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# Natural Compounds Inhibiting *Pseudomonas aeruginosa* Biofilm Formation by Targeting Quorum Sensing Circuitry

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## Abstract

The biofilm lifestyle mode certainly represents one of the most successful behaviors to facilitate bacterial survival in diverse inhospitable environments. Conversely, the ability of bacteria to develop effective biofilms represents one of the major obstacles in the fight against bacterial infections. In *Pseudomonas aeruginosa*, the biofilm formation is intimately connected to the quorum sensing (QS) mechanisms, a mode of cell-to-cell communication that allows many bacteria to detect their population density in order to coordinate common actions. In this chapter, we propose an overview (i) on *P. aeruginosa* QS mechanisms and their implication in biofilm formation, and (ii) on natural products that are known to interfere with these QS mechanisms, subsequently disrupting biofilm formation. The concluding remarks focus on perspectives of these compounds as possible antibiotherapy adjuvants.

**Keywords:** biofilm, *las*, natural products, PQS, *pseudomonas*, quorum sensing, *rhl*

## 1. Introduction

Bacterial infections are mainly related to the ability of bacteria to invade and disseminate through their hosts by using different types of motility, by releasing a myriad of virulence factors, by building structured biofilm which lead to host cell and tissue damage but also allow bacteria to evade the immune system and conventional antimicrobial agents [1]. For decades, antibiotics, although less effective in biofilm-growing bacteria [2], have represented our best weapon against bacterial diseases. However, the on-going emergence and worldwide spreading of resistant bacteria is considerably reducing the antibiotic pallet available for the treatment of bacterial infections [3]. This alarming situation forces researchers to consider other strategies to combat bacterial infections, notably the use of phages [4] or the use of alternative agents, such as essential oils [5], silver nanoparticles [6], bacteriocins [7], and antimicrobial peptides [8]. Some interesting strategies propose original compounds that disrupt biofilm formation without affecting the viability of invading bacteria; this strategy is expected (i) to reduce the bacterial aptitude to build protective barriers, but without exerting a selective pressure *per se* [4]; (ii) to allow

sufficient time for the immune defenses to effectively destroy invaders; and (iii) to minimize the use of effective antibiotics.

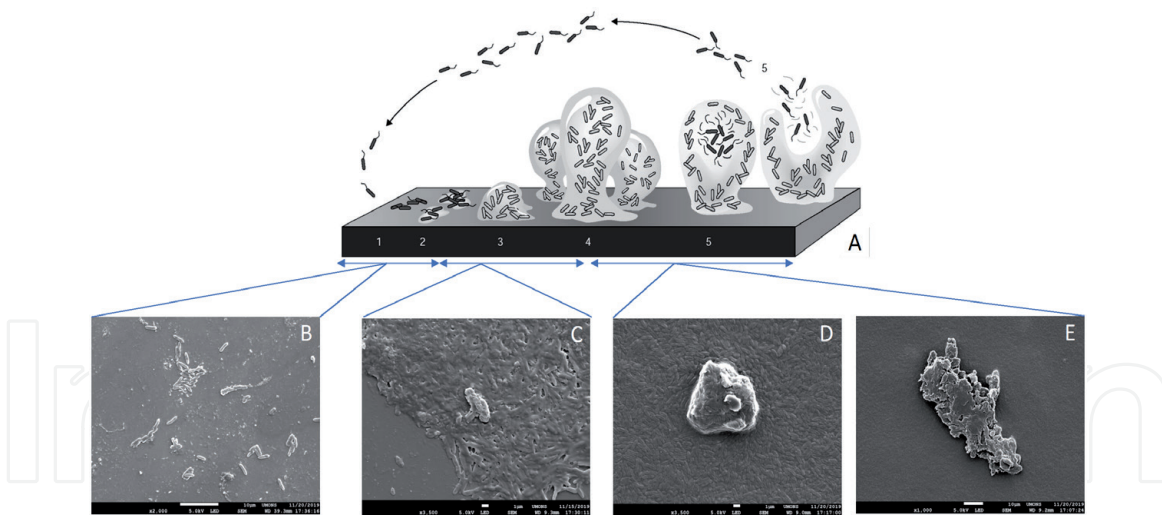
In most bacteria, the expressions of virulence factors are coordinated by quorum sensing (QS) mechanisms, a cell-to-cell communication which allows bacteria to detect their population density by producing and perceiving diffusible signal molecules to synchronize common actions [9]. This cell-to-cell communication has been largely investigated in *Pseudomonas aeruginosa*, an opportunistic pathogen which mainly affects people who are severely immunocompromised, in part due to its ability to evade from both innate and acquired immune defenses through adhesion, colonization, and biofilm forming and to produce various virulence factors that cause significant tissue damage [10, 11]. In this bacterium, QS regulates virulence factors production, motilities and, in particular, biofilm formation for which QS is one of the relevant key actors. Interestingly, within the two past decades, study papers reporting natural and synthetic compounds that interfere with QS and/or biofilm formation are regularly published; QS circuitry and biofilm formation control mechanisms indeed constitute promising targets to struggle against *P. aeruginosa* infection with potential huge clinical interests [12]. The present chapter covers the scope of natural compounds from both prokaryote and eukaryote organisms that have been identified to disrupt the biofilm lifestyle cycle in *P. aeruginosa* via modulation of QS mechanisms. An overview of the entanglement between QS circuitry and biofilm formation is reported as a prerequisite for a better understanding of the mechanisms of action proposed for some of the identified compounds. The concluding remarks focus on the perspectives of these compounds as possible antibiotherapy adjuvants for possible eradication of resistant infections caused by *P. aeruginosa*.

## 2. *P. aeruginosa* biofilm lifestyle

Like most bacteria, *P. aeruginosa* can develop two distinct lifestyles, planktonic and sessile cells. The planktonic state is encountered when *P. aeruginosa* evolves freely in a liquid suspension, whereas on natural or synthetic surfaces, *P. aeruginosa* can form sticky clusters in permanent rearrangements characterized by the secretion of an adhesive and protective matrix [13]. Defined as “biofilm,” this set of bacterial community adherent to a surface appears as an adaptive response to an environment more or less unsuited to growth in planktonic form [14].

The biofilm formation can be delimited in five main stages (**Figure 1**, image A). A first reversible phase corresponds to the initial adhesion of bacteria to surfaces; this adhesion becomes irreversible in the second stage (image B). Then, thanks to a proliferation period corresponding to the third stage, microcolonies are built concomitantly with the production of extracellular matrix (image C), leading to the fourth stage of biofilm structuration and organization in which the growth of three dimensional communities is observed with amplified extracellular matrix production (image D). This biofilm cycle is completed by a dispersion step (image E) [12].

The secreted extracellular matrix mainly consists of proteins, nucleic acids, lipids, and exopolysaccharides (EPS). These account for 50–90% of total organic matter [16]. *P. aeruginosa* produces at least three types of EPS that are required for biofilm formation and architecture [17]. (i) Alginate a linear polysaccharide composed of L-guluronic and D-mannuronic acids linked by  $\beta$ -1,4 bonds [18], (ii) Pel polysaccharide, a glucose-rich matrix material, with unclarified composition, and (iii) Psl polysaccharide, a repeating pentasaccharide consisting of D-mannose, L-rhamnose, and D-glucose. In mucoid strains, EPS are predominantly characterized by the presence of alginate. The alginate participates in the structuring of the



**Figure 1.**

Sketch of the different steps of a biofilm development (A) [15]. Several representative scanning electron microscopy (SEM-JEOL JSM-7200F) images of the *P. aeruginosa* biofilm at different steps of development and with different magnifications (B = reversible and irreversible stages at 8 h growth, C = microcolonies stage at 30 h growth, D = mature biofilm stage at 120 h growth, and E = dispersion stage at 144 h growth). *P. aeruginosa* PAO1 colonies were grown at 37°C with Centers for Disease Control and Prevention (CDC) biofilm reactor (biosurface technologies, MT) on tryptone soy broth (TSB).

biofilm [19], but its real importance is still controversial since some authors claim that it is not essential; indeed architecture and antibiotic resistance profiles of wild-type and alginate-deficient biofilms are identical [20, 21]. Nevertheless, the overexpression of alginate was shown to protect *P. aeruginosa* from phagocytosis and host responses [22]. In “nonmucoid” *P. aeruginosa* strains, such as the PAO1 strain isolated from an infected wound [23], alginate is even considered poorly produced at the expense of exopolysaccharides rich in glucose and mannose [24], Pel and Psl, which have been described as being more important in the formation and maintenance of the biofilm [25].

Extracellular DNA (eDNA) is an important component of *P. aeruginosa* biofilm matrix, which particularly intervenes in the establishment, maintenance, and perpetuation of structured biofilms [26]. Its importance has been demonstrated since *P. aeruginosa* biofilm formation is prevented by exposition to DNase I [27] and biofilms that are deficient in eDNA have been shown to be more sensitive to the detergent sodium dodecyl sulfate [28]. It has been established that eDNA plays roles in bacterial adhesion and in the structural stability of biofilms by maintaining coherent cell alignments [29]; interestingly, its contribution to antimicrobial resistance has also been proposed as eDNA, a highly anionic polymer, is believed to bind cationic antibiotics, such as aminoglycosides and antimicrobial peptides [30].

### 3. QS mechanisms and their implication in biofilm formation

The complex regulation of biofilm formation involves multiple bacterial machineries including the QS systems. In *P. aeruginosa*, this mechanism is involved in the development of various common bacterial behaviors, including virulence factors expression and biofilm formation, which are mostly implicated in infection success. Three QS systems have been clearly characterized: (i) the *las* system and the *rhl* system, two LuxI/R type systems using the signal molecules of the family of acyl-homoserine lactones (AHLs); and (ii) the PQS (pseudomonas quinolone signal) system based on molecules of the 2-alkyl-4-quinolone class [10, 31]. The mechanisms of QS in *P. aeruginosa* are summarized in **Figure 2** while the main



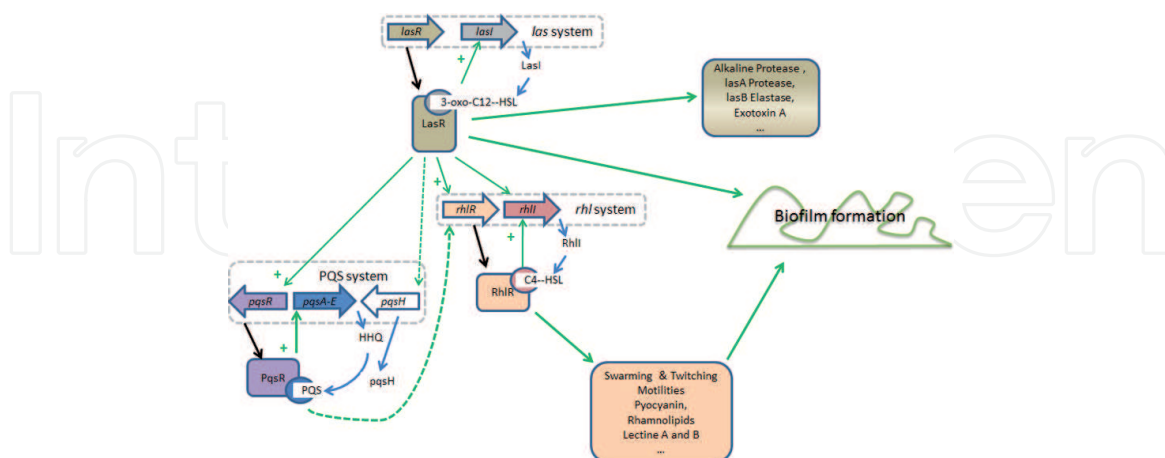
functions regulated by QS systems and involved in the pathogenesis of *P. aeruginosa* are presented in **Figure 3**.

Evidence that the *las* system is implicated in biofilm formation has been firstly established when Davies et al. [32] demonstrated that the biofilm formed by *lasI* mutant appears flat, undifferentiated, and quickly dispersed from the surface upon exposure to sodium dodecyl sulfate, compared to wild type biofilms.

Furthermore, Gilbert et al. [33] observed the binding of the QS regulator LasR to the promoter region of the *psl* operon, suggesting that the *psl* expression may be regulated by the QS. Considering that the *psl* operon is implied in biofilm modulation, the QS then plays a role in the biofilm formation and architecture. The transcription of the *pel* operon seems to be reduced in *rhlI* mutant, suggesting that the *rhl* system plays a biofilm formation role in *P. aeruginosa* by modulating the biosynthesis of the Pel polysaccharide [34]. The *pqsA* mutant produces a biofilm with less eDNA than the wild type biofilm, suggesting that the PQS system also plays a role in biofilm formation, more particularly in the eDNA releasing [34].

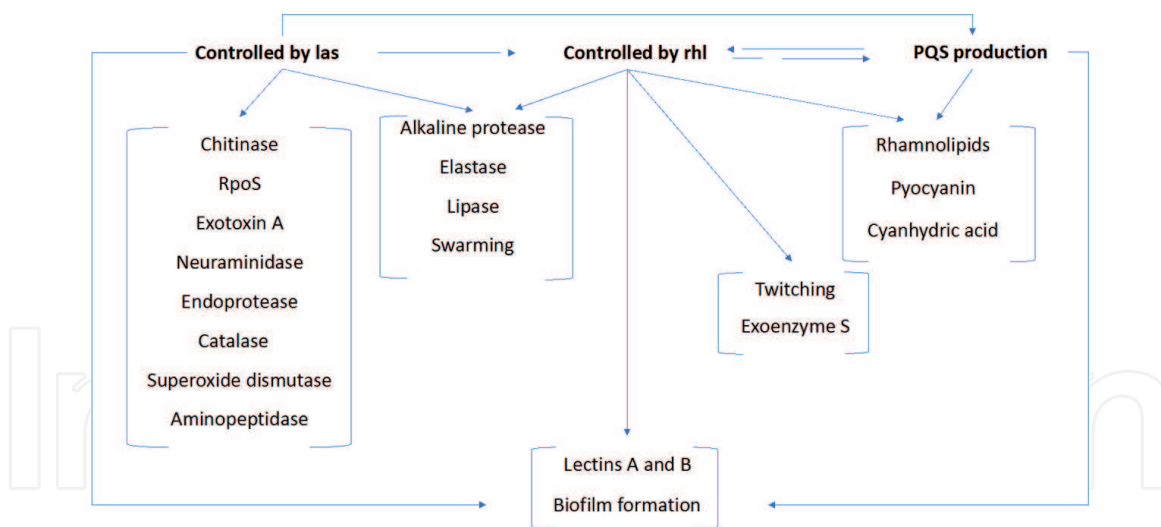
Notably, the production of rhamnolipids and lectins is under QS control, indicating a further indirect link between biofilm formation/degradation and QS.

Indeed, the *rhl* system controls the production of rhamnolipids [35], that play multiple roles in *P. aeruginosa* biofilm formation: (i) as biosurfactant and virulence factor [36]; (ii) in the formation of microcolonies [37]; (iii) in the maintenance of open channel structures necessary for nutrient circulation [38]; (iv) in the development of biofilm mushroom-shaped structures [37]; and (v) in cell dispersion from the biofilm [39]. Indeed, a hyper-detaching property has been observed in the *P. aeruginosa* mutants that produce more rhamnolipids compared to wild type strains [40]. Moreover, the *rhl* system also controls the expression of the cytotoxic virulence factors LecA and LecB. Data obtained on mutant strains indicate that these galactophilic lectins probably contribute to the biofilm development [41, 42]. Similarly, two types of *P. aeruginosa* motilities implicated in biofilm formation are also QS-regulated. The first movement, swarming motility, accomplishes an organized surface translocation, dependent on cell-to-cell



**Figure 2.**

*Systems involved in P. aeruginosa QS circuitry. The main QS systems in P. aeruginosa are the las, rhl, and PQS systems. The las system consists of a lasR regulatory gene coding for the LasR protein, a lasI gene coding for a LasI synthase involved in the synthesis of a signal molecule of the acyl-homoserine lactone (AHL) family, the 3-oxo-C12-HSL. The LasR/3-oxo-C12-HSL complex is a transcriptional activator of virulence genes (protease, elastase, and exotoxin) and lasI gene. According to the same model, the rhl system consists of rhlR, rhlI genes, and another AHL, the C4-HSL. This system activates genes in common with the las system and also specific genes, such as those coding for the synthesis of rhamnolipids, pyocyanin, and swarming/twitching motilities. The las system controls the rhl system. The third PQS system is interposed between the two main systems. The PqsABCDE operon produces the precursor 2-heptyl-4-quinolone (HHQ), and PqsH catalyzes conversion of HHQ to 2-heptyl-3-hydroxy-4-quinolone (PQS), detected by the receptor PqsR [10, 31].*



**Figure 3.**  
 Functions positively regulated by QS in *P. aeruginosa* [10, 31].

contacts and extensive flagellation [43]; this has been observed during the first stage of *P. aeruginosa* biofilm development and seems to be regulated by the *rhl* system [44]. Flat and uniform biofilms are formed when the strains grow under conditions promoting swarming motility, for example, a growth medium with glutamate or succinate as carbon sources; by contrast, a biofilm without confluent cell aggregates is formed by strains with limited swarming motility [45]. The second movement, a flagella-independent form of translocation, is described as a successive extension and retraction of polar type IV pili [46]. This kind of movement, regulated by the *rhl* system on a Fe-limited minimal medium [47], is necessary to assemble bacteria in monolayers that form microcolonies [38].

#### 4. Other mechanisms implied in biofilm formation

The QS systems are not the sole key actors intervening in biofilm formation by *P. aeruginosa*. Indeed, the complex regulation of biofilm formation involves multiple bacterial machineries that also include the membrane-bound sensor kinase GacS, the transcriptional response regulator GacA (GacS/GacA two-component regulatory system), and the intracellular second messenger bis-(3'-5')-cyclic dimeric guanosine monophosphate (c-di-GMP). Briefly, the GacS/GacA system acts as a super-regulator of the *las* and *rhl* systems [48], whereas c-di-GMP is important for the biosynthesis of alginate and Pel polysaccharides and for the switch from planktonic to biofilm lifestyle [49].

#### 5. Natural products that affect QS and biofilm formation by *Pseudomonas aeruginosa*

##### 5.1 From prokaryotes

###### 5.1.1 Enzymes

Microorganisms known to have the ability to produce anti-QS enzymes are still limited to a few bacteria from the families of (i) *Actinobacteria* (*Rhodococcus* and *Streptomyces*); (ii) *Firmicutes-Arthrobacter* (*Bacillus* and *Oceanobacillus*); (iii) *Cyanobacteria* (*Anabaena*); (iv) *Bacteroidetes* (*Tenacibaculum*); (v) *Proteobacteria*

(*Acinetobacter*, *Agrobacterium tumefaciens*, *Alteromonas*, *Comomonas*, *Halomonas*, *Hyphomonas*, *Klebsiella pneumoniae*, *P. aeruginosa*, *Ralstonia*, *Stappia*, and *Variovorax paradoxus*) [50–56].

Four types of enzymes are known to degrade AHLs [57, 58], a phenomenon sometimes described as “quorum quenching” (QQ) [59]; these include AHL-lactonases and decarboxylases that attack the lactone ring (*Bacillus indicus*, *B. pumilus*, and *B. sp.* SS4 cause significant inhibition of QS-dependent activities in Gram-negative bacteria such as *P. aeruginosa* PAO1, *Serratia marcescens*, and *Vibrio*), AHL-acylases that cleave the acyl side chain (*B. pumilus* S8-07 degrades 3-oxo-C12-HSL into the corresponding lauric acid [60]), and deaminases that separate the lactone ring from the acyl side chain. Recently, lactonases and acylases were identified in *Erythrobacter*, *Labrenzia*, and *Bacterioplanes* found in Red Sea sediments; these both degrade AHLs of different acyl chain lengths, particularly the 3-oxo-C12-HSL, and inhibit the formation *P. aeruginosa* PAO1 biofilm [59].

*Mycobacteroides abscessus* subspecies, emerging pathogens, are capable of degrading both PQS and HHQ. *M. abscessus* subsp. *abscessus*, in coculture with *P. aeruginosa* PAO1, reduced PQS levels through a PQS dioxygenase (encoded by the *aqdC* gene), *M. abscessus* subsp. *massiliense*, a recombinant strain overexpressing the *aqdC* gene, reduces the level of the virulence factors pyocyanin, pyoverdine, and rhamnolipids, suggesting that AqdC is a QQ enzyme [61]. Its impact on biofilm formation would have been interesting to investigate as another dioxygenase, the 2-alkyl-3-hydroxy-4(1H)-quinolone 2,4-dioxygenase (HodC), was described to cleave PQS, attenuate the production of virulence factors but conversely increase the viable biomass, in both newly formed and established biofilms, by increasing iron availability [62].

### 5.1.2 Organic acids

The acetic and phenyl lactic acids, found in the supernatant of probiotic strains *Lactobacillus paracasei* subsp. *paracasei* CMGB isolated from newborn feces, were shown to inhibit, at nonbacteriostatic/bactericide levels, the expression of QS genes in *P. aeruginosa*, preventing adherence of bacteria to an inert substratum [63, 64]. Similarly, the lactic acid produced by a potential probiotic *Pediococcus acidilactici* M7 strain, also isolated from newborn feces, inhibits the production of *P. aeruginosa* short-chain AHLs, elastase, protease, pyocyanin, and biofilm as well as the swarming-swimming-twitching motilities [65].

## 5.2 From fungi

### 5.2.1 Antibiotics and mycotoxins

Penicillin produced by *Penicillium* spp. has been shown to be effective in controlling a bacterial infection. Recently, about 33 *Penicillium* spp. have been recognized as producers of QS inhibitors such as the small lactone mycotoxins patulin and penicillic acid. The use of patulin can significantly reduce lung infection caused by *P. aeruginosa* on a mouse model. Interestingly, a synergy has been shown *in vitro* between patulin and tobramycin toward *P. aeruginosa* PAO1 biofilms, whereas patulin alone does not affect the development of biofilm [66]. Although the anti-infective property of patulin has been demonstrated, its genotoxicity and potential carcinogenic properties [67] probably preclude clinical applications.

Erythromycin, a macrolide antibiotic isolated from *Saccharopolyspora erythraea*, has been recently demonstrated to reduce virulence factors in *P. aeruginosa* PAO1, including various motilities, biofilm formation, and production of rhamnolipids, total protease, elastase, and pyocyanin at nonmicrobicidal level (1.6 µg/mL) [68]. Comparably,



the erythromycin derivate, azithromycin, shows a strong *P. aeruginosa* QS and biofilm inhibitory effect [69–71] with inhibition of alginate synthesis [69], a reduction of each type of bacteria movement [72] and a diminution of *gacA* gene expression [73]. At weak antibiotic concentration (2 µg/mL), a biofilm inhibition is observed, probably explained by a lower production of both AHL signal molecules, C4-HSL and 3-oxo-C12-HSL, and of virulence factors [74–76].

### 5.2.2 Alkylcyclopentanone

Recently, Kim et al. [77] indicated that the alkylcyclopentanone terrein, isolated from *Aspergillus terreus*, reduced virulence factors (elastase, pyocyanin, and rhamnolipids) and biofilm formation via antagonizing QS receptors without affecting *P. aeruginosa* cell growth. Beyond a negative impact on the production of QS signaling molecules and expression of QS-related genes, terrein also reduced c-di-GMP levels, an important secondary messenger for the switch from planktonic to biofilm lifestyle mode, by decreasing the activity of a diguanylate cyclase required for c-di-GMP biosynthesis [78].

## 5.3 From Plants

### 5.3.1 Derivatives of shikimic acid, phenols, and polyphenols

Many phenolic compounds and derivatives with anti-QS and antibiofilm activities have been isolated from plants [79]. Cinnamaldehyde [the dominant compound of certain essential oils, in particular *Cinnamomum camphora* (L.) J. Presl] and its derivatives modulate a wide range of anti-QS and antibiofilm activities of *P. aeruginosa* [80–82]. *Curcuma longa* L. produces curcumin, which inhibits the expression of virulence genes of *P. aeruginosa* PAO1 [83].

Ellagic acid derivatives from *Terminalia chebula* Retz. downregulate *lasIR* and *rhlIR* genes expression and decrease AHLs production, leading to an attenuation of virulence factor production and to an enhanced sensitivity of biofilm facing a tobramycin treatment [84].

Flavonoids have been investigated for their roles as QS modulating compounds. From these, naringenin and taxifolin reduced the expression of several QS-controlled genes (i.e., *lasI*, *lasR*, *rhlI*, *rhlR*, *lasA*, *lasB*, *phzA1*, and *rhlA*) in *P. aeruginosa* PAO1. Similarly, the flavan-3-ol catechin, extracted from the bark of *Combretum albiflorum* (Tul.) Jongkind, reduces the production of QS-dependent virulence factors, such as pyocyanin, elastase, and the formation of biofilm by *P. aeruginosa* PAO1 [85]. Interestingly, baicalin, an active natural compound extracted from the traditional Chinese medicinal *Scutellaria baicalensis*, has been demonstrated to inhibit the formation of *P. aeruginosa* biofilms and enhance the bactericidal effects of antibiotics such as amikacin. Moreover, at sub-minimal inhibitory concentration (256 µg/mL), this flavonoid has been shown to reduce LasA protease, LasB elastase, pyocyanin, rhamnolipids, and exotoxin A production and to downregulate the three QS-regulatory genes, including *lasI*, *lasR*, *rhlI*, *rhlR*, *pqsR*, and *pqsA* [86]. Consistently, *in vivo* experiments indicated that baicalin treatment reduces *P. aeruginosa* pathogenicity in *Caenorhabditis elegans* and enhances the clearance of *P. aeruginosa* from the peritoneal implants of infected mice.

Furocoumarins from grapefruit can inhibit the QS signaling (AHLs and AI-2) of *V. harveyi* BB886 and BB170 strains as well as biofilm formation in pathogens such as *E. coli* O157:H7, *Salmonella typhimurium* and *P. aeruginosa* [87]. These purified furocoumarins (dihydroxybergamottin and bergamottin), tested at the concentration of 1 µg/mL, cause 94% inhibition of autoinducers (AHLs) without affecting



bacterial viability. Biofilm inhibition was up to 58.3 and 72%, respectively, for *E. coli* O157:H7 but modest for *P. aeruginosa* (27.3 and 18.1%, respectively).

Malabaricone C, a diarylnonanoid isolated from the bark of *Myristica cinnamomea* King inhibited the QS-regulated pyocyanin production and biofilm formation in *P. aeruginosa* PAO1 [88].

A screening of various herbs revealed that a clove extract [*Syzygium aromaticum* (L.) Merr. Et Perry] inhibits QS-controlled gene expression (*las* and PQS systems) in *P. aeruginosa* with eugenol as major active constituent [89]. Recently, the effects of eugenol and its nanoemulsion on *P. aeruginosa* QS-mediated virulence factors and biofilm formation have been identified by Lou et al. [90] at a 0.2 mg/mL concentration. Similarly, the anthraquinone emodin from *Rheum palmatum* L., a traditional Chinese medicinal plant, was found to inhibit the *P. aeruginosa* biofilm formation at 20  $\mu$ M, increasing the antibiotic activity of ampicillin [91]. Finally, the 6-gingerol, isolated from fresh ginger oil, reduces the production of several virulence factors, decreasing the mortality induced in mice by *P. aeruginosa*. A DNA microarray analysis revealed that the application of the 6-gingerol on biofilm-encapsulated cells down-regulates several QS-related genes, notably those involved in the production of rhamnolipids, elastase, pyocyanin, all of which are involved in biofilm formation [92].

### 5.3.2 Alkaloids

Recently, caffeine (a purine alkaloid) has been shown to inhibit AHLs production and swarming mobility in *P. aeruginosa* PAO1 without causing AHLs degradation [93].

### 5.3.3 Terpenoids and Triterpenoids

The pentacyclic triterpenoid ursolic acid was identified as an inhibitor of biofilm formation from *Diospyros dendo* Welw, the tree used for ebony from Gabon, Africa [94]. Tested at a dose of 10  $\mu$ g/mL, ursolic acid reduces biofilm formation by 79% in *E. coli* and 57–95% in *V. harveyi* and *P. aeruginosa* PAO1. Similarly, oleanolic acid inhibits the *in vitro* biofilm formation by *S. aureus* and *P. aeruginosa* [95]. However, these triterpenoids showed no inhibitory effect on QS mechanisms contrarily to triterpenoid coumarate esters isolated from *Dalbergia trichocarpa*, a tropical legume from Madagascar. Indeed, oleanolic aldehyde coumarate at 200  $\mu$ M inhibits the formation/maintenance of *P. aeruginosa* PAO1 biofilm and the expression of the *las* and *rhl* QS systems as well as *gacA* gene [96]. Consequently, the production of QS-controlled virulence factors, including, rhamnolipids, pyocyanin, elastase, and extracellular polysaccharides, as well as twitching and swarming motilities is reduced. Other African plants harbor terpenoids and triterpenoids with antivirulence properties. Indeed, cassipourol and  $\beta$ -sitosterol (both at 100  $\mu$ M), isolated from *Platostoma rotundifolium* (Briq.) A. J. Paton, a Burundian medicinal plant, inhibit quorum sensing-regulated and -regulatory gene expression in *las* and *rhl* systems. These triterpenoids can still disrupt the formation of biofilms at concentrations down to 12.5 and 50  $\mu$ M [97].

### 5.3.4 Isothiocyanates and organosulfur compounds

Isothiocyanates produced by many plants are also QS inhibitors in *P. aeruginosa* PAO1. For example, iberin, isolated from horseradish (*Armoracia rusticana* G. Gaertn et al.), specifically blocks the expression of QS-regulated genes in *P. aeruginosa* PAO1 at the concentration of 100  $\mu$ M; its impact on biofilm formation has not been investigated [98]. Sulforaphane and erucin, two isothiocyanates isolated from

broccoli, inhibit the *P. aeruginosa* PAO1 *las* and *rhl* system as well as biofilm formation at concentrations of 50 and 100  $\mu$ M, respectively [99].

A further compound known to affect the QS-regulated genes in *P. aeruginosa*, including the rhamnolipids production, is ajoene, an allyl sulfide isolated from *Allium sativum* L. Ajoene, at the concentration of 100  $\mu$ g/mL and combined with the antibiotic tobramycin, leads to killing of biofilm-encapsulated *P. aeruginosa*. In a mouse model of pulmonary infection, this synergy improves the clearance of *P. aeruginosa* from lungs [100]. The S-phenyl-L-cysteine sulfoxide and its derivatives, notably diphenyl disulfide, have shown a significant impact on the biofilm formation by *P. aeruginosa* [101]; the sulfoxide derivative seems to interfere with both *las* and *rhl* systems whereas the diphenyl sulfide only disturbs the *las* system.

## 5.4 From marine organisms

### 5.4.1 Furanones

A series of studies have indicated that marine organisms are a potential source of anti-QS [102–104]. The halogenated furanones produced by the red alga *Delisea pulchra* inhibit QS-induced activities in bacteria by competing with AHL signals related to their receptor site (LuxR) [104]. This protein-ligand binding is destabilized, causing rapid receptor recycling [102]. Inspired from natural compounds, the halogenated furanones C-30 and C-56 have been demonstrated to exhibit biofilm reduction and target the *las* and *rhl* systems in *P. aeruginosa* [105].

### 5.4.2 Terpenoids

Following a screening of 284 extracts from the marine sponge *Luffariella variabilis*, 36 extracts were revealed as inhibitors of *P. aeruginosa* QS, targeting the *las* system [103]; from these, the sesterterpenoids manoalide displays anti-biofilm activities. Note that this molecule does not generate bactericidal effects on *P. aeruginosa* [103], but presents an antibiotic activity against Gram-positive bacteria [106].

## 5.5 From animals and human

### 5.5.1 Enzymes

Type I porcine kidney acylase inactivates QS signals such as C6-HSL and 3-oxo-C12-HSL but not C4-HSL [50]. This type I acylase moderately reduces biofilm formation in *Aeromonas hydrophila*, *P. putida*, and probably *P. aeruginosa* [107]. This degradation is dependent on the length of the acyl chain, since only C6-HSL and 3-oxo-C12-HSL are degraded [108].

Mammalian cells release enzymes called paraoxonases 1 (extracted from human and murine sera) that have lactonase activity; degrading *P. aeruginosa* AHLs. They prevent, in an indirect way, QS and biofilm formation [109]. Similarly, human epithelial cells and particularly human respiratory epithelia have the capacity to inactivate a *P. aeruginosa* QS signal by inactivating AHLs (3-oxo-C12HSL) produced by *P. aeruginosa* [108, 110]. However, the enzyme or enzyme-like compound involved in acyl-homoserine lactone inactivation have not been identified and characterized yet. Recently, Losa et al. [111] demonstrated that polarized airway epithelial monolayers, in contrast to nonpolarized cells, are also able to degrade 3-oxo-C12-HSL using membrane-associated paraoxonase 2 that catalyzes the opening of the lactone ring.

### 5.5.2 Alkaloids

The *P. aeruginosa* pyocyanin production is inhibited by a molecule found and isolated from the ant *Solenopsis invicta*, the piperidine alkaloid Solenopsin A alkaloid. The biofilm formation is also reduced in a dose-dependent manner. This molecule probably disrupts the signals from the *rhl* system [112].

## 6. Concluding remarks

This review presents natural compounds reported to exhibit anti-QS and anti-biofilm properties against *P. aeruginosa* (summarized in **Table 1**); these highlight the great potentiality of living organisms as reservoir of compounds susceptible to modulate virulence mechanisms without affecting bacterial viability. Overall, it appears that prokaryotes as well as animals and humans are sources for enzymes that degrade or antagonize AHLs, whereas plants harbor larger panels of anti-QS and antibiofilm compounds with very diverse chemical structures, including alkaloids, organosulfurs, phenolics, and terpenoids. Contrarily to animals and humans, plants are not able to deploy elaborate defense through humoral and cell-mediated immunity (antibodies and phagocytes) to struggle against bacterial invasions [113]. Plants immune defenses rely on the secretion of antibacterial compounds (bactericide and/or bacteriostatic agents [114]), including resistance modulating compounds [115] (e.g., inhibitors of efflux pumps [116]), and mostly on their ability to recognize molecules released from pathogens through plant cell surface receptors. This recognition triggers specific signaling cascades, activating series of defense responses, including the synthesis of antimicrobial lytic proteins, enzymes, phytoalexins, and other secondary metabolites. Some of these exert nonmicrobicidal antivirulence properties [117, 118]. Finally, marine organisms and fungi produce also bioactive secondary metabolites (halogenated furanones and antibiotics, respectively) and other original and promising compounds, such as terrein which was identified as the first dual inhibitor of QS and c-di-GMP signaling at 30  $\mu$ M.

The increasing presence of antibiotic-resistant bacteria certainly pushes scientists to reorient the strategy of fight against bacterial infections to defer entry into a post-antibiotic era where major antibiotics would not be effective even for banal infections. Antivirulence approaches and antivirulence drugs are being increasingly considered as potential therapeutic alternatives and/or adjuvants to currently failing antibiotics. For example, oleanolic aldehyde coumarate and cassipourol, anti-QS compounds, exert interesting antibiofilm properties, restoring the effectiveness of the antibiotic tobramycin in the clearance of biofilm-encapsulated *P. aeruginosa* (**Figure 4**); also the association between biofilm formation and antimicrobial resistance has been highlighted in carbapenem-resistant *P. aeruginosa* [119]. Such nonmicrobicidal drugs inhibit virulence factors essential for establishing infection and pathogenesis through targeting nonessential metabolic pathways which should not lead to activation of bacterial evasion mechanisms. This approach should reduce the selective pressure and consequently could slow down the development of resistance. Compounds that target QS may be particularly interesting as they impact planktonic and biofilm lifestyles, by reducing at the same time the production of virulence factors and the generation of biofilms. This should lead to less severe infections at levels that can be cleared by the host's immune defense and with increased activity of antibiotics.

Despite these important prospects, however, the big breakthrough in anti-bacterial strategies is still out of reach. This is probably due to a very complex

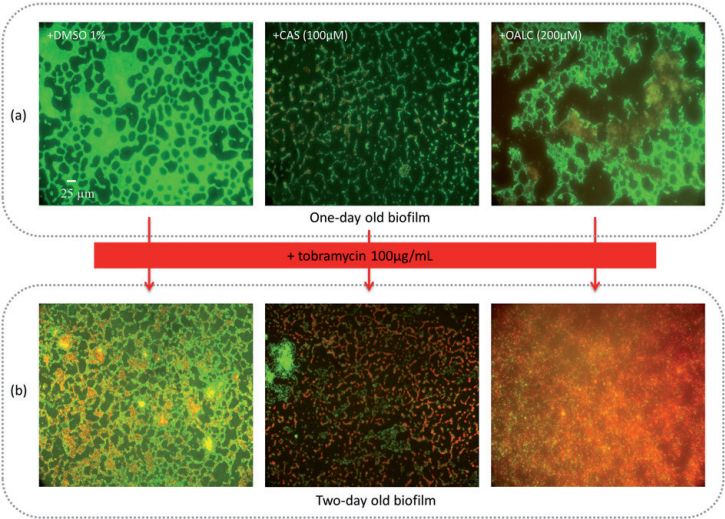
	Origin	Compounds (class)	Target (QS)	Synergy with antibiotics
Prokaryotes	<i>Bacillus indicus</i> , <i>B. pumilus</i> , <i>B. sp.</i> [60]; <i>Erythrobacter</i> , <i>Labrenzia</i> , <i>Bacterioplanes</i> [59]	AHL-acylase (Enzyme)	AHL degradation	NC
		AHL-lactonase (Enzyme)		NC
	<i>Lactobacillus paracasei</i> subsp. <i>Paracasei</i> [64]; <i>Pediococcus acidilactici</i> M7 [65]	Acetic acid, lactic acid, phenyl lactic acid	AHL antagonist	NC
Fungi	<i>Penicillium</i> species [66]	Penicillic acid (Furanone)	LasR and RhlR	NC
		Patulin (Furopyranone)	LasR and RhlR <sup>†</sup>	+ <sup>1</sup>
	<i>Saccharopolyspora erythraea</i> [68]	Erythromycin (Macrolide)	<i>rhl</i> system and <i>GacA</i>	NC
	<i>Aspergillus terreus</i> [77]	Terrein (alkylcyclopentanone)	LasR and RhlR antagonist; c-di-GMP	NC
marine organisms	<i>Delisea pulchra</i> [102, 104]	halogenated furanones and derivative	AHL antagonist	+ <sup>1</sup>
	<i>Luffariella variabilis</i> (Polejaeff, 1884) [103]	Manoalide (Sesterterpenoid)	<i>las</i> system	NC
Plants	<i>Platostoma rotundifolium</i> (Briq.) A, J, Paton [97]	Cassipourol (terpenoid), $\beta$ -sitosterol (terpenoid)	<i>las</i> and <i>rhl</i> systems	+ <sup>1</sup>
	<i>Combretum albiflorum</i> (Tul.) Jongkind [85]	Catechin (Flavonoid)	<i>las</i> and <i>rhl</i> systems	NC
	<i>Dalbergia trichocarpa</i> Baker. [96]	Oleanolic aldehyde Coumarate (Phenolic compound)	<i>las</i> and <i>rhl</i> systems	+ <sup>1</sup>
	<i>Allium sativum</i> L. [100]	Ajoene (Organosulfur)	<i>las</i> and <i>rhl</i> systems	+ <sup>1</sup>
	<i>Armoracia rusticana</i> G. Gaertn et al. [98]	Iberin (Isothiocyanate)	<i>las</i> and <i>rhl</i> systems	NC
	<i>Terminalia chebula</i> Retz. [84]	Ellagic acid derivatives (Phenolic compound)	<i>las</i> and <i>rhl</i> systems	+ <sup>1</sup>
	<i>Syzygium aromaticum</i> (L.) Merr. Et Perry [89, 90]	Eugenol (Phenylpropanoid)	<i>las</i> and PQS systems	NC
	<i>Curcuma longa</i> L. [83]	Curcumin (Phenolic compound)	AHLs inhibition	NC
	<i>Citrus paradisi</i> Macfad. (Rio Red and Marsh White grapefruits) [87]	Bergamottin and dihydroxybergamottin (Furocoumarins)	AHLs inhibition	NC
	<i>Rheum palmatum</i> L. [91]	Emodin (Anthraquinone)	docking traR <sup>*</sup>	+ <sup>2</sup>
	<i>Scutellaria baicalensis</i> Georgi. [86]	Baicalin (Flavonoid)	<i>las</i> , <i>rhl</i> and PQS systems	+ <sup>1</sup>
	<i>Zingiber officinale</i> Rosc. [92]	6-gingerol (Phenolic compound)	docking lasR	NC



	Origin	Compounds (class)	Target (QS)	Synergy with antibiotics
Animals and Human	Porcine kidney [50, 107]	Type I acylase	AHL degradation	NC
	Human and murine sera [109, 110]	Paraoxonases 1 Enzyme (lactonase)	AHL degradation	NC
	<i>Solenopsis invicta</i> (insect; ant) [112]	Solenopsin A (Alkaloid)	<i>rhl</i> system	NC

+, yes; NC, not communicated.  
<sup>†</sup>Patulin alone does not affect the development of biofilm.  
<sup>\*</sup>*LuxR*-type transcription factor of *Agrobacterium tumefaciens*.  
<sup>1</sup>Aminoglycosides.  
<sup>2</sup>Ampicillin.

**Table 1.**  
Natural compounds inhibiting *P. aeruginosa* QS and biofilm formation.



**Figure 4.**  
*P. aeruginosa* biofilm phenotypes and effectiveness of tobramycin treatment in presence of dimethyl sulfoxide (DMSO 1%) or, cassipourol (CAS: 100 μM) or oleanolic aldehyde coumarate (OALC: 200 μM). (a) After 1 day of incubation, *P. aeruginosa* fails to form structured confluent aggregate in presence of CAS or OALC as compared to DMSO treatment. (b) CAS and OALC considerably increase the susceptibility of *P. aeruginosa* to tobramycin (100 μg/mL), as shown by the increased proportion of dead cells compared with DMSO. Similar results are observed when tobramycin is added simultaneously with CAS or OALC to one-day old untreated biofilms. The bacterial viability was assessed by staining the cells with SYTO-9 (green areas zones—live living bacteria) and propidium iodide (red areas zones—dead bacteria) furnished in the LIVE/DEAD BacLight kit. Cells were visualized using a LeicaDMIRE2 inverted fluorescence microscope using equipped with a 40× objective lens and colored images were assembled using Adobe Photoshop.

entanglement between different QS systems, to the ability of *Pseudomonas* to compensate deficient systems and to the intervention of key actors involved in biofilm formation, outside of QS circuitry [12]. Millenia of coevolution between plants and bacteria have led to complex defense strategies, with plants producing cocktails of bioactive compounds with multiple targets [114] and/or compounds such as terrein that impact dual inhibitory targets. In the current state of research, much remains to be done in understanding these mechanisms and the real impact of such combinations before arriving at a commercial use. Nevertheless, following a combined approach for “adjuvant antibiotherapy” and “combined antibiotherapy” will undeniably lead to a renewed concept of “complex drugs for complex diseases,” a well-known presupposed in traditional medicines [120].

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## Conflict of interest

The authors declare that there is no conflict of interests regarding the publication of this paper.

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## References

- [1] Wu H-J, Wang AHJ, Jennings MP. Discovery of virulence factors of pathogenic bacteria. *Current Opinion in Chemical Biology*. 2008;**12**:93-101
- [2] Ciofu O, Tolker-Nielsen T. Tolerance and resistance of *Pseudomonas aeruginosa* biofilms to antimicrobial agents-how *P. aeruginosa* can escape antibiotics. *Frontiers in Microbiology*. 2019;**10**:913
- [3] Pashang R, Yusuf F, Zhao S, Deljoomanesh S, Gilbride KA. Widespread detection of antibiotic-resistant bacteria from natural aquatic environments in southern Ontario. *Canadian Journal of Microbiology*. 2018;**65**(4):322-331
- [4] Torres-Barceló C, Hochberg ME. Evolutionary rationale for Phages as complements of antibiotics. *Trends in Microbiology*. 2016;**24**(4):249-256. DOI: 10.1016/j.tim.2015.12.011
- [5] Valdivieso-Ugarte M, Gomez-Llorente C, Plaza-Díaz J, Gil Á. Antimicrobial, antioxidant, and Immunomodulatory properties of essential oils: A systematic review. *Nutrients*. 2019;**11**(11):2786. DOI: 10.3390/nu11112786
- [6] Rai M, Paralikar P, Jogee P, Agarkar G, Ingle AP, Derita M, et al. Synergistic antimicrobial potential of essential oils in combination with nanoparticles: Emerging trends and future perspectives. *International Journal of Pharmaceutics*. 2017;**519** (1-2):67-78. DOI: 10.1016/j.ijpharm.2017.01.013
- [7] Cotter PD, Ross RP, Hill C. Bacteriocins- a viable alternative to antibiotics? *Nature Reviews Microbiology*. 2013;**11**(2):95-105. DOI: 10.1038/nrmicro2937
- [8] Nuti R, Goud NS, Saraswati AP, Alvala R, Alvala M. Antimicrobial peptides: A Promising therapeutic strategy in tackling antimicrobial resistance. *Current Medicinal Chemistry*. 2017;**24**(38):4303-4314. DOI: 10.2174/0929867324666170815102441
- [9] Rutherford ST, Bassler BL. Bacterial quorum sensing: Its role in virulence and possibilities for its control. *Cold Spring Harb, Perspect, Med*. 2012;**2**:a012427
- [10] Turkina MV, Vikström E. Bacteria-host crosstalk: Sensing of the quorum in the context of *Pseudomonas aeruginosa* infections. *Journal of Innate Immunity*. 2019;**11**(3):263-279
- [11] Bricha S, Ounine K, Oulkheir S, Haloui N, Attarassi B. Virulence factors and epidemiology related to *Pseudomonas aeruginosa*. *Tunisian Journal of Infectious Diseases*. 2009;**2**:7-14
- [12] Rasamiravaka T, Labtani Q, Duez P, El Jaziri M. The formation of biofilms by *Pseudomonas aeruginosa*: A review of the natural and synthetic compounds interfering with control mechanisms. *BioMed Research International* 2015; 2015: a759348
- [13] Filloux A, Vallet I. [biofilm: Set-up and organization of a bacterial community] article in french. *Médecine Sciences*. 2003;**19**:77-83. DOI: 10.1051/medsci/200319177
- [14] Vu B, Chen M, Crawford RJ, Ivanova EP. Bacterial extracellular polysaccharides involved in biofilm formation. *Molecules*. 2009;**14**(7):2535-2554. DOI: 10.3390/molecules14072535
- [15] Sauer K. The genomics and proteomics of biofilm formation. *Genome Biology*. 2003;**4**:219. DOI: 10.1186/gb-2003-4-6-219

- [16] Häussler S, Parsek MR. Biofilms 2009: New perspectives at the heart of surface-associated microbial communities. *Journal of Bacteriology*. 2010;**192**(12):2941-2949. DOI: 10.1128/JB.00332-10
- [17] Ghafoor A, Hay ID, Rehm BHA. Role of exopolysaccharides in *Pseudomonas aeruginosa* biofilm formation and architecture. *Applied and Environmental Microbiology*. 2011;**77**(15):5238-5246
- [18] Evans LR, Production LA. Characterization of the slime polysaccharide of *Pseudomonas aeruginosa*. *Journal of Bacteriology*. 1973;**116**(2):915-924
- [19] Hentzer M, Wu H, Andersen JB. Attenuation of *Pseudomonas aeruginosa* virulence by quorum sensing inhibitors. *EMBO Journal*. 2003;**22**(15):3803-3815
- [20] Wozniak DJ, Wyckoff TJO, Starkey M, Keyser R, Azadi P, O'Toole GA, et al. Alginate is not a significant component of the extracellular polysaccharide matrix of PA14 and PAO1 *Pseudomonas aeruginosa* biofilms. *Proceedings of the National Academy of Sciences of the United States of America*. 2003;**100**(13):7907-7912
- [21] Stapper AP, Narasimhan G, Ohman DE, Barakat J, Hentzer M, Molin S, et al. Alginate production affects *Pseudomonas aeruginosa* biofilm development and architecture, but is not essential for biofilm formation. *Journal of Medical Microbiology*. 2004;**53**(7):679-690
- [22] Leid JG, Willson CJ, Shirtliff ME, Hassett DJ, Parsek MR, Jeffers AF. The exopolysaccharide alginate protects *Pseudomonas aeruginosa* biofilm Bacteria from IFN- $\gamma$ -mediated macrophage killing. *Journal of Immunology*. 2005;**175**(11):7512-7518
- [23] Friedman L, Kolter R. Genes involved in matrix formation in *Pseudomonas aeruginosa* PA14 biofilms. *Molecular Microbiology*. 2004;**51**(3):675-690
- [24] Holloway BW, Krishnapillai V, Morgan AF. Chromosomal genetics of *pseudomonas*. *Microbiological Reviews*. 1975;**43**(1):73-102
- [25] Yang L, Liu Y, Wu H. Combating biofilms. *FEMS Immunology & Medical Microbiology*. 2012;**65**(2):146-157
- [26] Okshevsky M, Meyer RL. The role of extracellular DNA in the establishment, maintenance and perpetuation of bacterial biofilms. *Critical Reviews in Microbiology*. 2015;**41**(3):341-352
- [27] Swartjes JJTM, Das T, Sharifi S. A functional DNase coating to prevent adhesion of bacteria and the formation of biofilm. *Advanced Functional Materials*. 2013;**23**(22):2843-2849
- [28] Yang L, Barken KB, Skindersoe ME, Christensen AB, Givskov M, Tolker-Nielsen T. Effects of iron on DNA release and biofilm development by *Pseudomonas aeruginosa*. *Microbiology*. 2007;**153**(5):1318-1328
- [29] Gloag ES, Turnbull L, Huang A. Self-organization of bacterial biofilms is facilitated by extracellular DNA. *Proceedings of the National Academy of Sciences of the United States of America*. 2013;**110**(28):11541-11546
- [30] Mulcahy H, Charron-Mazenod L, Lewenza S. Extracellular DNA chelates cations and induces antibiotic resistance in *Pseudomonas aeruginosa* biofilms. *PLoS Pathogens*. 2008;**4**(11):e1000213
- [31] Jimenez PN, Koch G, Thompson JA, Xavier KB, Cool RH, Quax WJ. The multiple signaling systems regulating



virulence in *Pseudomonas aeruginosa*. *Microbiology and Molecular Biology Reviews*. 2012;**76**(1):46-65

[32] Davies DG, Parsek MR, Pearson JP, Iglewski BH, Costerton JW, Greenberg EP. The involvement of cell-to-cell signals in the development of a bacterial biofilm. *Science*. 1998;**280**(5361):295-298

[33] Gilbert KB, Kim TH, Gupta R, Greenberg EP, Schuster M. Global position analysis of the *Pseudomonas aeruginosa* quorum-sensing transcription factor LasR. *Molecular Microbiology*. 2009;**73**(6):1072-1085

[34] Sakuragi Y, Kolter R. Quorum-sensing regulation of the biofilm matrix genes (*pel*) of *Pseudomonas aeruginosa*. *Journal of Bacteriology*. 2007;**189**(14):5383-5386

[35] Davey ME, Caiazza NC, O'Toole GA. Rhamnolipid surfactant production affects biofilm architecture in *Pseudomonas aeruginosa* PAO1. *Journal of Bacteriology*. 2003;**185**(3):1027-1036

[36] Pamp SJ, Tolker-Nielsen T. Multiple roles of biosurfactants in structural biofilm development by *Pseudomonas aeruginosa*. *Journal of Bacteriology*. 2007;**189**(6):2531-2539

[37] Dusane DH, Zinjarde SS, Venugopalan VP, Mclean RJC, Weber MM, Rahman PKSM. Quorum sensing: Implications on Rhamnolipid biosurfactant production. *Biotechnology and Genetic Engineering Reviews*. 2010;**27**:159-184

[38] O'Toole GA, Kolter R. Flagellar and twitching motility are necessary for *Pseudomonas aeruginosa* biofilm development. *Molecular Microbiology*. 1998;**30**(2):295-304

[39] Boles BR, Thoendel M, Singh PK. Rhamnolipids mediate detachment

of *Pseudomonas aeruginosa* from biofilms. *Molecular Microbiology*. 2005;**57**(5):1210-1223

[40] Schooling SR, Charaf UK, Allison DG, Gilbert P. A role for rhamnolipid in biofilm dispersion. *Biofilms*. 2004;**1**:91-99

[41] Diggle SP, Stacey RE, Dodd C, Camara M, Williams P, Winzer K. The galactophilic lectin, LecA, contributes to biofilm development in *Pseudomonas aeruginosa*. *Environmental Microbiology*. 2006;**8**(6):1095-1104

[42] Tielker D, Hacker S, Loris R. *Pseudomonas aeruginosa* lectin LecB is located in the outer membrane and is involved in biofilm formation. *Microbiology*. 2005;**151**(5):1313-1323

[43] Fraser GM, Hughes C. Swarming motility. *Current Opinion in Microbiology*. 1999;**2**(6):630-635

[44] Daniels R, Vanderleyden J, Michiels J. Quorum sensing and swarming migration in bacteria. *FEMS Microbiology Reviews*. 2004;**28**(3):261-289

[45] Shrout JD, Chopp DL, Just CL, Hentzer M, Givskov M, Parsek MR. The impact of quorum sensing and swarming motility on *Pseudomonas aeruginosa* biofilm formation is nutritionally conditional. *Molecular Microbiology*. 2006;**62**(5):1264-1277

[46] Mattick JS. Type IV pili and twitching motility. *Annual Review of Microbiology*. 2002;**56**:289-314

[47] Patriquin GM, Banin E, Gilmour C, Tuchman R, Greenberg EP, Poole K. Influence of quorum sensing and iron on twitching motility and biofilm formation in *Pseudomonas aeruginosa*. *Journal of Bacteriology*. 2008;**190**(2):662-671

- [48] Parkins MD, Ceri H, Storey DG. *Pseudomonas aeruginosa* GacA, a factor in multihost virulence, is also essential for biofilm formation. *Molecular Microbiology*. 2001;**40**(5):1215-1226
- [49] Merighi M, Lee VT, Hyodo M, Hayakawa J, Lory S. The second messenger bis-(3'-5')-cyclic-GMP and its PilZ domain-containing receptor Alg44 are required for alginate biosynthesis in *Pseudomonas aeruginosa*. *Molecular Microbiology*. 2007;**65**(4):876-895
- [50] Dong YH, Zhang LH. Quorum sensing and quorum-quenching enzymes. *The Journal of Microbiology*. 2005;**43**(Spec):101-109
- [51] Huma N, Pratap S, Jyoti K, Ashish B, Jayadev J, Tanmoy M, et al. Diversity and polymorphism in AHL-Lactonase gene (*aiiA*) of *Bacillus*. *Journal of Microbiology and Biotechnology*. 2011;**21**(10):1001-1011
- [52] Kalia VC, Purohit HJ. Quenching the quorum sensing system: Potential antibacterial drug targets. *Critical Reviews in Microbiology*. 2011;**37**(2):121-140
- [53] Kang Y, Durfee T, Glasner JD, Qiu Y, Frisch D, Winterberg KM, et al. Systematic mutagenesis of the *Escherichia coli* genome. *Journal of Bacteriology*. 2004;**186**:4921-4930
- [54] Park JK, Jung JY, Park YH. Cellulose production by *Gluconacetobacter hansenii* in a medium containing ethanol. *Biotechnology Letters*. 2003;**25**(24):2055-2059
- [55] Romero D, Aguilar C, Losick R, Kolter R. Amyloid fibers provide structural integrity to *Bacillus subtilis* biofilms. *Proceedings of the National Academy of Sciences of the United States of America*. 2010;**107**:2230-2234
- [56] Uroz S, Dessaux Y, Oger P. Quorum sensing and quorum quenching : The Yin and Yang of bacterial communication. *Chembiochem*. 2009;**10**(2):205-216
- [57] Musthafa KS, Ravi AV, Annapoorani A, Packiavathy ISV, Pandian SK. Evaluation of anti-quorum-sensing activity of edible plants and fruits through inhibition of the N-acyl-homoserine lactone system in *Chromobacterium violaceum* and *Pseudomonas aeruginosa*. *Chemotherapy*. 2010;**56**:333-339
- [58] Nithya C, Pandian SK. The in vitro antibiofilm activity of selected marine bacterial culture supernatants against *Vibrio* spp. *Archives of Microbiology*. 2010;**192**:10
- [59] Rehman ZU, Leiknes T. Quorum quenching bacteria isolated from Red Sea sediments reduces biofilm formation by *Pseudomonas aeruginosa*. *Frontiers in Microbiology*. 2018;**9**:1354
- [60] Nithya C, Begum MF, Pandian SK. Marine bacterial isolates inhibit biofilm formation and disrupt mature biofilms of *Pseudomonas aeruginosa* PAO1. *Applied Microbiology and Biotechnology*. 2010;**88**(1):341-358
- [61] Birmes FS, Säring R, Hauke MC, Ritzmann NH, Drees SL, Daniel J, et al. Interference with *Pseudomonas aeruginosa* quorum sensing and virulence by the mycobacterial *Pseudomonas* quinolone signal Dioxygenase Aqdc in combination with the N-Acylhomoserine lactone Lactonase QsdA. *Infection and Immunity*. 2018;**87**(10):e00278-e00219
- [62] Tettmann B, Niewerth C, Kirschhöfer F, Neidig A, Dötsch A, Brenner-Weiss G, et al. Enzyme-mediated quenching of the *Pseudomonas* quinolone signal (PQS) promotes biofilm formation of *Pseudomonas aeruginosa* by increasing iron

availability. *Frontiers in Microbiology*. 2016;**7**:1978

[63] Cotar A, Chifiriuc MC, Dinu S, Pelinescu D, Banu O. Quantitative real-time PCR study of the influence of probiotic culture soluble fraction on the expression of *Pseudomonas aeruginosa* quorum sensing genes. *Roumanian Archives of Microbiology and Immunology*. 2010;**69**:213-223

[64] Chifiriuc MC, Ditu ML, Banu O, Bleotu C, Dracea O. Subinhibitory concentrations of phenyl lactic acid interfere with the expression of virulence factors in *Staphylococcus aureus* and *Pseudomonas aeruginosa* clinical strains. *Roumanian Archives of Microbiology and Immunology*. 2009;**68**:27-33

[65] Kiyimaci ME, Altanlar N, Gumustas M, Ozkan SA, Akin A. Quorum sensing signals and related virulence inhibition of *Pseudomonas aeruginosa* by a potential probiotic strain's organic acid. *Microbial Pathogenesis*. 2018;**121**:190-197

[66] Rasmussen TB, Skindersoe ME, Bjarnsholt T. Identity and effects of quorum-sensing inhibitors produced by *Penicillium* species. *Microbiology*. 2005;**151**(5):1325-1340

[67] Glasser N. Patulin: Mechanism of genotoxicity. *Food and Chemical Toxicology*. 2012;**50**(5):1796-1801. DOI: 10.1016/j.fct.2012.02.096

[68] Shusaku S, Yoko M, Katsumi F, Nobuhiko F. Effects of long-term, low-dose macrolide treatment on *Pseudomonas aeruginosa* PAO1 virulence factors *In vitro*. *Archives in Clinical Microbiology*. 2017;**8**(4):50. DOI: 10.21767/1989-8436.100050

[69] Skindersoe ME, Alhede M, Phipps R. Effects of antibiotics on quorum sensing in *Pseudomonas*

*aeruginosa*. *Antimicrobial Agents and Chemotherapy*. 2008;**52**(10):3648-3663

[70] Tateda K, Comte R, Pechere J-C, Köhler T, Yamaguchi K, van Delden C. Azithromycin inhibits quorum sensing in *Pseudomonas aeruginosa*. *Antimicrobial Agents and Chemotherapy*. 2001;**45**(6):1930-1933

[71] Ichimiya T, Takeoka K, Hiramatsu K, Hirai K, Yamasaki T, Nasu M. The influence of azithromycin on the biofilm formation of *Pseudomonas aeruginosa* *in vitro*. *Chemotherapy*. 1996;**42**(3):186-191

[72] Bala A, Kumar R, Harjai K. Inhibition of quorum sensing in *Pseudomonas aeruginosa* by azithromycin and its effectiveness in urinary tract infections. *Journal of Medical Microbiology*. 2011;**60**(3):300-306

[73] Pérez-Martínez I, Haas D. Azithromycin inhibits expression of the GacA-dependent small RNAs RsmY and RsmZ in *Pseudomonas aeruginosa*. *Antimicrobial Agents and Chemotherapy*. 2011;**55**(7):3399-3405

[74] Pechère J-C. Azithromycin reduces the production of virulence factors in *Pseudomonas aeruginosa* by inhibiting quorum sensing. *Japanese Journal of Antibiotics*. 2001;**54**:87-89

[75] Sofer D, Gilboa-Garber N, Belz A, Garber NC. 'Subinhibitory' erythromycin represses production of *Pseudomonas aeruginosa* lectins, autoinducer and virulence factors. *Chemotherapy*. 1999;**45**(5):335-341

[76] Favre-Bonté S, Köhler T, van Delden C. Biofilm formation by *Pseudomonas aeruginosa*: Role of the C4-HSL cell-to-cell signal and inhibition by azithromycin. *Journal of Antimicrobial Chemotherapy*. 2003;**52**(4):598-604



- [77] Kim B, Park J-S, Choi HY, Yoon SS, Kim WG. Terrein is an inhibitor of quorum sensing and c-di-GMP in *Pseudomonas aeruginosa*: A connection between quorum sensing and c-di-GMP. *Scientific Reports*. 2018;**8**(1):8617
- [78] Merighi M, Lee VT, Hyodo M, Hayakawa Y, Lory S. The second messenger bis-(3'-5')-cyclic-GMP and its PilZ domain containing receptor Alg44 are required for alginate biosynthesis in *Pseudomonas aeruginosa*. *Molecular Microbiology*. 2007;**65**(4):876-895
- [79] Silva LN, Zimmer KR, Macedo AJ, Trentin DS. Plant natural products targeting bacterial virulence factors. *Chemical Reviews*. 2016;**116**:9162-9236
- [80] Brackman G, Defoirdt T, Miyamoto C, Bossier P, Van Calenbergh S, Nelis H, et al. Cinnamaldehyde and cinnamaldehyde derivatives reduce virulence in vibrio spp, by decreasing the DNA-binding activity of the quorum sensing response regulator LuxR. *BMC Microbiology*. 2008;**8**:149. DOI: 10.1186/1471-2180-8-149
- [81] Niu C, Gilbert ES. Colorimetric method for identifying plant essential oil components that affect biofilm formation and structure. *Applied and Environmental Microbiology*. 2004;**70**(12):6951-6956
- [82] Niu C, Afre S, Gilbert ES. Subinhibitory concentrations of cinnamaldehyde interfere with quorum sensing. *Letters in Applied Microbiology*. 2006;**43**(5):489-494
- [83] Rudrappa T, Bais HP. Curcumin, a known phenolic from *Curcuma longa*, attenuates the virulence of *Pseudomonas aeruginosa* PAO1 in whole plant and animal pathogenicity models. *Journal of Agricultural and Food Chemistry*. 2008;**56**(6):1955-1962
- [84] Sarabhai S, Sharma P, Capalash N. Ellagic acid derivatives from *Terminalia chebula* Retz, Downregulate the expression of quorum sensing genes to attenuate *Pseudomonas aeruginosa* PAO1 virulence. *PLoS One*. 2013;**8**(1):e53441
- [85] Vandeputte OM, Kiendrebeogo M, Rajaonson S, Diallo B, Mol A, El Jaziri M, et al. Identification of catechin as one of the flavonoids from *Combretum albiflorum* bark extract that reduces the production of quorum-sensing-controlled virulence factors in *Pseudomonas aeruginosa* PAQ1. *Applied and Environmental Microbiology*. 2010;**76**(1):243-253
- [86] Luo J, Dong B, Wang K, Cai S, Liu T, Cheng X, et al. Baicalin inhibits biofilm formation, attenuates the quorum sensing-controlled virulence and enhances *Pseudomonas aeruginosa* clearance in a mouse peritoneal implant infection model. *PLoS One*. 2017;**12**(4):e0176883
- [87] Girennavar B, Cepeda ML, Soni KA. Grapefruit juice and its furocoumarins inhibits autoinducer signaling and biofilm formation in bacteria. *International Journal of Food Microbiology*. 2008;**125**(2):204-208
- [88] Chong YM, Yin WF, Ho CY, Mustafa MR, Hadi AH, Awang K. Malabaricone C from *Myristica cinnamomea* exhibits anti-quorum sensing activity. *Journal of Natural Products*. 2011;**74**:2261-2264
- [89] Zhou L, Zheng H, Tang Y, Yu W, Gong Q. Eugenol inhibits quorum sensing at sub-inhibitory concentrations. *Biotechnology Letters*. 2013;**35**(4):631-637
- [90] Lou Z, Letsididi KS, Yu F, Pei Z, Wang H, Letsididi R. Inhibitive effect of eugenol and its nanoemulsion on quorum sensing-mediated virulence



factors and biofilm formation by *Pseudomonas aeruginosa*. *Journal of Food Protection*. 2019;**82**(3):379-389

[91] Ding X, Yin B, Qian L. Screening for novel quorum sensing inhibitors to interfere with the formation of *Pseudomonas aeruginosa* biofilm. *Journal of Medical Microbiology*. 2011;**60**(12):1827-1834

[92] Kim H-S, Lee S-H, Byun Y, Park H-D. 6-Gingerol reduces *Pseudomonas aeruginosa* biofilm formation and virulence via quorum sensing inhibition. *Scientific Reports*. 2015;**5**:8656

[93] Maisarah Norizan SN, Chan KG, Yin W-F, Ping TS, Nafiah MA. The study of caffeine as novel quorum sensing inhibitor. *The Open Conference Proceedings Journal*. 2013;**4**:185. DOI: 10.2174/22102892013040100185

[94] Ren D, Zuo R, González-Barrios AF. Differential gene expression for investigation of *Escherichia coli* biofilm inhibition by plant extract ursolic acid. *Applied and Environmental Microbiology*. 2005;**71**(7):4022-4034

[95] Kiplimo JJ, Koorbanally NA, Chenia HY. Triterpenoids from *Vernonia auriculifera* Hiern exhibit antimicrobial activity. *African Journal of Pharmacy and Pharmacology*. 2011;**5**:1150-1156

[96] Rasamiravaka T, Vandeputte OM, Pottier L, Huet J, Rabemanantsoa C, Kiendrebeogo M, et al. *Pseudomonas aeruginosa* biofilm formation and persistence, along with the production of quorum sensing dependent virulence factors, are disrupted by a triterpenoid coumarate ester isolated from *Dalbergia trichocarpa*, a tropical "legume". *PLoS One*. 2015;**10**:e0132791

[97] Rasamiravaka T, Ngezahayo J, Pottier L, Oliveira Ribeiro S, Souard F, Hari L, et al. Terpenoids from *Platostoma rotundifolium* (Briq.)

a, J, Paton Alter the expression of quorum sensing-related virulence factors and the formation of biofilm in *Pseudomonas aeruginosa* PAO1. *International Journal of Molecular Sciences*. 2017;**18**:1270

[98] Jakobsen TH, Bragason SK, Phipps RK, Christensen LD, van Gennip M, Alhede M, et al. Food as a source for quorum sensing inhibitors: Iberin from horseradish revealed as a quorum sensing inhibitor of *Pseudomonas aeruginosa*. *Applied Environmental Microbiology*. 2012;**78**(7):2410-2421

[99] Ganin H, Rayo J, Amara N, Levy N, Krief P, Meijler MM. Sulforaphane and erucin, natural isothiocyanates from broccoli, inhibit bacterial quorum sensing. *Medicinal Chemistry Communications*. 2013;**4**:175-179

[100] Jakobsen TH, van Gennip M, Phipps RK. Ajoene, a sulfur-rich molecule from garlic, inhibits genes controlled by quorum sensing. *Antimicrobial Agents and Chemotherapy*. 2012;**56**(5):2314-2325

[101] Cady NC, McKean KA, Behnke J. Inhibition of biofilm formation, quorum sensing and infection in *Pseudomonas aeruginosa* by natural products-inspired organosulfur compounds. *PLoS One*. 2012;**7**(6):e38492

[102] Manefield M, de Nys R, Naresh K, Roger R, Givskov M, Peter S, et al. Evidence that halogenated furanones from *Delisea pulchra* inhibit acylated homoserine lactone (AHL)-mediated gene expression by displacing the AHL signal from its receptor protein. *Microbiology*. 1999;**145**(2):283-291

[103] Skindersoe ME, Ettinger-Epstein P, Rasmussen TB, Bjarnsholt T, de Nys R, Givskov M. Quorum sensing antagonism from marine organisms. *Marine Biotechnology*. 2008;**10**(1):56-63

- [104] Manefield M, Rasmussen TB, Hentzer M, Andersen JB, Steinberg P, Kjelleberg S, et al. Halogenated furanones inhibit quorum sensing through accelerated LuxR turnover. *Microbiology*. 2002;**148**(4):1119-1127
- [105] Hentzer M, Riedel K, Rasmussen TB. Inhibition of quorum sensing in *Pseudomonas aeruginosa* biofilm bacteria by a halogenated furanone compound. *Microbiology*. 2002;**148**(1):87-102
- [106] Ebada SS, Lin W, Proksch P. Bioactive sesterterpenes and triterpenes from marine sponges: Occurrence and pharmacological significance. *Marine Drugs*. 2010;**8**(2):313-346
- [107] Paul D, Kim YS, Ponnusamy K, Kweon JH. Application of quorum quenching to inhibit biofilm formation. *Environmental Engineering Science*. 2009;**26**(8):1319-1324
- [108] Chun CK, Ozer EA, Welsh MJ, Zabner J, Greenberg EP. Inactivation of a *Pseudomonas aeruginosa* quorum-sensing signal by human airway epithelia. *Proceedings of the National Academy of Sciences*. 2004;**101**(10):3587-3590
- [109] Ozer EA, Pezzulo A, Shih DM. Human and murine paraoxonase 1 are host modulators of *Pseudomonas aeruginosa* quorum-sensing. *FEMS Microbiology Letters*. 2005;**253**(1):29-37
- [110] Stoltz DA, Ozer EA, Ng CJ, Yu JM, Reddy ST, Lusi AJ, et al. Paraoxonase-2 deficiency enhances *Pseudomonas aeruginosa* quorum sensing in murine tracheal epithelia. *American Journal of Physiology-Lung Cellular and Molecular Physiology*. 2007;**292**:852-860
- [111] Losa D, Kohler T, Bacchetta M, Saab JB, Frieden M, van Delden C, et al. Airway epithelial cell integrity protects from cytotoxicity of *Pseudomonas aeruginosa* quorum-sensing signals. *American Journal of Respiratory Cell and Molecular Biology*. 2015;**53**(2):265-275
- [112] Park J, Kaufmann GF, Bowen JP, Arbiser JM, Janda KD. Solenopsin a, a venom alkaloid from the fire ant *Solenopsis invicta*, inhibits quorum-sensing signaling in *Pseudomonas aeruginosa*. *Journal of Infectious Diseases*. 2008;**198**(8):1198-1201
- [113] Villena J, Kitazawa H, Van Wees SC, Pieterse CM, Takahashi H. Receptors and signaling pathways for recognition of bacteria in livestock and crops: Prospects for beneficial microbes in healthy growth strategies. *Frontiers in Immunology*. 2018;**9**:2223. DOI: 10.3389/fimmu.2018.02223
- [114] Ngezahayo J, Pottier L, Ribeiro SO, Delporte C, Fontaine V, Hari L, et al. *Plastotoma rotundifolium* aerial tissue extract has antibacterial activities. *Industrial Crops and Products*. 2016;**86**:301-310
- [115] Okusa PN, Penge O, Devleeschouwer M, Duez P. Direct and indirect antimicrobial effects and antioxidant activity of *Cordia gillettii* De wild (Boraginaceae). *Journal of Ethnopharmacology*. 2007;**112**(3):476-481
- [116] Okusa PN, Stévigny C, Névraumont M, Gelbcke M, Van Antwerpen P, Braekman J-C, et al. Ferulaldehyde and lupeol as direct and indirect antimicrobial compounds from *Cordia gillettii* (Boraginaceae) root barks. *Natural Product Communications*. 2014;**9**(5):619-622
- [117] Nobori T, Mine A, Tsuda K. Molecular networks in plant-pathogen holobiont. *FEBS Letters*. 2018;**592**(12):1937-1953
- [118] Ahmed SA, Rudden M, Smyth TJ, Dooley JS, Marchant R, Banat IM. Natural quorum sensing

inhibitors effectively downregulate gene expression of *Pseudomonas aeruginosa* virulence factors. *Applied Microbiology and Biotechnology*. 2019;**103**(8):3521-3535

[119] Cho HH, Kwon KC, Kim S, Park Y, Koo SH. Association between biofilm formation and antimicrobial resistance in carbapenem-resistant *Pseudomonas aeruginosa*. *Annals of Clinical and Laboratory Science*. 2018;**48**(3):363-368

[120] Xu Q, Bauer R, Hendry BM, Fan T-P, Zhao Z, Duez P, et al. The quest for modernisation of traditional Chinese medicine. *BMC Complementary and Alternative Medicine*. 2013;**13**:132-143