodies mainly target HSLs in *P. aeruginosa* for signal inactivation. On the other hand, other factors playing a role in signal synthesis can also be targeted. Immunopharmacotherapeutic approaches employing monoclonal or polyclonal antibodies have been investigated to attenuate QS, controlling virulence regulation and biofilm formation, as shown in Table 1.

Table 1 Quorum quenching antibodies

Antibody	Study	Target	References
3-oxo-C12-HSL-bovine serum albumin (BSA) conjugate	3-oxo-C12-HSL-carrier protein conjugate against pulmonary tumour necrosis factor (TNF)-alpha and apoptosis in macrophages	*P. aeruginosa* C12HSL	Miyairi et al. (2006)
HSL-2 and HSL-4 monoclonal antibodies (mAbs)	MAbs	*P. aeruginosa* HSLs	Palliyil et al. (2014)
MAbs	MAbs targeting DNA-binding tips of DNABII proteins	Biofilm disruption of *P. aeruginosa,* MAbs-antibiotic therapy	Novotny et al. (2016)
RS2-1G9	Monoclonal antibody	*P. aeruginosa* C12HSL	Kaufmann et al. (2006)
XYD-11G2	Monoclonal antibody	*P. aeruginosa* C12HSL	De Lamo Marin et al. (2007)

Miyairi et al. (2006) investigated the effects of active immunization with OdDHL-carrier protein conjugate on mice with *P. aeruginosa* lung infections. Their results show that mice with specific antibodies to OdDHL in serum have a higher survival rate against *P. aeruginosa* infections. Immune serum also increased the cell viability of OdDHL-induced apoptosis in macrophages. Palliyil et al. (2014) used sheep immunization and recombinant antibody technology to generate monoclonal antibodies (MAbs) and detect *P. aeruginosa* HSL molecules. They achieved nanomolar sensitivity to detect HSLs in urine. They used nematode and mouse models to compare survival rates of infected groups treated with HSL MAbs with control groups, presenting a significant increase. These studies present that antibodies against QS signal molecules can be a viable approach for supplemental treatment methods.

2.3.3 Natural Quorum Sensing Inhibitor Compounds and Extracts

Natural QSIs are produced by several organisms, such as bacteria, algae, animals, plants, or fungi, and their QS inhibitory effects have been demonstrated by many studies. These inhibitors exhibit high diversity in their biochemical structure. Unfortunately, there is a lack of excessive information about the functions of molecular structures or chemical groups of the QSIs on QS-mediated pathways (LaSarre and Federle, 2013).

Bacteria

Some potential QSIs have been reported from the members of various phyla of bacteria such as *Actinobacteria, Firmicutes, Cyanobacteria, Bacteroidetes, and Proteobacteria.* It is not uncommon to isolate bacteria from other organisms and study their QSI activities. This approach seems plausible, considering the competitive environment. A number of bacteria show their QSI activities via enzymes as previously explained. Known AHL lactonase enzymes with QSI activity are MomL, *hqiA,* $AiiA_{AI96}$, *Sso*Pox, SsoPox-W263I, AiiM, AttM, AiiB, Ahl, AhlD, MLR6805, DlhR, Qsd, AidC, AhlS, Aii20J, QsdA, GKL, MCP, AidH, AiiO, QsdH, QlcA, BpiB01, BpiB04, BpiB05, and BpiB07. MacQ, penicillin V acylases, penicillin G acylase KcPGA, AiiD, PvdQ QuiP HacA, HacB, AibP, AhlM, AiiC, and Aac are acylases. LsrK and LsrG are inhibitors for AI-2 mediated QS. Hod and CarAB targets PQS and DSF pathways, respectively. CYP102A1 is an oxidoreductase enzyme (LaSarre and Federle, 2013; Grandclement et al., 2016).

Devaraj et al. (2017) reported the QSI potentials of 147 soil actinobacterial strains against swarming motility and pyocyanin production in *P. aeruginosa* PAO1, with positive results. They observed that three actinobacterial strains (*Micromonospora, Rhodococcus,* and *Streptomyces*) inhibited violacein production of *Chromobacterium violaceum* CV026, and also swarming and pyocyanin production of *P. aeruginosa* PAO1. Yaniv et al. (2017) tested the QSI potentials of library clones including 2500 bacterial artificial chromosomes (BAC) from the Red Sea planktonic microbiome against the indicator organism, *C. violaceum*. They found that

an active compound, 14-A5, can inhibit the QS pathways, and it can also reduce biofilm formation in *P. aeruginosa*.

Chang et al. (2017) screened the marine bacterial strains from the surface waters of the North Atlantic Ocean and evaluated for their anti-QS potentials. *Rhizobium* sp. NAO1 extracts were found to have AHL-based QS analogue molecules. They observed that *Rhizobium* sp. NAO1 has inhibitory effects on QS system and also biofilm formation on *C. violaceum* and *P. aeruginosa* PAO1, respectively. They also studied the virulence factors such as siderophores and elastase activity of *P. aeruginosa* PAO1, and certain inhibitions were noted. The researchers detected an increased susceptibility to aminoglycoside antibiotics when applied with secondary metabolite products of *Rhizobium* sp. NAO1 due to the inhibition of *P. aeruginosa* biofilm formation.

An ethyl acetate (EtOAc) extract of *Rheinheimera aquimaris* QSI02 was tested for QQ activity against *C. violaceum* CV026. They utilized bioassay-guided isolation protocol and detected an active diketopiperazine factor, cyclo (Trp-Ser) from *R. aquimaris*. The diketopiperazine factor was shown to suppress production of pyocyanin, elastase activity, and biofilm formation in *P. aeruginosa* PAO1 (Sun et al., 2016).

Muller et al. (2014) isolated and identified *Rhodococcus erythropolis* as a PQS-degrading bacterium from soil. They reported that *R. erythropolis* strain BG43 had a potential to degrade HHQ and PQS, which are QS molecules of *P. aeruginosa*, into anthranilic acid and also can transform 2-heptyl-4-hydroxyquinoline-*N*-oxide to PQS. Two sets of PQS-inducible genes, responsible for encoding enzymes in pathways of HHQ hydroxylation to PQS and the degradation of PQS to anthranilate, were identified on a plasmid pRLCBG43 of strain BG43—namely, aqdA1B1C1 and aqdA2B2C2. It is assumed that these genes play an important role in the expression of dioxygenases for PQS cleavage in *P. aeruginosa*. The AqdC proteins firstly identified enzymes that cleave PQS. The potential for inhibition of pyocyanin, rhamnolipid, and pyoverdine production was demonstrated by expression of PQS dioxygenase gene aqdC1 or aqdC2 in *P. aeruginosa* PAO1 (Muller et al., 2015).

Algae

Several active compounds such as phlorotannins regarding QS inhibition can be obtained from macroalgae and microalgae. Numerous marine-derived QSIs have been reported from various marine organisms. Moreover, some macroalgae are believed to defend themselves against surface-associated bacteria (Saurav et al., 2017).

Rajamani et al. (2008) evaluated the effect of lumichrome, a natural compound from the green algae *Chlamydomonas reinhardtii* CC-2137, on *P. aeruginosa*. The lumichrome is a derivative of the vitamin riboflavin. They detected an increase in the *luxCDABE* gene expression by riboflavin and lumichrome, indicating the specificity of these compounds to the LasR receptor, and they reported that activated LasR by lumichrome could bind to LasI promoter.

Also, riboflavin and lumichrome could bind to the same binding pocket of LasR, which was confirmed by docking methods.

Delisea pulchra, red Australian macroalgae, was shown to have antimicrobial effects due to its secondary metabolites. They produce brominated and chlorinated furanones, which are similar to AHL molecules in structure, and they may bind easily to the signal receptors (Shannon and Abu-Ghannam, 2016).

Animals

Several QSIs have been identified from animals, most of which are utilized in antibiofouling. Sesterterpene metabolites manoalide, manoalide monoacetate, and secomanoalide from *Luffariella variabilis* and ethyl acetate extract of *Hyalinella punctate* are known for their QSI effects against *P. aeruginosa* (Skindersoe et al., 2008; Pejin et al., 2016).

Costantino et al. (2017) isolated a γ-lactone called plakofuranolactone, which is responsible for LasI/R system from Indonesian marine sponge *Plakortis* cf. *Lita*. extracts. This compound was tested on *Escherichia coli* pSB401 and *C. violaceum* CV026 against short acyl chain signals for QQ potential, but no inhibitory effect was detected. On the other hand, they observed an inhibition in AHL-induced bioluminescence of C6-HSL detecting pSB401 and C12-HSL detecting pSB1075 monitor strains, and a decrease in protease activity of *P. aeruginosa* PAO1.

Skindersoe et al. (2008) screened 284 marine samples from the Great Barrier Reef for their QSI activities via two QSI selector systems (QSIS1 and QSIS2). The three C25 sesterterpene metabolites (manoalide, manoalide monoacetate, and secomanoalide) from *Luffariella variabilis* were demonstrated to have A QSI effect on *lasB::gfp* [ASV] fusion.

Hyalinella punctate, a freshwater bryozoan, was tested for its antibiofilm and anti-QS activities (Pejin et al., 2016). The ethyl acetate extracts of *H. punctata* had significant antibiofilm activity for *P. aeruginosa* PAO1. These extracts also were effective in twitching motility and in the inhibition of pyocyanin production by *P. aeruginosa* PAO1.

Quintana et al. (2015) evaluated QSI activity of 39 extracts belonging to 26 sponges, 7 soft corals, 5 algae, and 1 zooanthid. QS inhibition was found to be considerably effective in the crude extracts of *Eunicea laciniata*, *Svenzea tubulosa, Ircinia felix*, and *Neopetrosia carbonaria*. The researchers isolated furanosesterterpenes from the crude extract from the sponge *I. felix* with moderate anti-QS potential.

Mai et al. (2015) isolated the compounds isonaamine A, isonaamidine A, isonaamine D, leucettamine D, and di-isonaamidine A from the crude extracts of *Leucetta chagosensis*. Isonaamine D and isonaamidine A inhibited three QS pathways of *Vibrio harveyi*. Isonaamidine A had the greatest inhibitory effect on the AI-2 biosensor.

QQ potentials of 78 natural products from marine organisms (sponges, algae, fungi, tunicates, cyanobacteria, terrestrial plants) were evaluated by utilizing *C. violaceum* CV017. Demethoxy encecalin, midpacamide, tenuazonic acid, hymenialdisin, microcolins A and B, and kojic acid were found to be potent and abundant compounds for QS inhibition. Midpacamide and tenuazonic acid were found to be toxic to *E. coli* pSB401 and *E. coli* pSB1075. QS-dependent luminescence in *E. coli* pSB1075 (C12-HSL monitor) was reduced by demethoxy encecalin and hymenialdisin, whereas in *E. coli*, pSB401 was reduced by hymenialdisin, demethoxy encecalin, microcolins A and B, and kojic acid (Dobretsov et al., 2011).

Díaz et al. (2015) isolated five lipid compounds from soft coral *Eunicea* sp., and three terpenoids and sterols from *Eunicea fusca*. These compounds were tested against *Ochrobactrum pseudogringnonense, Alteromonas macleodii, V. harveyi, P. aeruginosa,* and *S. aureus*. They showed effectiveness depending on the bacteria. The batyl alcohol 1 and fuscoside E peracetate 6 were found to have potent antibiofilm effect with less toxicity.

Plants

Many natural QSIs can be provided from various plants some of which are vegetables and edible fruits. These plant-derived QSI molecules can act as agonists or antagonists. The opinion that plants can control the QS system has arisen from the knowledge that plants do not have any immune mechanism similar to humans. The idea is that they might fight against other QS pathogens via anti-QS compounds, most of which are secondary metabolites (Kalia, 2013). It is possible to extract these molecules from plant tissues (root, leaf, etc.), but the production quantities differ among plants due to their development phase. The chemical nature of these molecules is sometimes similar to the QS signaling molecules. To decide the safety of these molecules, the toxicity parameter must also be taken into consideration.

Secondary metabolites obtained from plants are of great importance due to their antimicrobial, antifungal, and antitumor properties (and more). The antimicrobial activity—exhibiting plant compounds are listed as phenolics, phenolic acids, quinones, saponins, FLs, tannins, coumarins, terpenoids, and alkaloids. Also, antibiofilm activities of plant compounds are provided by naringenin, oroidin, salicylic acid, ursolic acid, cinnamaldehyde, methyl eugenol, extracts of garlic, and edible fruits (Asfour, 2018).

Quercetin is a commonly known flavonoid with antioxidant and supplementary effect. Gopu et al. (2015) evaluated the anti-QS potential of a quercetin on *P. aeruginosa*, and they reported the inhibition of several virulence factors such as alginate, pyocyanin, protease, elastase and exopolysaccharide (EPS) production, biofilm formation, and motility.

Ursolic acid is a known, nontoxic compund with various pharmacological effects. Ren et al. (2005) created a library of 13,000 compounds and screened them for their potential to inhibit biofilm formation against *V. harveyi* and *P. aeruginosa* PAO1. They observed that ursolic acid (10 μg/mL) obtained from *Diospyros dendo* inhibited biofilm formation.

The possible inhibitory effect of methanolic leaf extracts of *Acer palmatum*, *Acer pseudosieboldianum*, and *Cercis chinensis* were tested on biofilm formation, swarming motility, pyocyanin production, and *Caenorhabditis elegans* killing activity of *P. aeruginosa* PAO1. These extracts were found to be successful in the inhibition of biofilm formation, swarming motility, and AI production (Niu et al., 2017).

Jakobsen et al. (2012a) isolated ajoene (4,5,9-trithiadodeca-1,6,11-triene 9-oxide), the sulfur-containing compound, from garlic extract. The researchers developed synthetic ajoene to evaluate QSI potential in vitro and in vivo. They indicated that this synthetic ajoene was successful in the inhibition of virulence genes involved in *P. aeruginosa*. Also, ajoene was reported to have synergistic effect with tobramycin in *P. aeruginosa* biofilms.

Packiavathy et al. (2014) investigated the anti-QS activity of curcumin from *Curcuma longa* against *Escherichia coli*, *P. aeruginosa* PAO1, *Proteus mirabilis*, and *Serratia marcescens*. The biofilm formation, exopolysaccharide (EPS) and alginate production, swimming and swarming motility were inhibited, and biofilm susceptibility enhancement to conventional antibiotics were detected.

The inhibitors of ENR, triclosan, and green tea epigallocatechin gallate (EGCG) were tested for QS inhibitory potentials by Yang et al. (2010). EGCG was reported to have higher binding affinity to ENR of *P. aeruginosa*, indicating its QQ effect. Moreover, this compound inhibited the swarming motility and biofilm formation of *P. aeruginosa*.

Vandeputte et al. (2011) evaluated the inhibitory effects of commercially available flavonoids (apigenin, luteolinflavonols, kaempferol, quercetin, myricetin, naringenin, naringin, eriodictyol, taxifolin, trans-benzylideneacetophenone) on *P. aeruginosa* PAO1 and *C. violaceum* CV026. Naringenin inhibited elastase activity, biofilm formation, *lasB, lasI, lasR, rhlA, rhlI, rhlR* gene expressions, production of *N*-(3-oxododecanoyl)-L-homoserine lactone (3-oxo-C12-HSL) and *N*-butanoyl-L-homoserine lactone (C4-HSL) in *P. aeruginosa* PAO1. Also, taxifolin was found to inhibit pyocyanin production and elastase activity *of P. aeruginosa* PAO1 in this study.

Phenylacetic acid is a compound known for its antifungal, antioxidant, and antiinflammatory properties. Musthafa et al. (2012b) evaluated the anti-QS potential of phenylacetic acid and observed an inhibitory effect on protease and elastase activities, EPS production, and swimming motility of PAO1.

Zingerone, from ginger root, has been shown to have inhibitory effects on swimming, swarming and twitching motility, biofilm formation, production of rhamnolipid, elastase, protease, and pyocyanin in *P. aeruginosa* by Kumar et al. (2015). Moreover, anti-QS potential of zingerone was also evaluated by molecular docking, and this compound was found to block QS signal receptors by binding to them (TraR, LasR, RhlR, and PqsR).

Fungi

Numerous secondary metabolites with QSI potentials are produced by fungi. The amount of this metabolite production can vary according to environmental conditions. Fungal QSIs are considerably important for drug and food industry. Patulin and penicillic acid are reported secondary metabolites with QSI potentials from *Penicillium radicicola* and *Penicillium coprobium* by Rasmussen et al. (2005). These mycotoxins were found to modulate QS related genes in *P. aeruginosa*, indicating their QSI activities (45% and 60% inhibition by patulin and penicillic acid, respectively). Also, they detected that patulin could increase the potential of tobramycin against biofilm forms. This compound was also found to be considerably effective in the clearance of *P. aeruginosa* in a mouse model of pulmonary infection when compared with a control group.

Agaricus blazei water extracts were also evaluated on *P. aeruginosa*. PAO1 QS-regulated virulence factors and biofilm formation. Sub-MIC concentrations of the extract (without inhibiting the growth of bacteria) exhibited reduction in virulence factors of *P. aeruginosa*, such as pyocyanin production, twitching, and swimming motility. Also, the biofilm formation was inhibited in a concentration-dependent manner at sub-MIC values (Soković et al., 2014).

Farnesol, a sesquiterpene alcohol, and tyrosol, a phenylethanoid, isolated from *Candida albicans* are reported to be QQ molecules and biofilm inhibitors. It is known that phenazine compounds are secreted by several bacteria such as *Pseudomonas* spp. These compounds play important roles, especially in bacterial virulence. For example, pyocyanin from *P. aeruginosa* is responsible from colonization in patients' lungs suffering from cystic fibrosis. Cugini et al. (2010) reported that farnesol increased the levels of PQS and C4HSL in *lasR* mutant strains. As a result, the high levels of PQS led to increase in the phenazine (pyocyanin) production from *P. aeruginosa* in the co-culture of *C. albicans* and *P. aeruginosa*. This increased PQS was reported to be dependent on *rhlR*, *rhlI*, and *pqsH*. Farnesol induced the *pqsH* transcription in *lasR* mutants by RhlR activation. Also, they observed that PqsR activity was inhibited by farnesol at low cell densities.

sRNAs

It may also be possible to achieve QS inhibition by targeting small RNAs (sRNAs), which are posttranscriptional regulators. PhrS was identified as an activator of PqsR synthesis by Sonnleitner et al. (2011). Jakobsen et al. (2017) demonstrated that previously known QSI ajoene may also act as an inhibitor against sRNAs RsmY and RsmZ in *P. aeruginosa*. sRNAs are presented as a new target for antivirulence approaches.

There's an abundance of QSIs reported from natural sources against *P. aeruginosa*, many of which are listed in Table 2.

Table 2 Natural QSI compounds against *P. aeruginosa*

QSI	Inhibition	References
[6]-Gingerol, [6]-shogaol and zingerone from *Zingiber officinale*	Biofilm formation, pyocyanin production	Kumar et al. (2015) and Kim et al. (2015)
Allicin and ajoene from *Allium sativum*	Virulence, LasI, LasR, sRNAs RsmY, and RsmZ	Jakobsen et al. (2012a)
Baicalein (flavonoid) Scutellaria baicalensis Georgi.	Biofilm formation	Zeng et al. (2008)
Baicalin hydrate, cinnamaldehyde and hamamelitannin	Increase biofilm susceptibility to treatment with antibiotics	Brackman et al. (2011)
Casbane diterpene (diterpenoid) *Croton nepetaefolius* Baill.	Biofilm formation	Carneiro et al. (2010)
Cassipourol, β-sitosterol and α-amyrin, terpenoids from *Platostoma rotundifolium*	Biofilm formation, EPS production, motility	Rasamiravaka et al. (2017)
Catechin	Pyocyanin production, elastase activity, biofilm production	Vandeputte et al. (2010)
Chrysophanol, nodakenetin, shikonin and emodin from traditional Chinese herbs	Biofilm formation	Ding et al. (2011)
Coumarate ester (phenolic compound) *Dalbergia trichocarpa*	Biofilm formation, pyocyanin, LasB elastase production, proteolytic activity, motility	Rasamiravaka et al. (2013)
Curcumin from *Curcuma longa* L.	Biofilm formation, EPS and alginate production, swimming and swarming motility, biofilm susceptibility enhancement to antibiotics	Packiavathy et al. (2014)
Delftia tsuruhatensis	Biofilm formation	Singh et al. (2017)
Dihydroxybergamottin and bergamottin	Biofilm formation	Girennavar et al. (2008)
Ellagic acid derivatives	Biofilm formation	Sarabhai et al. (2013)
Epigallocatechin gallate from green tea, triclosan (5-Chloro-2-(2,4-dichlorophenoxy)phenol)	Biofilm formation and swarming motility, show synergistic activity with ciprofloxacin	Yang et al. (2010)
Eriodictyol	Pyocyanin production, elastase activity	Vandeputte et al. (2011)
Eugenol from *Syzygium aromaticum*	Biofilm formation	Zhou et al. (2013)
Evernic acid	LasB, RhlA, biofilm formation	Gokalsin and Sesal (2016)
Farnesol	Pyocyanin production, PQS	Cugini et al. (2010)
Iberin	RhlA, LasB	Jakobsen et al. (2012b)
Malabaricone C from *Myristica cinnamomea*	Pyocyanin production, elastase activity, biofilm formation	Chong et al. (2011)
Manolide, manolide monoacetate and secomanoalide from marine sponge *Luffariella variabilis*	Biofilm formation	Skindersoe et al. (2008)
Methyl eugenol from *Cuminum cyminum*	Biofilm formation, motility	Packiavathy et al. (2012)
Naringenin (flavonoid) commercial	Biofilm formation, elastase activity, *lasB, lasI, lasR, rhlA, rhlI, rhlR* gene expression, AHL production	Vandeputte et al. (2011)

Table 2 Natural QSI compounds against *P. aeruginosa*—Cont'd

QSI	Inhibition	References
p-Coumaroyl-hydroxy-ursolic acid (coumarate ester of triterpen) *Diospyros dendo* Welw.	Biofilm formation	Hu et al. (2006)
Patulin (furopyranone) and penicillic acid (furanone) from *Penicillium* species	LasR, RhlR	Rasmussen et al. (2005)
Phenylacetic acid	Pyocyanin production, elastase activity, biofilm production, swimming motility	Musthafa et al. (2012b)
Riboflavin and its derivative lumichrome	LasR	Rajamani et al. (2008)
Rosmarinic acid	Biofilm formation	Walker et al. (2004)
Rosmarinic acid, naringin, chlorogenic acid, morin and mangiferin	LasR, RhlR, biofilm formation, protease, elastase and haemolysin production	Annapoorani et al. (2012)
Salicylic acid	AHL production, twitching and swimming motility, protease activity, LasR, RhI, PQS activity, rhamnolipid production pyoverdine production, biofilm formation	Yang et al. (2009) and Bandara et al. (2006)
Salicylic acid, tannic acid and trans-cinnamaldehyde	Swarming activity and pyocyanin production	Chang et al. (2014)
Saponins, ginsenosides, and polysaccharides from Panax ginseng	AHL synthesis, LasA, LasB	Song et al. (2010)
Sesquiterpenoid viridiflorol and triterpenoids, ursolic and betulinic acids, from the liverwort *Lepidozia chordulifera*	Biofilm formation and elastolytic activity	Gilabert et al. (2015)
Solenopsin A (alkaloid) *Solenopsis invicta* (insect; ant)	Biofilm formation	Park et al. (2008)
Taxifolin	Pyocyanin production, elastase activity	Vandeputte et al. (2011)
Ursolic acid (triterpenoid) *Diospyros dendo* Welw.	Biofilm formation	Ren et al. (2005)
Zeaxanthin (carotenoid)	LasB, RhlA, biofilm formation	Gokalsin et al. (2017)

2.3.4 Bacteriophage

Bacteria are lysed through phage infections and therefore developed various defense mechanisms against these viruses such as clustered regularly interspaced short palindromic repeats (CRISPRs). Although information on CRISPR-Cas regulation is limited, some studies suggest that QS systems play an active role (Patterson et al., 2016; Hoyland-Kroghsbo et al., 2017).

Hoyland-Kroghsbo et al. (2017) reported that QS modulators can activate or suppress the CRISPR-Cas system in *P. aeruginosa*. Accordingly, they refer to the possibility of QSI-phage combined multitherapies. Qin et al. (2017) also report that QS should be involved in *P. aeruginosa* defense mechanism against phage K5 infections. On the contrary, temperate bacteriophage D3112 and JBD30 were determined to prefer QS capable *P. aeruginosa* instead of QS deficient strains in a competitive population in *Galleria mellonella*. Nevertheless, Mumford and Friman (2017) revealed that lytic PT7 phage causes a decrease in the population of LasR deficient *P. aeruginosa*, whereas an increase in PAO1 strain, in the presence of competitors *Staphylococcus aureus* and *Stenotrophomonas maltophilia*.

2.4 Synthetic Quorum Sensing Modulators

Although most QSIs are obtained from natural products, another approach is the development of synthetic QSIs. Such compounds are sometimes derived from natural ligands. In addition, there are structural mimics of HSLs and structurally unrelated compounds (Table 3).

AHL molecules have a head and a tail part in their structure: a HSL moiety with a tail of *N*-acyl residue. Usually a synthesized AHL mimic has one part intact and the other part derived, hoping to create a more robust molecule with antagonistic effect. In their study, Biswas et al. (2017) synthesized and tested several AHL mimics acetoxy-glucosamides, hydroxy-glucosamides, and 3-oxo-glucosamides against the *P. aeruginosa* MH602 strain. They showed that the strongest QSI compound 9b, a hydroxy-glucosamide, can inhibit the *P. aeruginosa las* system by 79.1%. Docking studies also revealed the binding poses of these compounds. Morkunas et al. (2012) have synthesized a collection of abiotic OdDHL mimics and have shown that some are capable of inhibiting QS and pyocyanin production. Hodgkinson et al. (2012) also synthesized and evaluated OdDHL mimics and found a number of new compounds that can modulate the *las* system of *P. aeruginosa*.

AHL analogues are mostly studied for their ability to bind with the receptors active site, but prevent the detection of QS signals. The idea is to synthesize compounds that act as antagonists. However, some modifications might end up with causing agonistic effects. For example, meta-bromo-thiolactone (mBTL) acts as an agonist in the absence of AHLs but antagonizes both LasR and RhlR in *P. aeruginosa* when in the presence of natural AIs (O'loughlin et al., 2013).

PQS signaling has been demonstrated to be responsible for virulence factor production and biofilm maturation in *P. aeruginosa*. The precursor HHQ, which is produced from anthranilate and a β-keto-fatty acid, is later converted into PQS (LaSarre and Federle, 2013). Depending on ligand-based or fragment-based approaches, agonists/antagonists are employed for their QSI properties by researchers. Targeting anthranilate is presented as an efficient approach for inhibition of PQS. The methyl anthranilate and halogenated anthranilate analogues were demonstrated to inhibit PQS biosynthesis (Calfee et al., 2001). Another alternative

Table 3 Synthetic QSI compounds, including nanoparticles

Compound	Inhibition	References
(*z*)-5-Octylidenethiazolidine-2, 4-dione (TZD-C8)	LasI	Lidor et al. (2015)
2,5-Piperazinedione	LasR	Musthafa et al. (2012a)
2-Amino-oxadiazoles	PqsR	Zender et al. (2013)
2-Nitrophenyl derivatives	PqsD	Storz et al. (2013)
3-Nitro phenylacetanoyl HL (C14)	LasR	Geske et al. (2008a)
4-I N-phenylacetyl-L-homoserine lactones (PHL)	LasR	Geske et al. (2007)
4-Nitro-pyridine-*N*-oxide (NPO)	Virulence	Rasmussen et al. (2005)
5-Aryl-ureidothiophene-2-carboxylic acids	PqsD	Sahner et al. (2013)
Benzamidobenzoic acids	PqsD	Hinsberger et al. (2014) and Weidel et al. (2013)
Chloro-pyridine pharmacophore	LasR	Marsden et al. (2010)
Compound 1	PqsR	Lu et al. (2014)
Furanone C-30	Virulence factors	Hentzer et al. (2003)
Furanone F2, F3 and F4	QscR	Liu et al. (2010)
Fusaric acid analogues	*las*, *rhl* systems	Tung et al. (2017)
Hydroxy-glucosamides	*las*, *rhl* systems	Biswas et al. (2017)
Long-chain 4-aminoquinolines	PQS, biofilm formation	Aleksić et al. (2017)
Meta-bromo-thiolactone	LasR and RhlR	O'loughlin et al. (2013)
Mycofabricated silver nanoparticles	Biofilm formation, LasA protease, LasB elastase, pyocyanin, pyoverdin, pyochelin, rhamnolipid, and alginate	Singh et al. (2015)
N-Decanoyl cyclopentylamide	LasR and RhlR	Ishida et al. (2007)
OdDHL-mimics	LasR, pyocyanin production, biofilm formation	Morkunas et al. (2012)
OdDHL-mimics which incorporated an (hetero)aromatic head group	LasR	Hodgkinson et al. (2012)
Phenylpropionyl-homoserine lactones	LasR	Geske et al. (2008b)
Quinazolinone (QZN)	PqsR	Ilangovan et al. (2013)
Selenium nanoparticles with honey phytochemicals	LasR, biofilm formation, elastase	Prateeksha et al. (2017)
Silver nanoparticles	Biofilm formation	Barapatre et al. (2016)
Silver nanoparticles impregnated in dressing	Biofilm formation	Velazquez-Velazquez et al. (2015)
ZnO nanoparticles	Biofilm formation, elastase, pyocyanin	Garcia-Lara et al. (2015)

approach is to target the PqsD, a key enzyme in the biosynthesis of HHQ and PQS. Recently, *S*-phenyl-L-cysteine sulfoxide was reported to inhibit kynureninase, an enzyme that catalyzes the cleavage of kynurenine into anthranilic acid and 3-hydroxyanthranilic acid (Kasper et al., 2016).

There are also synthetic modulators that are unrelated to AHLs. For example, Lidor et al. (2015) have synthesized and studied thiazolidinedione type molecules, and observed that the compound named (*z*)-5-octylidenethiazolidine-2, 4-dione (TZD-C8) has strong motility and biofilm inhibition properties. Furthermore, they explored the QSI properties and in silico structural affinity, finding out that TZD-C8 has significant inhibition potential for the *las* system.

Tung et al. (2017) have synthesized 40 novel fusaric acid analogues via microwave assisted synthesis and investigated their QSI potentials against *las* and *rhl* Qs systems of *P. aeruginosa*. They found that one of the analogues is capable of inhibiting the *las* system and related virulence factors.

Moreover, several studies investigated the QSI potentials of nanoparticles with promising results. Singh et al. (2015) evaluated the QS inhibition properties of mycofabricated silver nanoparticles (Ag-NPs) employing metabolites of *Rhizopus arrhizus* BRS-07 against *P. aeruginosa*. It was shown that these Ag-NPs can inhibit QS-regulated virulence factors, including LasA protease, LasB elastrase, pyocyanin, pyoverdin, pyochelin, rhamnolipid, and alginate. Barapatre et al. (2016) biosynthesized Ag-NPs via lignin-degrading fungi *Aspergillus flavus* and *Emericella nidulans*. They observed antimicrobial effects as well as strong antibiofilm effects. On the other hand, Velazquez-Velazquez et al. (2015) have impregnated Ag-NPs in dressings and tested them against *P. aeruginosa* biofilms. They suggest that Ag-NP impregnated dressings can reduce or prevent bacterial growth in wound environments.

In 2017, Prateeksha et al. studied selenium nanoparticles (Se-NPs) with honey phytochemicals against *P. aeruginosa* biofilm and QS. They utilize Se-NPs as vectors for a drug delivery system. They have shown that the nano-scaffold demonstrated a greater QS and biofilm inhibition in both in vitro and in vivo, compared to its counterparts. Molecular dockings suggested that nano-scaffold assists honey phytochemicals in binding to the LasR receptor cavity.

As expected, the number of candidate compounds can be too much to handle easily, and they can be modified in various ways. Since there are many options on how these modifications can be made, many novel synthetic QSIs are based on structural scaffolds that are determined by high-throughput and virtual screens of small molecules. Another idea to modulate QS is to inhibit AHL synthase enzymes by deriving synthetic analogues of their intermediates as previously described. SAM is an intriguing intermediate, considering it is a precursor for both AI1 and AI2 (Kalia, 2013).

3 Conclusion and Opinion

There is a growing concern that antibiotics will soon be ineffective against common infections in the near future due to the increase in bacterial antibiotic resistance. Therefore, the subsequent response is antivirulence strategy with the leading application of QQ. A multitude of QSIs against *P. aeruginosa* have been discovered to date with natural compounds in abundance, as

presented in this chapter. Without a doubt, there are many more natural compounds, and their synthetic derivatives are waiting to be investigated for their QSI potentials. Modern technology allows us to perform screens swiftly by employing carefully developed monitor strains. In silico methods like building compound libraries for virtual screenings by computer-aided simulations also pick up speed. These methods save time, costs, and labor in laboratories; therefore it is essential to analyze and deal with their limitations.

Compared with planktonic bacteria, biofilm forms have a complex structure. Therefore, it should be assumed that QS signal production and concentration is not homogenous. The diffusion of signal molecules would also vary, causing some bacteria to detect more AHL molecules than others. Moreover, flat or mushroom structures of biofilms must have different signal gradients. All these factors and more should be investigated for a better understanding of the system flow and selecting the inhibitors accordingly.

Many studies nowadays focus on multitherapies with QQ approach. Multitherapies can be performed in combination with two or more QSIs, enzymes, antibodies, or antibiotics. QSIs are known to increase bacterial susceptibility to antibiotics and phage infections. Moreover, infection models and applications against multispecies and strains keep this approach more realistic. Certainly there are some disadvantages and unknown parameters regarding QQ therapies, and it even might be possible for bacteria to gain resistance against QSIs. Further research will confidently allow us to utilize QSIs effectively and sort out the drawbacks.

Glossary

Acyl-homoserine lactone A small and diffusible signaling molecule involved in quorum sensing mechanism.

Antibiotic-resistance The ability of microorganisms to resist the effects of antibiotics that could successfully treat them before.

Antivirulence therapy Alternative treatment approach to inhibit bacterial virulence factors without any extermination of pathogens.

Biofilm A *layer* of prokaryotic microorganisms that attach to surfaces and secrete exopolysaccharides.

Cystic fibrosis A disease that is encountered frequently and affects approximately 70,000 people in the world, known to degrade lung functions by affecting respiratory system.

Multidrug resistance Antimicrobial resistance exhibited by microorganisms against multiple antimicrobial drugs.

Quorum quenching Alternative strategy to disrupt bacterial communication without killing bacteria or preventing their growth.

Quorum sensing inhibitors Natural or synthetic compounds inhibiting a quorum sensing mechanism.

Quorum sensing Bacterial communication system controlling numerous behaviors, including bioluminescence, swarming, conjugation, protease activity, and biofilm formation, and so on, via small and diffusible chemical signaling molecules called autoinducers.

Virtual screening A computer-aided technique that is used in drug discovery to search chemical libraries, including compounds having particular properties.

Abbreviations

3OC6HSL	*N*-3-(oxohexanoyl)-homoserine lactone
3-oxo-C12-HSL, OdDHL	*N*-3-oxododecanoyl-homoserine lactone
Ag-NP	Silver Nanoparticles
AHL	*N*-acylated L-homoserine lactone
AI	autoinducer
AI-2	autoinducer-2
AiiA	autoinducer inactivation gene
AIP	autoinducing Peptide
AQ	2-alkyl4-quionolones
BAC	bacterial artificial chromosomes
C4-HSL, BHL	*N*-butanoyl-L-homoserine lactone
CDC	The Centers for Disease Control and Prevention
CF	cystic fibrosis
CRISPR	clustered regularly interspaced short palindromic repeat
DPD	4,5-dihydroxy 2,3 pentanedione
EGCG	epigallocatechin gallate
ENR	enoyl-acyl-carrier-protein (ACP) reductase
EPS	exopolysaccharide
FabI	NADH-dependent ENR
HHQ	2-heptyl-4-hydroxyquinolone
Hod	2,4-dioxygenase
hPON1	human serum paraoxonase 1
mBTL	meta-bromo-thiolactone
MDR	multidrug resistant
PON1	paraoxonase 1
PON2	paraoxonase 2
PON3	paraoxonase 3
PQS	pseudomonas quinolone signal
PVA	penicillin V acylase
QQ	quorum quenching
QS	quorum sensing
QSI	QS inhibitor

QSIS	QSI selector system
SAM	*S*-adenoysl methionine
Se-NP	selenium nanoparticles
sRNA	small RNA
***Sso*Pox**	hyperthermostable lactonase
TZD-C8	(*z*)-5-octylidenethiazolidine-2, 4-dione
VAI	AI of *V. fischeri*

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