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Exploiting quorum sensing inhibition for the control of *Pseudomonas aeruginosa* and *Acinetobacter baumannii* biofilms

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Abstract: *Pseudomonas aeruginosa* and *Acinetobacter baumannii* are two of the main bacteria responsible for nosocomial infections; both organisms are resistant to several classes of antibiotics making their infections very difficult to treat. Moreover, they possess a remarkable ability to form biofilms, which further enhances their antimicrobial resistance. Both organisms coordinate their formation of biofilms and their expression of virulence factors through quorum sensing, a system that regulates gene expression at high cell densities and that plays a key role in the establishment of bacterial infections. Hence, interfering with these quorum-sensing systems has been proposed as an alternative to traditional antibiotics for the eradication of bacterial infections. In this review, we describe the quorum sensing systems of both organisms, the way they coordinate the formation of biofilms, the recent advances in biofilm disruption by quorum sensing interference, and the advantages and limitations of the implementation of these novel therapeutic options in the clinic.

Keywords: Quorum quenching, homoserine lactone, in vivo biofilm models, stress response, resistance.

1. INTRODUCTION

Pseudomonas aeruginosa is a cosmopolitan and remarkably adaptable bacterium able to survive in a wide range of environments including soil, water, and multiple hosts, and is an opportunistic pathogen of plants and animals including humans. For humans, it is one of the main microorganisms responsible of nosocomial infections, usually attacking those patients that are immunocompromised, intubated, have prosthetic devices, have severe burns, or suffer from cystic fibrosis [1]. It has an intrinsic high antimicrobial tolerance and a remarkable ability to become resistant against new antibiotics [2] as well as the ability to synthesize robust biofilms on abiotic and biotic surfaces [3]. *P. aeruginosa* virulence is a complex multifactorial phenomenon [4] influenced by environmental cues from the host, such as temperature [5], iron [6], phosphate [7] and calcium levels [8], by the presence of metabolites like adenosine [9] and hormones [10], by other bacterial species [11], as well as by information about the status of the *P. aeruginosa* population *per se*. Arguably the main system that coordinates the expression of its virulence factors is quorum sensing (QS), a process that allows the cells to evaluate their population levels and to reprogram their gene expression accordingly [1].

Acinetobacter baumanni is an emerging opportunistic human pathogen belonging to the complex *calcoaceticus-baumanii*, which also includes *A. calcoaceticus*, *A. pittii* and *A. nosocomialis.* This bacterium is implicated in multiple infections such as septicemia, pneumonia, endocarditis, meningitis, skin and wound infections, burn wound and urinary tract infections [12, 13]. It has become an important pathogen in intensive care units (ICUs), probably as a result of the increasingly invasive diagnostic and therapeutic procedures used in these hospital settings [14]. Infections caused by *A. baumannii* are difficult to treat as a result of different factors including resistance to multiple antibiotics and the virulence and pathogenesis of the microorganism [15]. This results in mortalities of 43% for patients in ICUs and of 23% for hospitalized patients [16].

QS systems rely on the constant production of small diffusible molecules called autoinducers which are produced at a basal lower rate in low density populations; however, when the population density increases, autoinducers reach their threshold concentration, bind their receptors and then activate the expression of the genes encoding for the enzymes that produce them, creating a positive feedback loop, and hence, auto-inducing their synthesis [17]. In addition to the auto inducing phenomena, the QS receptors form a complex with the autoinducers and bind to the promoters of many other genes (between 3% and 7% of the

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genome), activating and occasionally repressing them [18].

These QS systems link the expression of costly phenotypes such as light production in marine vibrios as well as siderophore production, virulence factors production and biofilm formation in several bacterial species [19-21]. The expression of these costly phenotypes (when bacteria reach a high cellular densities or otherwise low diffusion rates) collectively increases the fitness of the bacterial population. Presumably, QS expression at low cell densities will probably result in a waste of the individual resources without conferring an advantage to the population; in contrast, when bacteria reach a high density, the population has enough critical mass to utilize the resources efficiently [22].

This is illustrated by the fact that although light production in *Vibrio fischeri* consumes high amounts of ATP, it is useful for the bacteria when it is produced inside the light organ of *Euprymna scolopes*, which utilizes this bioluminescent organ to hide its shadow during nocturnal hunting. In return, the squid provide shelter and nutrients that allow the bacteria to grow to high densities [23]. In the case of bacterial pathogens such as *P. aeruginosa* and *A. baumannii*, the maximization of the production of virulence factors at high cell densities increases their chances to overcome the host defenses and to invade, propagate and establish infections.

1.1. Quorum sensing systems in *P. aeruginosa* and *A. baumannii*

P. aeruginosa possesses an intricate hierarchical QS network governed by the LasRI system. In this system, the transcriptional activator LasR binds the autoinducer N-3-oxo-dodecanoyl-L-homoserine lactone (3O-C12-HSL), produced by the LasI enzyme, and subsequently forms a dimer and binds to the promoter of the *lasI* gene as well as several others, activating the expression of genes such as those that code for elastase B and alkaline protease (collagenase); this dimer with 3O-C12-HSL also activates a second homoserine lactone QS system, RhlRI, that produces and senses N-butyryl-L-homoserine lactone (C4-HSL). RhlR/C4-HSL in turn activates the expression of multiple virulence genes like those encoding rhamnolipids and pyocyanin. In addition, *P. aeruginosa* possesses a third QS system based on hydroxy-alkyl-quinolone (HAQ) autoinducers that is also activated by LasRI and antagonized by RhlRI. This system utilizes quinolones as signals, specifically 2-heptyl-4-quinolone (HHQ) and 2-heptyl-3-hydroxy-4-quinolone (PQS), which bind the MvfR (PqsR) transcriptional activator [24]. MvfR/HHQ or MvfR/PQS activate the transcription of the genes involved in pyocyanin production, although indirectly, possibly by stabilizing RhlR/C4-HSL, and influence the expression of approximately 60 small secreted molecules [25]. In addition, the synthesis of antioxidant enzymes such as catalase is controlled by QS in *P. aeruginosa* [26],

The QS transcriptional regulators LasR, RhlR and MvfR, have been defined as receptors of their cognate autoinducers.

The QS system effectors of *Acinetobacter spp*. are homologues of the LuxR (receptor) and LuxI (synthase) proteins of *Vibrio fischeri* and are named AbaR and AbaI respectively. Similar to the case of other bacterial pathogens, in *A. baumannii*, its QS system regulates biofilm formation [27] and motility [28] by the production of N-(3-hydroxydodecanoyl)-L-HSL (3-hydroxy-C12-HSL) [29, 30]). Accordingly, the disruption of *abaI*, which produces the A-HSL molecule, results in a 30–40% reduction in biofilm production relative to that of the isogenic parental strain [31]. In agreement, the addition of exogenous purified *Acinetobacter* A-HSL restored biofilm maturation in the *abaI* mutant [32].

Several strains of *Acinetobacter* (63%) produce more than one A-HSL. However, none of the A-HSL signals can be specifically assigned to a particular species of the genus [17]. *Acinetobacter* quorum signals are not homogeneously distributed, and therefore distinction between virulent and non-virulent strains on the basis of QS signals is difficult. [33].

Bhargava et al. showed that catalase and superoxide dismutase (SOD), which participate in the elimination of reactive oxygen species (ROS), are positively controlled by a QS system in *A. baumannii* [34]. These authors studied the mechanisms associated with the stable coexistence of mixed-culture biofilms of *A. baumannii* and *P aeruginosa*. The pyocyanin produced by *P. aeruginosa*, which generally eliminates competition from other pathogens, leads to the generation of ROS in *A. baumannii*, which in response, shows a significant increase ($P \leq 0.05$) in catalase and SOD production. Also, a QS mutant of *A. nosocomialis* M2 (*abaI*::Km), lacking the autoinducer synthase gene, has significantly lower levels of catalase and SOD enzymes (even on exposure to pyocyanin) than the wild type.

1.2. Influence of QS in biofilm formation

Biofilm formation is a complex developmental process consisting of several steps, beginning with initial reversible attachment mediated by weak electrostatic interactions such as van der Waals forces and hydrophobic interactions, followed by irreversible attachment by the action of specific adhesion proteins and complexes including extracellular polymeric substances (EPS) [35] and fimbria. The developmental process that leads to the formation of biofilms involves changes in expression of a large number of genes [36]. The formation of micro colonies and the maturation of biofilms, is a process that involves the synthesis and secretion (or otherwise liberation by cell lysis) of a complex extracellular matrix that includes the exopolysacharides alginate [37], Psl, [38], and Pel [39], specific proteins, and extracellular DNA for *P. aeruginosa* [40, 41].

For *A. baumannii*, Anbazhagan et al. [42] analyzed the A-HSL production in biofilm-forming clinical isolates and found about 60% (n = 30) of the clinical strains tested formed biofilms under prolonged incubation. Of the 30 strains of biofilm-forming isolates tested, only 7 produced long-chain AHLs. This may be because biofilm formation is a multifactorial event and QS signals are only one of the factors involved. Two molecules were particularly abundant in these strains: 3-hydroxy-C12-HSL and N-(3-hydroxydecanoyl)-L-HSL (3-hydroxy-C10-HSL).

Multidrug efflux pumps play a central role in drug resistance, cell division and pathogenicity, and, more recently, in the secretion of QS signals [43, 44]. In *A. baumannii*, the AdeFGH efflux pump is involved in the synthesis and transport of autoinducer molecules during biofilm formation in clinical strains [43]. Notably, biofilm induction was most intense in combination with the consistent upregulation of *abaI* and *adeG* in *A. baumannii* clinical strains, suggesting a potential effect of the combined upregulation of AdeFGH and AbaI on biofilm development [43]. Moreover, the overexpression of the AdeABC efflux pump by delection of AdeRS (a two-component regulation system) has been associated with biofilm formation and virulence phenotype in this pathogen [45].

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1.3. Synthesis and release of biofilm matrix components is regulated by QS

The main glycolipids that are produced by *P. aeruginosa* are the biosurfactants known as rhamnolipids (Rhl), that influence biofilm formation, mainly through the promotion of bacterial detachment from the biofilm [46-48]. *P. aeruginosa* produces two types of Rhls, and the first is mono-Rhls that are synthetized by the concerted activity of the enzymes encoded by the *rhlAB* operon. RhlA uses as substrate ACP-β-hydroxyacids and produces a fatty-acid dimer that is one of the substrates of RhlB, rhamnosyl-transferase, which catalyzes the formation of mono-Rhls using this fatty acid-dimer and TDP-L-rhamnose as the second substrate. The second type of Rhls, are the di-Rhls, which contain two rhamnose moieties that are produced by RhlC rhamnosyl-transferase that uses mono-Rhls and TDP-L-rhamnose as substrates [49]. Rhl production is regulated at the transcription level in a coordinated manner with different virulence-related traits such as elastase and pyocyanin by the QS system. These Rhls have a role in the dispersion of cells in mature biofilms.

Extracellular DNA (eDNA) is one of the main components of the *P. aeruginosa* biofilm matrix, and it is liberated mainly by cell lysis mediated by the QS controlled quinolone 2-N-heptyl-4-hydroxyquinoline-N-oxide (HQNO) [41]. This quinolone is one of the PQS controlled secretion factors but unlike the specific ligands of the MvfR receptor (HHQ and its derivative PQS), HQNO has no cognate QS receptor. Instead HQNO, acts directly by inhibiting the Q cycle at the level of the bc1 cytochrome, hence promoting the generation of reactive oxygen species (ROS) such as superoxide and hydrogen peroxide, which in turn damage the membrane, promoting cell lysis and eDNA release. This process is similar to apoptosis and apparently is widespread in clinical *P. aeruginosa* strains. Moreover the presence of eDNA in the biofilm matrix increase their robustness and their antibiotic tolerance [41]. An alternative mechanism for providing eDNA for biofilm formation not involving quinolones also involves lysis as a result of explosive lysis mediated by cryptic phage endolysins and the formation of membrane vesicles [50].

2. Qs inhibition and its influence in biofilm formation: targeting of the QS receptors

QS inhibition, also known as quorum quenching (QQ), consists of disruption of QS systems in order to block high cell density collective behaviours such as biofilm formation and virulence factor production and can be achieved by blocking the QS receptors with antagonistic molecules, by interfering with the autoinducer production and signal propagation (e.g., by blocking the synthases or receptors) or by degrading the autoinducers by the action of hydrolytic enzymes. These approaches then lead to a reduction in biofilm formation.

One of the early examples of QS inhibition is the utilization of non-functional signal analogues such as brominated furanones that resemble A-HSL´s and thus, are able to bind to their receptors [51]. These quorum quenchers were first isolated from the marine red algae *Delisea pulchra*, which produces a cocktail of these compounds, presumably to prevent QS-mediated colonization of bacterial biofilms in its marine niche [51]. Accordingly, synthetic derivatives of these furanones, like C-30 and C-56 (Figure 1A B), are able to inhibit QS dependent phenotypes in *P. aeruginosa* including the production of virulence factors and biofilm formation. Importantly, and in agreement with its action through QQ, these furanones do not interfere with initial attachment of cells but instead affect the architecture of biofilms as well as promote detachment [52].

Also, similar to *P. aeruginosa*, some non-native A-HSLs are able to inhibit *A. bumannii* QS by blocking the AbaR receptor (LasR homologue protein). Notably, the most effective AbaR antagonists contain aromatic acyl groups, and remarkably, the 10 most potent compounds also inhibited *A. baumannii* motility, and five of them inhibited biofilm formation by up to 40% [53].

Moreover, treatment with synthetic furanones also renders biofilms more sensitive to diverse antimicrobials such as hydrogen peroxide, detergents like SDS, antibiotics like tobramycin, and even immune effector cells like polymorphonuclear leukocytes [54]. Importantly, QS deficient mutants such as those with *lasR/rhlR* mutations also produce labile biofilms [51, 54], and planktonic cells of these mutants are much more sensitive to several kinds of stressors, like toxic compounds, high salt concentrations, heavy metals and heat [55]. The effect of QQ over *P. aeruginosa* biofilm architecture and robustness must be at least partially explained by deficient synthesis of important matrix components. Accordingly, the destabilization of biofilms with agents that disrupt biofilm components, such as DNAse, increase biofilm susceptibility against antibiotics [41, 56].

It was initially assumed that QS inhibitors would be impervious to the generation of resistance; however, it was demonstrated that in conditions in which growth is directly linked to QS, its disruption promotes the development of resistance against brominated furanones, primarily via efflux through efflux pumps like MexAB-OmpR [57, 58].

Moreover, resistance against furanone C-30 has also been found in clinical isolates from diverse origins [57, 59], and recent experiments suggest that resistance against C-30 in clinical isolates may be also mediated by a lack of permeability for the compound rather than by efflux. Critically, low C-30 doses (1 μM), which are unable to interfere with QS, severely decrease the growth of some clinical isolates [60]. Also, all known QS inhibitors affect growth and bacterial fitness to some degree (especially during infection); hence, it is possible that resistance against these kind of compounds and against any other quorum quencher, may arise and eventually spread [61]. Therefore, the use of QQ compounds is not a panacea, but is part of an important arsenal to thwart pathogens.

Another way to interfere with the binding of the autoinducers to their receptors is by using synthetic nonfunctional homoserine lactones as well as native homoserine lactones with inhibitory activity [62]. Although the effects of these kind of compounds has been mostly studied in reporter strains by evaluating their effect in LasR transcriptional activity, some studies have evaluated their effects on QS dependent virulence factors and phenotypes. For example Smith and coworkers in 2003 tested a battery of A-HSL analogues with modified head groups that replaced the lactone ring while maintaining the native 3-oxo-C12 acyl group; some of the tested compounds were able to significantly inhibit elastase and biofilm formation at micromolar concentrations [63].

Several other attempts to obtain A-HSL with QS inhibitory activities have been done by modifying the aliphatic acyl tail of native autoinducers, and hundreds of modified A-HSL have been synthesized [64, 65]. Some of these molecules are potent LasR antagonists, with remarkable inhibitory activities at the nanomolar concentrations. Nevertheless, there is a lack of evaluation of the activities of most of these compounds on the production of virulence factors and on biofilm formation, and there has been no *in vivo* evaluation of their activity in animal or plant models.

Inhibitors that target MvfR, the receptor of the PQS and HHQ quinolones signals, inhibit QS both *in vitro* and *in vivo* with laboratory as well as with clinical strains. In 2014, Starkey and coworkers [66], through an extensive whole-cell high-throughput screen and structure-activity relationship analysis, identified compounds with a benzamide-benzimidazole backbone that are able to bind MvfR and decrease the concentration of signaling quinolones as well as decrease the production of pyocyanin which increased mice survival in burn and lung infections models. In addition, an advantage of these kinds of compounds is that they decrease the fraction of antibiotic persister cells [66]. Persisters are bacterial cells with no genetic changes respective to their parental strains but that enter a dormant stage that enables them to tolerate high doses of antibiotics and that likely contributes to the establishment of chronic infections [67].

A third way to inhibit QS by targeting the receptors is to inactivate them via covalent modification. In this regard, in 2009 Amara and coworkers designed a set of electrophilic probes containing isothiocyanate that covalently binds the cysteine 79 residue in the LasR binding pocket and inactivates them. The most potent of them, itc-12 (Figure 1C), is able to decrease pyocyanin production and biofilm formation although moderately [68].

2.1 QS Inhibition via targeting autoinducer synthesis

Another important suitable target for the development and implementation of QS inhibitors are the enzymes that produce autoinducers; in the case of *P. aeruginosa*, these enzymes are those that produce the A-HSL 3-oxo-C12-HSL and C4-HSL, and that produce HAQ, PQS and HHQ. The synthesis of A-HSLs has a common substrate, the donor molecule of the amino group, *S*-adenosylmethionine (SAM), and the molecule that varies is the donor acyl group attached to the acyl carrier protein (acyl-ACP). For the synthesis of C4-HSL, butyryl-ACP and SAM are the substrates of the RhlI enzyme, and after four reaction steps, the products are free ACP, N-butyryl-L-homoserine lactone and 5'-methyladenosine (MTA). For the synthesis of 3-oxo-C12-HSL, LasI uses the acyl group β-ketoacyl-ACP as the donor molecule, and SAM to generate N-(3-oxo-dodecanoyl)-L-homoserine lactone, MTA and ACP [69]. For the QS quinolone system of *P. aeruginosa*, HHQ synthesis is carried out by six enzymes (PqsA, PqsB, PqsC and PqsD, PqsS and PqsM) using anthranilic acid (Figure 1L) and β-keto-ACP as substrates [70]; HHQ is converted to PQS by the activity of PqsH mono-oxygenase.

Regarding the inhibition of *P. aeruginosa* QS signal synthesis, in 2001 Calfe and coworkers identified that anthranilic acid is a precursor for the synthesis 4-hydroxy-2-alkylquinolones QS autoinducers, and that the anthranilate analogue methyl anthranilate inhibits PQS production and QS dependent elastase production [71]. In a subsequent study, Lesic and coworkers demonstrated that other analogues of anthranilic acid (6FABA (Figure 1M), 6CABA (Figure 1N), 4CABA (Figure 1O), 2-amino-6-fluorobenzoic acid, 2-amino-6-chloro benzoic acid, and 2-amino-4 chlorobenzoic acid, were able to compete with anthranilic acid for binding to the enzyme PqsA, a coenzyme A ligase, which is the first step in the HAQ biosynthetic pathway. These analogues inhibited the production of pyocyanin and elastase and were able to increase the survival of infected mice [72]. Nevertheless, their effects on biofilm formation were not characterized.

Interference of the synthesis of A-HSL autoinducers has also been attempted, and compounds that inhibit RhlI synthase like MTA and substrate analogs such as holo-ACP, sinefungin, D/L-*S*-adenosylhomocysteine, L-*S*-adenosylcysteine, and butyryl-SAM, can inhibit A-HSL synthesis *in vitro* but have variable effects *in vivo* [69, 73]. In addition, other A-HSL inhibitors with remarkable inhibition activity have been identified, but they also interfere with metabolically important processes such as amino acid and fatty acid metabolism, and hence have direct effects over bacterial viability [74].

2.2 QS Inhibition via degradation of the autoinducer signals

QQ enzymes include three groups, A-HSL-lactonases, A-HSL-acylases and paraoxonases (PON). Roughly, their mechanisms of action are the hydrolysis of the lactone ring by the lactonases, the disruption of the acylated chain by the

acylases and the degradation of the lactone ring by the paraoxonases.

2.2.1 A-HSL-lactonases

Lactonases can be classified as metallo-ß-lactamase-like lactonase, or phosphotriesterases like lactonases and paraoxonases. Lactolysis is a process, which is favored by alkaline pH and can be reversed in acidic pH. *In vitro* assays have shown that long acylated chains (C ≥ 8) are less prone to degradation than short chains, i.e., *N*-hexanoyl-homoserine lactone has shown different rates of degradation under different experimental conditions: under acidic pH conditions and low temperatures (pH 5.5, 4° C), it has a half-life of 21 days, while under alkaline pH and physiological temperatures (8.5, 37° C), its half-life is less than 30 min [75-77].

The first lactonase described was AiiA, which was isolated from the soil inhabitant *Bacillus sp.* 240B1il. AiiA has QQ activity against *Erwinia carotovora* and is able to decrease its production of virulence factors [78, 79].

Metallo-ß-lactamases-like lactonases constitute a superfamily [78, 80]. Sequence alignments of amino acids reveal that there is a conserved Zn^{2+}- binding HXHXDH motif, which is also present in the metallo-ß-lactamases superfamily of proteins [78, 80]. This superfamily has activity against A-HSL with medium or large chains, regardless of the C3-substitutions present in the molecules.

Lactonases can be classified in two clusters, the most extensive is the AiiA cluster, and the members of this group share 90% identity at the amino acid level; all members of this cluster belong to the Gram positive bacilli of the genus *Bacillus*. Members of this cluster are *B. anthracis* Ames (Ba ames), *B. cereus* (Bc 14579), *B. thuringiensis* serovar oswaldocruzi (Bt osw), *B. cereus* ATCC 10987 (Bc 10987), *B. thuringiensis serovar thompsoni* (Bt tho), *Bacillus sp*. COT1 (B. sp. COT1), *B. thuringiensis serovar toumanoffi* (Bt. tou), *Bacillus sp*. A24 (B. sp. A24) and *Bacillus sp*. 240B1 (B. sp. 240B1) [78, 81, 82]. The second cluster is known as the AttM cluster. Their members share between 30-50% of identity in their protein sequences. Lactonases from this cluster are present in *Klebsiella pneumoniae* (Kp ahlK), *Agrobacterium tumefaciens* (At attM), *A. tumefaciens* (At aiiB, gi16119885), and *Arthrobacter sp*. IBN110 (Art ahlD) [83, 84]. Both clusters share less than 25% of identity between them, but all these enzymes have the same conserved HXDH~H~D motif [85].

Another lactonase group is the phosphotriesterase-like [86]. The members of this lactonase group belong to the amidohydrolase superfamily. They have a broad range of action against A-HSL; however, they have higher hydrolyzing activity towards lactones with hydrophobic characteristics. Initially, these enzymes were classified as paraoxonases because they had activity against paraoxon (paraoxon is an active metabolite of the insecticide parathion which is an organophosphated pesticide). The main feature of these members is that they have a binuclear metal center within a $(ß/\ \alpha)_8$-barrel structural scaffold [87, 88]. SisLac and SsoPox are canonical examples of phosphotriesterase-like lactonases; these enzymes were isolated from *Sulfolobus islandicus* and *S. solfataricus* respectively [88]. Other microorganisms that produce phosphotriesterase-like lactonases are *Rhodococcus erythropolis* [89], *Vulcanisaeta moutnovskia* [90], *Mycobacterium tuberculosis* [86].

Another example of successful QS inhibition that leads to the disruption of biofilm formation is the utilization of an experimentally-evolved, thermostable QQ lactonase from *Geobacillus kaustophilus*, which possess enhanced catalytic activity and broadened substrate range against A-HSLs. The modified enzyme (E101G/R230C) presented good catalytic efficiency for both 3-OH-C12-HSL and 3-OH-C10-HSL (an autoinducer produced by the clinical strain S1 included in their study). Accordingly, treatment with this lactonase significantly decreases biofilm biomass, thickness and surface area of the laboratory M2 strain (to levels comparable to those produced by the QS-deficient mutant Δ*abaI*) and was as effective with other strains including clinical isolates; in addition, the treatment was also effective against preformed biofilms [91].

2.2.2 A-HSL-acylases

The first acylase enzyme was isolated from *Variovorax paradoxus* VAI-C [92], and this QQ enzyme hydrolyzes the amide linkage of A-HSL, which is located between the acyl chain and the homoserine moiety, in an irreversible way, then the fatty acid is released, and the produced molecules have no signaling activity [93]. The fatty acid released can be used by the bacteria as a nutritional source and the homoserine lactone can be used as a nitrogen source [94]. Acylases have been identified in several microorganisms such as *P. aeruginosa* [95], *Ralstonia spp.* [96], *P. syringae* [97], *Comamonas sp.* [98], *Ochrobactrum sp.* [99] and *Streptomyces sp.* M664 [100]. The first crystal structure of an acylase was reported in 2010 [101] and it was isolated from *P. aeruginosa*; this enzyme is PvdQ which shows a typical α/β-heterodimeric Ntn-hydrolase fold with an unusually-large hydrophobic-binding pocket adapted to the long acyl chain of the A-HSL substrates. Its catalytic mechanism proceeds via a covalently bound intermediate [75].

2.2.3 Paraoxonases

This family of QQ enzymes includes three members, paraoxonases (PON) 1, 2 and 3 and are located in tandem on the long arm of the human chromosome 7 (7q21-22) [102]. The name of these enzymes is due to their activity against paraoxon. The first time that the activity of these enzymes was detected was through the inactivation of A-HSL lactone in human epithelial cells [103]. After that initial observation, serum from rabbit, mouse, horse, sheep, bovine and humans were tested and in all of them, A-HSL inactivation was detected [104]. These enzymes are Ca^{2+} dependent and have a broad spectrum of physiologically hydrolytic activities [104, 105]. PON´s have activity against different A-HSLs, and they display strong degradation activity, especially if the acylated chain is long [103, 104]. Particularly, PON 1 has shown remarkably good activity hydrolyzing the *P. aeruginosa* autoinducer 3-oxo-C12-HSL.

2.3 QS Inhibitors with a non-characterized mechanism

A number of bacteria and some plants produce indole and some of its derivatives; indole (Figure 1 D) plays some beneficial roles in several indole-producing organisms by acting as an intercellular signaling molecule [106-108]. In contrast, indole and its related compounds have considerable potential as QQ agents and antivirulence compounds against non-indole-producing pathogenic bacteria [109, 110]. Indole was found to inhibit A-HSL-mediated QS signaling in various bacteria, including *P. aeruginosa*, wherein the gene expression was altered in a manner contrary to that produced by A-HSLs [111]. Although the precise mechanisms of QS inhibition by indole are still not completely understood, recent evidence in *Acinetobacter oleivorans* indicate that it binds and destabilizes QS receptors, hence promoting their degradation and therefore QQ[112]. Hence, indole and its derivatives can modulate QS-regulated phenotypes, such as biofilm formation and toxin production, in various bacteria, including *Acinetobacter oleivorans*, *Chromobacterium violaceum*, *Pseudomonas chlororaphis*, and *Serratia marcescens* [110]. It is notable that non-indole-producing pathogens have developed defense systems against indole by oxidizing it and inducing biofilm formation and antibiotic resistance [111, 113-115]. Hence, it is important to identify more potent, stable, and non-toxic indole derivatives without these contradictory effects. For example, plant auxin 3-indolylacetonitrile (Figure 1E) [116] and 7-fluoroindole (Figure 1F) [117] show anti-QS and anti-biofilm activities without antibiotic tolerance in *P. aeruginosa* or *Escherichia coli* O157:H7. Also, several synthetic indole derivatives, such as indole derived flustramine (Figure 1G) [118], indole/triazole conjugates (Figure 1H) [119], 2-aminobenzimidazole (Figure 1I) [120], desformylflustrabromine (Figure 1J) [121], and indole/*N*-acylated *L*-homoserine lactone conjugates (Figure 1K) [122] also show anti-QS and/or anti-biofilm activities more potent than those of indole itself. In addition, indole and its derivatives were found to inhibit the attachment of the fungal pathogen *Candida albicans* to intestinal epithelial HT-29 cells [123] and to reduce the production of the virulence factor staphyloxanthin in *Staphylococcus aureus* [124].

Indole is a simple *N*-heterocyclic compound and its derivatives are widespread in the microbial community and plant extracts. Also, thousands of synthetic indole derivatives are commercially available and more can be easily developed. Hence, it would be interesting to investigate the structure-activity relationships of indole derivatives and how indole derivatives control bacterial QS and biofilm formation.

Although several other compounds that inhibit *P. aeruginosa* QS and QS-dependent virulence factors have been identified, the mechanism for many is uncharacterized. Among the most interesting is 5-fluorouracil (5-FU) (Figure 1P), a compound used in cancer chemotherapy, which has also has classical antimicrobial activity. In addition, in 2009, Ueda and coworkers [125] discovered that a functional uracil biosynthetic pathway is essential for robust biofilm formation in *P. aeruginosa* and that mutants impaired in this pathway also express low levels of several QS controlled virulence factors such as elastase, pyocyanin, rhamnolipids and HAQ such as PQS. Hence, it is likely that uracil or some related metabolite is important for the regulation of QS systems [125]. In agreement with these findings, the uracil analogue 5-FU is a potent inhibitor of the expression of QS in this bacterium, as it is able to disrupt biofilm formation and the virulence factor production and to reduce virulence *in vivo* in a barley germination model [125]. Remarkably, 5-FU has been used for the coating of venous catheters in clinical trials with human patients, demonstrating even better properties than the commonly used chlorhexidine and silver sulfadiazine in the abolishment of microbial colonization and biofilm formation in the devices [126]; hence, it is the first quorum-quenching compound to be found to be effective with humans. Since 5-FU is a compound already in use in the clinic, it may be eventually repurposed to treat bacterial infections and prevent biofilm formation in catheters and prosthetic devices [127].

Another class of promising QS inhibitors is Zn oxide based nanoparticles, which are able to inhibit QS dependent virulence factors (including biofilms) in *P. aeruginosa* laboratory and clinical strains [128, 129]. Interestingly, these nanoparticles are effective even for strains that are resistant to other quorum quenchers like furanone C-30 [130] and that are resistant to novel metal-containing antimicrobials like gallium nitrate [131].

In addition, several natural compounds from plants and other sources are also QS inhibitors active against *P. aeruginosa*. Among these are compound like 6-gingerol (Figure 1Q) and some fatty acids like *cis*-2-dodecenoic acid (Figure 1R) that are able to inhibit QS controlled virulence phenotypes such as pyocyanin, exoprotease production and biofilm formation, presumably by binding and inactivating the LasR receptor [132, 133].

In contrast with *P. aeruginosa* QS research, the field of QS inhibition in *A. baumannii* is still in its early stage, but there are already examples of studies demonstrating that QS disruption either by specific molecules or by degrading its A-HSL autoinducers, is able to decrease the expression of virulence-linked phenotypes including biofilm formation. Interestingly some antibiotics such as the aminoglycoside streptomycin when administrated at low-growth, sub-inhibitory concentrations are able to inhibit the production of the signal molecule 3-OH-C12-HSL as well as surface motility, and although its action mechanism is yet unknown, its effect is specific since other aminoglycosides such as gentamicin lack those anti-QS effects [134].

3. QS inhibition and targeting of biofilm formation *in vivo*

Since the first report in 2004 of the positive effect on biofilm inhibition by QS interference *in vivo* in *P. aeruginosa* [135], corroboration of the success of QS-biofilm therapies in animal models is one of the greatest challenges for the successful implementation of these strategies in the short term. In this regard, some researchers have used *in vivo* models to assess the antibiofilm activity of molecules that inhibit the QS systems of *P. aeruginosa* [136-139].

Most of the tested biofilm-infection models have been developed in mice, in which biofilm formation by *P. aeruginosa* is favored. Specifically, the main models are (i) the *Pulmonary infection model* (PIM) in which the infection is performed with a substrate to which bacteria are adhered (mainly alginate beads) and simulates the conditions present in patients with cystic fibrosis., (ii) the *Foreign-body implant model* (FBM) in which a fragment of silicone or intravenous catheter is infected with *P. aeruginosa* and then is positioned inside the peritoneal cavity, and (iii) the *Urinary tract infection model* (UTI) in which the infection is induced by inoculating animals via polyethylene tubing.

Garlic is one of the natural products that have been evaluated *in vivo* for its potential QS inhibition dependent on its antibiofilm activity in *P. aeruginosa*. Using the PIM model, Bjarnsholt and coworkers reported that prophylactic treatment with 1.5% garlic extract maintained for 7 days reduces animal mortality by 39% and promotes the clearance of the bacteria from the lungs [54]. However, although the antibiofilm mechanism involved was not described, it was suggested that this effect is mediated by inhibition of QS, and by a synergistic effect with the activity of polymorphonuclear cells, which increase with the administration of garlic extract [54]. In addition, the authors indicate that to achieve an effective therapy it is important to identify the active compounds present in the extract, because, if the results obtained in the animal model are extrapolated to humans, the consumption of ~ 59 bulbs of garlic a day would be required to achieve the same effect [54]. In a later study, a positive effect of garlic extract was also recorded using the UTI model, since the prophylactic oral administration of the extract 14 days before the infection and during its course, significantly reduced the renal bacterial counts and decreased the presence of lesions [140]. Moreover, synthetic ajoene (ajoene 4,5,9,-trithiadodeca-1,6,11-triene-9-oxide) (Figure 1S) derived from garlic, is able to attenuate the production of various QS-dependent virulence factors in *P. aeruginosa* and using the PIM model, its prophylactic administration two days before the infection and during its course, reduced the number of viable bacteria in the lungs by 500-fold relative to the non-treated mice [141]. Like 5-FU, garlic extract has been tested in clinical trials in humans, however, in this study no significant improvement in the lung function of patients with cystic fibrosis who consumed garlic capsules was detected [142].

The brominated furanones are a group of QS inhibitors that have been evaluated for their antibiofilm potential in animal models. Using the FBM model, the intraperitoneal administration of furonone C-30 showed significantly faster clearing of the infection of implants as compared to the placebo-treated group [137]. A similar effect was observed using the PIM model in which furanone C-56 accelerated the removal of the bacterial biofilms from the lungs, reducing the severity of the damage and increasing the survival of mice [135]. Notwithstanding the positive results obtained with furanones *in vivo*, their application is restricted due to their high toxicity. Nevertheless, a recent study indicates that combining furanones with antibiotics or other QS disruptors may potentiate their effect, so it is feasible to reduce the doses of furanones and thus to decrease their toxicity. Specifically Christensen and coworkers reported that using the FBM model, the combination of furanone C-30 or ajoene with tobramycin reduced the number of viable bacteria in biofilms cells more efficiently than the groups that received a single-treatment [143].

Some authors have proposed the repurposing of drugs used in the clinic as an alternative to combat multi-resistant bacterial infections in the short term [127, 144]. In this context, in recent years, some antibiotics that exhibit QS inhibition properties and that have antibiofilm activity had been found. Such is the case of azithromycin (AZM) (figure 1T), an antibiotic used mainly to treat Gram-positive bacteria, and which does not exhibit significant bactericidal activity against *P. aeruginosa* but interferes with the formation of alginate polymers in biofilms [145, 146]. Similarly, in the clinic, AZM significantly improves the lung function of patients with cystic fibrosis who are infected by *P. aeruginosa* [147]. In agreement, Hoffman and colleagues reported that using the PIM model, treatment with AZM improves the clearance of the bacterium from alginate embedded biofilms, and consequently, the damage produced to the host lungs is reduced (Hoffmann et al., 2007). A similar effect was reported by Bala and collaborators using the UTI model, where AZM interferes with the QS systems of *P. aeruginosa*, and its oral and intravenous administration removes bacteria from the renal tissue [148]. Interestingly, the combination of AZM with ciprofloxacin (one of the most effective antibiotics against *P. aeruginosa*) inhibits biofilm formation more efficiently compared to the activity displayed by the single compounds. In agreement, the oral administration of AZM (500 mg/Kg) and ciprofloxacin (30 mg/Kg) promoted an accelerated clearance of the bacteria from the kidney and bladder tissues in mice [149]. Although the antibiofilm mechanisms of the compounds *in vivo* were not specified, likely immunomodulatory and anti-inflammatory responses may be also involved [149]. Recently, Das and colleagues reported that in the FBM model, the intravenous administration of AZM and gentamicin potentiates the activity of an isolated flavone from *Vitex peduncularis* called vitexin, which reduced more efficiently the number of viable bacteria, than the groups that received a single-treatment. The authors suggested that the antibiofilm mechanism of these compounds is related to QS inhibition [150].

Moreover, there are also reports of new sources of natural products that show promising antibiofilm activities *in vivo*. For example, Alasil and colleagues identified different extracts from bacterial cultures that were able to inhibit *P. aeruginosa* QS. Using the PIM model in rats, it was shown that several of these extracts prolonged the life of the animals and facilitated the clearance of biofilm infections [151]. Similarly, positive results have been obtained to prevent the biofilm formation of *P. aeruginosa* with the combination of QQ and biofilm matrix-degrading enzymes. In this regard, Ivanova and colleagues, using the UTI model with rabbits, showed a positive effect by reducing the formation of biofilms by up to 70% in certain regions of catheters coated with the quorum quenching acylases and amylases (which degrades exopolysaccharides) [152].

Finally, although the *in vivo* antibiofilm activity of some QS inhibitory compounds is promising, clinical studies remain scarce. Also, it should be noted that today, with the

exception of 5-FU, no antibiofilm drug has been implemented in the clinic.

4. Conclusion

Taken together, the evidence summarized here suggests a strong link between the QS systems and biofilm formation of two of the main bacterial pathogens responsible for nosocomial infections, *P. aeruginosa* and *A. baumannii*. The evidence discussed suggests that interrupting QS in *A. baumannii* severely impairs its ability to form biofilms. In the case of *P. aeruginosa*, the evidence obtained with QS mutants and from using some of the QS inhibitors, indicates that inactivating QS severely affects biofilm robustness and its resistance against different stressors including antibiotics, although both wild-type and QS mutant strains still produce comparable levels of biofilms in the absence of stress. Hence, it is likely that an optimal way to implement QS inhibition is to treat bacterial infections mediated by biofilms with co-administration of antibiotics, since biofilm cells treated with QS inhibitors will be less tolerant against them than untreated biofilms, and also since it is likely that planktonic cells dispersed from the biofilms will be also less tolerant to the antibiotics. Presumably, QS enhances stress tolerance by promoting the expression of anti-oxidant enzymes such as catalase and SOD in both species [34, 55]. Another plausible mechanism is that cells in biofilms modify bacterial antibiotic sensitivity by mediating the biochemical composition of their membranes by increasing fatty acid cyclopropanation and saturation with the concomitant reduction in the level of *cis*-unsaturated fatty acids; these changes in turn increase bacterial tolerance levels against several kinds of stresses [153].

In agreement, with these proposed mechanisms, there are several examples of successful antibiofilm therapies in animal models when antibiofilm molecules are combined with antibiotics [149, 150]. Nevertheless, current evidence demonstrating that the *in vivo* activity of biofilm inhibitors is mediated by QS inhibition is still indirect, and further studies are needed in order to fully demonstrate it.

It is therefore important to investigate the degree of bacterial resistance and the selection of resistance against QS inhibitors and biofilm inhibitors *in vivo* using animal models as well as designing microfluidic devices to evaluate the antibiofilm compounds in clinical isolates. This is especially important since well-known quorum quenchers such as furanone C-30 present variable effects in clinical strains, from a severe inhibition of their growth *in vitro*, to no effect in growth. Also, there is variability in the effect of furanone C-30 in clinical isolates from a significant inhibition of the production of QS-dependent virulence factors to even their activation [60].

ACKNOWLEDGEMENTS

I C-J was supported by Fideicomiso-COLPOS 167304, Cátedras-CONACyT program and CONACYT Problemas Nacionales 2015 Program 2015-01-402, IA H-B was supported by a scholarship for undergraduate students (24008) from the SEP/CONACYT 152794 grant, M T was supported by the Miguel Servet II Programme (SERGAS and ISCIII), P C-T was supported by a scholarship for master students provided by CONACYT (430164), RG-C was supported by SEP/CONACYT 152794, UNAM-PAPIIT IA201116 and CONACYT Problemas Nacionales 2015 Program 2015-01-402. RG-C is grateful for the help of Beatriz Meraz from the Faculty of Medicine UNMA with the organization of the manuscript. T.K.W. is the Biotechnology Endowed Professor at the Pennsylvania State University.

References

[1] Castillo-Juarez, I.; Maeda, T.; Mandujano-Tinoco, E.A.; Tomas, M.; Perez-Eretza, B.; Garcia-Contreras, S.J.; Wood, T.K.; Garcia-Contreras, R., Role of quorum sensing in bacterial infections. *World journal of clinical cases,* **2015**, *3*, (7), 575-598.

[2] Poole, K., *Pseudomonas aeruginosa*: resistance to the max. *Front Microbiol,* **2011**, *2*, 65.

[3] Drenkard, E., Antimicrobial resistance of Pseudomonas aeruginosa biofilms. *Microbes and infection / Institut Pasteur,* **2003**, *5*, (13), 1213-1219.

[4] Lee, D.G.; Urbach, J.M.; Wu, G.; Liberati, N.T.; Feinbaum, R.L.; Miyata, S.; Diggins, L.T.; He, J.; Saucier, M.; Deziel, E.; Friedman, L.; Li, L.; Grills, G.; Montgomery, K.; Kucherlapati, R.; Rahme, L.G.; Ausubel, F.M., Genomic analysis reveals that *Pseudomonas aeruginosa* virulence is combinatorial. *Genome Biol,* **2006**, *7*, (10), R90.

[5] Grosso-Becerra, M.V.; Croda-García, G.; Merino, E.; Servin-Gonzalez, L.; Mojica-Espinosa, R.; Soberón-Chávez, G., Regulation of *Pseudomonas aeruginosa* virulence factors by two novel RNA thermometers. *Proceedings of the National Academy of Sciences of the United States of America,* **2014**, *111*, (43), 15562-15567.

[6] Mittal, R.; Sharma, S.; Chhibber, S.; Harjai, K., Iron dictates the virulence of Pseudomonas aeruginosa in urinary tract infections. *J Biomed Sci,* **2008**, *15*, (6), 731-741.

[7] Zaborin, A.; Romanowski, K.; Gerdes, S.; Holbrook, C.; Lepine, F.; Long, J.; Poroyko, V.; Diggle, S.P.; Wilke, A.; Righetti, K.; Morozova, I.; Babrowski, T.; Liu, D.C.; Zaborina, O.; Alverdy, J.C., Red death in *Caenorhabditis elegans* caused by *Pseudomonas aeruginosa* PAO1. *Proceedings of the National Academy of Sciences of the United States of America,* **2009**, *106*, (15), 6327-6332.

[8] Sarkisova, S.; Patrauchan, M.A.; Berglund, D.; Nivens, D.E.; Franklin, M.J., Calcium-induced virulence factors associated with the extracellular matrix of mucoid *Pseudomonas aeruginosa* biofilms. *Journal of bacteriology,* **2005**, *187*, (13), 4327-4337.

[9] Sheng, L.; Pu, M.; Hegde, M.; Zhang, Y.; Jayaraman, A.; Wood, T.K., Interkingdom adenosine signal reduces *Pseudomonas aeruginosa* pathogenicity. *Microb Biotechnol,* **2012**, *5*, (4), 560-572.

[10] Hegde, M.; Wood, T.K.; Jayaraman, A., The neuroendocrine hormone norepinephrine increases Pseudomonas aeruginosa PA14 virulence through the las quorum-sensing pathway. *Applied microbiology and biotechnology,* **2009**, *84*, (4), 763-776.

[11] Frydenlund Michelsen, C.; Hossein Khademi, S.M.; Krogh Johansen, H.; Ingmer, H.; Dorrestein, P.C.; Jelsbak, L., Evolution of metabolic divergence in Pseudomonas

aeruginosa during long-term infection facilitates a proto-cooperative interspecies interaction. *The ISME journal*, **2015**.
[12] Bergogne-Berezin, E.; Towner, K.J., Acinetobacter spp. as nosocomial pathogens: microbiological, clinical, and epidemiological features. *Clin Microbiol Rev,* **1996**, *9*, (2), 148-165.
[13] del Mar Tomas, M.; Cartelle, M.; Pertega, S.; Beceiro, A.; Llinares, P.; Canle, D.; Molina, F.; Villanueva, R.; Cisneros, J.M.; Bou, G., Hospital outbreak caused by a carbapenem-resistant strain of *Acinetobacter baumannii*: patient prognosis and risk-factors for colonisation and infection. *Clin Microbiol Infect,* **2005**, *11*, (7), 540-546.
[14] Hartstein, A.I.; Rashad, A.L.; Liebler, J.M.; Actis, L.A.; Freeman, J.; Rourke, J.W., Jr.; Stibolt, T.B.; Tolmasky, M.E.; Ellis, G.R.; Crosa, J.H., Multiple intensive care unit outbreak of *Acinetobacter calcoaceticus* subspecies anitratus respiratory infection and colonization associated with contaminated, reusable ventilator circuits and resuscitation bags. *Am J Med,* **1988**, *85*, (5), 624-631.
[15] Falagas, M.E.; Karveli, E.A., The changing global epidemiology of *Acinetobacter baumannii* infections: a development with major public health implications. *Clin Microbiol Infect,* **2007**, *13*, (2), 117-119.
[16] Falagas, M.E.; Bliziotis, I.A.; Siempos, II, Attributable mortality of *Acinetobacter baumannii* infections in critically ill patients: a systematic review of matched cohort and case-control studies. *Crit Care,* **2006**, *10*, (2), R48.
[17] Waters, C.M.; Bassler, B.L., Quorum sensing: cell-to-cell communication in bacteria. *Annu Rev Cell Dev Biol*, **2005**, *21*, 319-346.
[18] Smith, R.S.; Iglewski, B.H., *Pseudomonas aeruginosa* quorum sensing as a potential antimicrobial target. *J Clin Invest,* **2003**, *112*, (10), 1460-1465.
[19] Lupp, C.; Ruby, E.G., Vibrio fischeri uses two quorum-sensing systems for the regulation of early and late colonization factors. *Journal of bacteriology,* **2005**, *187*, (11), 3620-3629.
[20] Antunes, L.C.; Ferreira, R.B.; Buckner, M.M.; Finlay, B.B., Quorum sensing in bacterial virulence. *Microbiology,* **2010**, *156*, (Pt 8), 2271-2282.
[21] Castillo-Juárez, I.; Maeda, T.; Mandujano-Tinoco, E.A.; Tomas, M.; Párez-Eretza, B.; Garcia-Contreras, S.J.; Wood, T.K.; García-Contreras, R., Role of quorum sensing in bacterial infections. *World J Clin Cases,* **2015**, *3*, (7), 575-598.
[22] Tomasz, M., Mitomycin C: small, fast and deadly (but very selective). *Chem Biol*, **1995**, *2*, (9), 575-579.
[23] Verma, S.C.; Miyashiro, T., Quorum sensing in the squid-Vibrio symbiosis. *International journal of molecular sciences,* **2013**, *14*, (8), 16386-16401.
[24] Lee, J.; Zhang, L., The hierarchy quorum sensing network in *Pseudomonas aeruginosa*. *Protein Cell,* **2015**, *6*, (1), 26-41.
[25] Deziel, E.; Lepine, F.; Milot, S.; He, J.; Mindrinos, M.N.; Tompkins, R.G.; Rahme, L.G., Analysis of Pseudomonas aeruginosa 4-hydroxy-2-alkylquinolines (HAQs) reveals a role for 4-hydroxy-2-heptylquinoline in cell-to-cell communication. *Proceedings of the National Academy of Sciences of the United States of America,* **2004**, *101*, (5), 1339-1344.
[26] Hassett, D.J.; Ma, J.F.; Elkins, J.G.; McDermott, T.R.; Ochsner, U.A.; West, S.E.; Huang, C.T.; Fredericks, J.; Burnett, S.; Stewart, P.S.; McFeters, G.; Passador, L.; Iglewski, B.H., Quorum sensing in *Pseudomonas aeruginosa* controls expression of catalase and superoxide dismutase genes and mediates biofilm susceptibility to hydrogen peroxide. *Mol Microbiol,* **1999**, *34*, (5), 1082-1093.
[27] Kang, Y.S.; Jung, J.; Jeon, C.O.; Park, W., Acinetobacter oleivorans sp. nov. is capable of adhering to and growing on diesel-oil. *J Microbiol*, *49*, (1), 29-34.
[28] Stacy, D.M.; Welsh, M.A.; Rather, P.N.; Blackwell, H.E., Attenuation of quorum sensing in the pathogen Acinetobacter baumannii using non-native N-Acyl homoserine lactones. *ACS Chem Biol*, *7*, (10), 1719-1728.
[29] Gonzalez, R.H.; Dijkshoorn, L.; Van den Barselaar, M.; Nudel, C., Quorum sensing signal profile of Acinetobacter strains from nosocomial and environmental sources. *Rev Argent Microbiol,* **2009**, *41*, (2), 73-78.
[30] Irie, Y.; Parsek, M.R., Quorum sensing and microbial biofilms. *Current topics in microbiology and immunology,* **2008**, *322*, 67-84.
[31] Niu, C.; Clemmer, K.M.; Bonomo, R.A.; Rather, P.N., Isolation and characterization of an autoinducer synthase from *Acinetobacter baumannii*. *Journal of bacteriology,* **2008**, *190*, (9), 3386-3392.
[32] Suppiger, A.; Schmid, N.; Aguilar, C.; Pessi, G.; Eberl, L., Two quorum sensing systems control biofilm formation and virulence in members of the *Burkholderia cepacia* complex. *Virulence*, **2013**, *4*, (5), 400-409.
[33] Anbazhagan, D.; Mansor, M.; Yan, G.O.; Md Yusof, M.Y.; Hassan, H.; Sekaran, S.D., Detection of quorum sensing signal molecules and identification of an autoinducer synthase gene among biofilm forming clinical isolates of Acinetobacter spp. *PLoS One*, *7*, (7), e36696.
[34] Bhargava, N.; Sharma, P.; Capalash, N., Pyocyanin stimulates quorum sensing-mediated tolerance to oxidative stress and increases persister cell populations in *Acinetobacter baumannii*. *Infection and immunity,* **2014**, *82*, (8), 3417-3425.
[35] Ritenberg, M.; Nandi, S.; Kolusheva, S.; Dandela, R.; Meijler, M.M.; Jelinek, R., Imaging Pseudomonas aeruginosa Biofilm Extracellular Polymer Scaffolds with Amphiphilic Carbon Dots. *ACS chemical biology,* **2016**.
[36] Folsom, J.P.; Richards, L.; Pitts, B.; Roe, F.; Ehrlich, G.D.; Parker, A.; Mazurie, A.; Stewart, P.S., Physiology of *Pseudomonas aeruginosa* in biofilms as revealed by transcriptome analysis. *BMC microbiology,* **2010**, *10*, 294.
[37] Wei, Q.; Ma, L.Z., Biofilm matrix and its regulation in *Pseudomonas aeruginosa*. *International journal of molecular sciences,* **2013**, *14*, (10), 20983-21005.
[38] Zegans, M.E.; DiGiandomenico, A.; Ray, K.; Naimie, A.; Keller, A.E.; Stover, C.K.; Lalitha, P.; Srinivasan, M.; Acharya, N.R.; Lietman, T.M., Association of Biofilm Formation, Psl Exopolysaccharide Expression, and Clinical Outcomes in *Pseudomonas aeruginosa* Keratitis: Analysis of Isolates in the Steroids for Corneal Ulcers Trial. *JAMA ophthalmology,* **2016**, *134*, (4), 383-389.
[39] Bouffartigues, E.; Moscoso, J.A.; Duchesne, R.; Rosay, T.; Fito-Boncompte, L.; Gicquel, G.; Maillot, O.;

Benard, M.; Bazire, A.; Brenner-Weiss, G.; Lesouhaitier, O.; Lerouge, P.; Dufour, A.; Orange, N.; Feuilloley, M.G.; Overhage, J.; Filloux, A.; Chevalier, S., The absence of the Pseudomonas aeruginosa OprF protein leads to increased biofilm formation through variation in c-di-GMP level. *Frontiers in microbiology,* **2015**, *6*, 630.

[40] Jennings, L.K.; Storek, K.M.; Ledvina, H.E.; Coulon, C.; Marmont, L.S.; Sadovskaya, I.; Secor, P.R.; Tseng, B.S.; Scian, M.; Filloux, A.; Wozniak, D.J.; Howell, P.L.; Parsek, M.R., Pel is a cationic exopolysaccharide that cross-links extracellular DNA in the Pseudomonas aeruginosa biofilm matrix. *Proceedings of the National Academy of Sciences of the United States of America,* **2015**, *112*, (36), 11353-11358.

[41] Hazan, R.; Que, Y.A.; Maura, D.; Strobel, B.; Majcherczyk, P.A.; Hopper, L.R.; Wilbur, D.J.; Hreha, T.N.; Barquera, B.; Rahme, L.G., Auto Poisoning of the Respiratory Chain by a Quorum-Sensing-Regulated Molecule Favors Biofilm Formation and Antibiotic Tolerance. *Curr Biol,* **2016**, *26*, (2), 195-206.

[42] Anbazhagan, D.; Mansor, M.; Yan, G.O.; Md Yusof, M.Y.; Hassan, H.; Sekaran, S.D., Detection of quorum sensing signal molecules and identification of an autoinducer synthase gene among biofilm forming clinical isolates of Acinetobacter spp. *PloS one,* **2012**, *7*, (7), e36696.

[43] He, X.; Lu, F.; Yuan, F.; Jiang, D.; Zhao, P.; Zhu, J.; Cheng, H.; Cao, J.; Lu, G., Biofilm Formation Caused by Clinical Acinetobacter baumannii Isolates Is Associated with Overexpression of the AdeFGH Efflux Pump. *Antimicrobial agents and chemotherapy,* **2015**, *59*, (8), 4817-4825.

[44] Kvist, M.; Hancock, V.; Klemm, P., Inactivation of efflux pumps abolishes bacterial biofilm formation. *Applied and environmental microbiology,* **2008**, *74*, (23), 7376-7382.

[45] Richmond, G.E.; Evans, L.P.; Anderson, M.J.; Wand, M.E.; Bonney, L.C.; Ivens, A.; Chua, K.L.; Webber, M.A.; Sutton, J.M.; Peterson, M.L.; Piddock, L.J., The *Acinetobacter baumannii* Two-Component System AdeRS Regulates Genes Required for Multidrug Efflux, Biofilm Formation, and Virulence in a Strain-Specific Manner. *mBio,* **2016**, *7*, (2).

[46] Wang, S.; Yu, S.; Zhang, Z.; Wei, Q.; Yan, L.; Ai, G.; Liu, H.; Ma, L.Z., Coordination of swarming motility, biosurfactant synthesis, and biofilm matrix exopolysaccharide production in Pseudomonas aeruginosa. *Applied and environmental microbiology,* **2014**, *80*, (21), 6724-6732.

[47] Kim, S.K.; Lee, J.H., Biofilm dispersion in Pseudomonas aeruginosa. *Journal of microbiology,* **2016**, *54*, (2), 71-85.

[48] Diaz De Rienzo, M.A.; Stevenson, P.S.; Marchant, R.; Banat, I.M., Effect of biosurfactants on Pseudomonas aeruginosa and Staphylococcus aureus biofilms in a BioFlux channel. *Applied microbiology and biotechnology,* **2016**.

[49] Soberon-Chavez, G.; Lepine, F.; Deziel, E., Production of rhamnolipids by Pseudomonas aeruginosa. *Applied microbiology and biotechnology,* **2005**, *68*, (6), 718-725.

[50] Turnbull, L.; Toyofuku, M.; Hynen, A.L.; Kurosawa, M.; Pessi, G.; Petty, N.K.; Osvath, S.R.; Carcamo-Oyarce, G.; Gloag, E.S.; Shimoni, R.; Omasits, U.; Ito, S.; Yap, X.; Monahan, L.G.; Cavaliere, R.; Ahrens, C.H.; Charles, I.G.; Nomura, N.; Eberl, L.; Whitchurch, C.B., Explosive cell lysis as a mechanism for the biogenesis of bacterial membrane vesicles and biofilms. *Nature communications,* **2016**, *7*, 11220.

[51] Hentzer, M.; Wu, H.; Andersen, J.B.; Riedel, K.; Rasmussen, T.B.; Bagge, N.; Kumar, N.; Schembri, M.A.; Song, Z.; Kristoffersen, P.; Manefield, M.; Costerton, J.W.; Molin, S.; Eberl, L.; Steinberg, P.; Kjelleberg, S.; Hoiby, N.; Givskov, M., Attenuation of *Pseudomonas aeruginosa* virulence by quorum sensing inhibitors. *Embo J,* **2003**, *22*, (15), 3803-3815.

[52] Hentzer, M.; Riedel, K.; Rasmussen, T.B.; Heydorn, A.; Andersen, J.B.; Parsek, M.R.; Rice, S.A.; Eberl, L.; Molin, S.; Hoiby, N.; Kjelleberg, S.; Givskov, M., Inhibition of quorum sensing in *Pseudomonas aeruginosa* biofilm bacteria by a halogenated furanone compound. *Microbiology,* **2002**, *148*, (Pt 1), 87-102.

[53] Stacy, D.M.; Welsh, M.A.; Rather, P.N.; Blackwell, H.E., Attenuation of quorum sensing in the pathogen *Acinetobacter baumannii* using non-native N-Acyl homoserine lactones. *ACS Chem Biol,* **2012**, *7*, (10), 1719-1728.

[54] Bjarnsholt, T.; Jensen, P.O.; Burmolle, M.; Hentzer, M.; Haagensen, J.A.; Hougen, H.P.; Calum, H.; Madsen, K.G.; Moser, C.; Molin, S.; Hoiby, N.; Givskov, M., *Pseudomonas aeruginosa* tolerance to tobramycin, hydrogen peroxide and polymorphonuclear leukocytes is quorum-sensing dependent. *Microbiology,* **2005**, *151*, (Pt 2), 373-383.

[55] García-Contreras, R.; Nunez-López, L.; Jasso-Chávez, R.; Kwan, B.W.; Belmont, J.A.; Rangel-Vega, A.; Maeda, T.; Wood, T.K., Quorum sensing enhancement of the stress response promotes resistance to quorum quenching and prevents social cheating. *The ISME journal,* **2015**, *9*, (1), 115-125.

[56] Tetz, G.V.; Artemenko, N.K.; Tetz, V.V., Effect of DNase and antibiotics on biofilm characteristics. *Antimicrobial agents and chemotherapy,* **2009**, *53*, (3), 1204-1209.

[57] Maeda, T.; García-Contreras, R.; Pu, M.; Sheng, L.; Garcia, L.R.; Tomas, M.; Wood, T.K., Quorum quenching quandary: resistance to antivirulence compounds. *The ISME journal,* **2012**, *6*, (3), 493-501.

[58] Moore, J.D.; Gerdt, J.P.; Eibergen, N.R.; Blackwell, H.E., Active efflux influences the potency of quorum sensing inhibitors in Pseudomonas aeruginosa. *Chembiochem,* **2014**, *15*, (3), 435-442.

[59] García-Contreras, R.; Maeda, T.; Wood, T.K., Resistance to quorum-quenching compounds. *Applied and environmental microbiology,* **2013**, *79*, (22), 6840-6846.

[60] Garcia-Contreras, R.; Perez-Eretza, B.; Jasso-Chavez, R.; Lira-Silva, E.; Roldan-Sanchez, J.A.; Gonzalez-Valdez, A.; Soberon-Chavez, G.; Coria-Jimenez, R.; Martinez-Vazquez, M.; Alcaraz, L.D.; Maeda, T.; Wood, T.K., High variability in quorum quenching and growth inhibition by furanone C-30 in *Pseudomonas aeruginosa* clinical isolates from cystic fibrosis patients. *Pathogens and disease,* **2015**.

[61] García-Contreras, R.; Maeda, T.; Wood, T.K., Can resistance against quorum-sensing interference be selected? *The ISME journal,* **2016**, *10*, (1), 4-10.

[62] Reverchon, S.; Chantegrel, B.; Deshayes, C.;

Doutheau, A.; Cotte-Pattat, N., New synthetic analogues of N-acyl homoserine lactones as agonists or antagonists of transcriptional regulators involved in bacterial quorum sensing. *Bioorg Med Chem Lett*, **2002**, *12*, (8), 1153-1157.
[63] Smith, K.M.; Bu, Y.; Suga, H., Induction and inhibition of Pseudomonas aeruginosa quorum sensing by synthetic autoinducer analogs. *Chem Biol*, **2003**, *10*, (1), 81-89.
[64] Geske, G.D.; Mattmann, M.E.; Blackwell, H.E., Evaluation of a focused library of N-aryl L-homoserine lactones reveals a new set of potent quorum sensing modulators. *Bioorg Med Chem Lett*, **2008**, *18*, (22), 5978-5981.
[65] Geske, G.D.; O'Neill, J.C.; Miller, D.M.; Mattmann, M.E.; Blackwell, H.E., Modulation of bacterial quorum sensing with synthetic ligands: systematic evaluation of N-acylated homoserine lactones in multiple species and new insights into their mechanisms of action. *J Am Chem Soc*, **2007**, *129*, (44), 13613-13625.
[66] Starkey, M.; Lepine, F.; Maura, D.; Bandyopadhaya, A.; Lesic, B.; He, J.; Kitao, T.; Righi, V.; Milot, S.; Tzika, A.; Rahme, L., Identification of anti-virulence compounds that disrupt quorum-sensing regulated acute and persistent pathogenicity. *PLoS pathogens*, **2014**, *10*, (8), e1004321.
[67] Wood, T.K., Combatting bacterial persister cells. *Biotechnol Bioeng*, **2016**, *113*, (3), 476-483.
[68] Amara, N.; Mashiach, R.; Amar, D.; Krief, P.; Spieser, S.A.; Bottomley, M.J.; Aharoni, A.; Meijler, M.M., Covalent inhibition of bacterial quorum sensing. *J Am Chem Soc*, **2009**, *131*, (30), 10610-10619.
[69] Parsek, M.R.; Val, D.L.; Hanzelka, B.L.; Cronan, J.E., Jr.; Greenberg, E.P., Acyl homoserine-lactone quorum-sensing signal generation. *Proceedings of the National Academy of Sciences of the United States of America*, **1999**, *96*, (8), 4360-4365.
[70] Diggle, S.P.; Matthijs, S.; Wright, V.J.; Fletcher, M.P.; Chhabra, S.R.; Lamont, I.L.; Kong, X.; Hider, R.C.; Cornelis, P.; Camara, M.; Williams, P., The Pseudomonas aeruginosa 4-quinolone signal molecules HHQ and PQS play multifunctional roles in quorum sensing and iron entrapment. *Chem Biol*, **2007**, *14*, (1), 87-96.
[71] Calfee, M.W.; Coleman, J.P.; Pesci, E.C., Interference with Pseudomonas quinolone signal synthesis inhibits virulence factor expression by Pseudomonas aeruginosa. *Proceedings of the National Academy of Sciences of the United States of America*, **2001**, *98*, (20), 11633-11637.
[72] Lesic, B.; Lepine, F.; Deziel, E.; Zhang, J.; Zhang, Q.; Padfield, K.; Castonguay, M.H.; Milot, S.; Stachel, S.; Tzika, A.A.; Tompkins, R.G.; Rahme, L.G., Inhibitors of pathogen intercellular signals as selective anti-infective compounds. *PLoS pathogens*, **2007**, *3*, (9), 1229-1239.
[73] Rasmussen, T.B.; Givskov, M., Quorum-sensing inhibitors as anti-pathogenic drugs. *Int J Med Microbiol*, **2006**, *296*, (2-3), 149-161.
[74] Chan, K.G.; Liu, Y.C.; Chang, C.Y., Inhibiting N-acyl-homoserine lactone synthesis and quenching Pseudomonas quinolone quorum sensing to attenuate virulence. *Front Microbiol*, **2015**, *6*, 1173.
[75] Grandclement, C.; Tannieres, M.; Morera, S.; Dessaux, Y.; Faure, D., Quorum quenching: role in nature and applied developments. *FEMS Microbiol Rev*, **2016**, *40*, (1), 86-116.
[76] Yates, E.A.; Philipp, B.; Buckley, C.; Atkinson, S.; Chhabra, S.R.; Sockett, R.E.; Goldner, M.; Dessaux, Y.; Camara, M.; Smith, H.; Williams, P., N-acylhomoserine lactones undergo lactonolysis in a pH-, temperature-, and acyl chain length-dependent manner during growth of Yersinia pseudotuberculosis and Pseudomonas aeruginosa. *Infect Immun*, **2002**, *70*, (10), 5635-5646.
[77] Delalande, L.; Faure, D.; Raffoux, A.; Uroz, S.; D'Angelo-Picard, C.; Elasri, M.; Carlier, A.; Berruyer, R.; Petit, A.; Williams, P.; Dessaux, Y., N-hexanoyl-L-homoserine lactone, a mediator of bacterial quorum-sensing regulation, exhibits plant-dependent stability and may be inactivated by germinating Lotus corniculatus seedlings. *FEMS Microbiol Ecol*, **2005**, *52*, (1), 13-20.
[78] Dong, Y.H.; Xu, J.L.; Li, X.Z.; Zhang, L.H., AiiA, an enzyme that inactivates the acylhomoserine lactone quorum-sensing signal and attenuates the virulence of Erwinia carotovora. *Proceedings of the National Academy of Sciences*, **2000**, *97*, (7), 3526-3531.
[79] Dong, Y.H.; Wang, L.H.; Xu, J.L.; Zhang, H.B.; Zhang, X.F.; Zhang, L.H., Quenching quorum-sensing-dependent bacterial infection by an N-acyl homoserine lactonase. *Nature*, **2001**, *411*, (6839), 813-817.
[80] Liu, D.; Lepore, B.W.; Petsko, G.A.; Thomas, P.W.; Stone, E.M.; Fast, W.; Ringe, D., Three-dimensional structure of the quorum-quenching N-acyl homoserine lactone hydrolase from Bacillus thuringiensis. *Proc Natl Acad Sci U S A*, **2005**, *102*, (33), 11882-11887.
[81] Dong, Y.H.; Gusti, A.R.; Zhang, Q.; Xu, J.L.; Zhang, L.H., Identification of quorum-quenching N-acyl homoserine lactonases from Bacillus species. *Appl Environ Microbiol*, **2002**, *68*, (4), 1754-1759.
[82] Lee, S.J.; Park, S.Y.; Lee, J.J.; Yum, D.Y.; Koo, B.T.; Lee, J.K., Genes encoding the N-acyl homoserine lactone-degrading enzyme are widespread in many subspecies of Bacillus thuringiensis. *Appl Environ Microbiol*, **2002**, *68*, (8), 3919-3924.
[83] Zhang, H.B.; Wang, L.H.; Zhang, L.H., Genetic control of quorum-sensing signal turnover in Agrobacterium tumefaciens. *Proc Natl Acad Sci U S A*, **2002**, *99*, (7), 4638-4643.
[84] Carlier, A.; Uroz, S.; Smadja, B.; Fray, R.; Latour, X.; Dessaux, Y.; Faure, D., The Ti Plasmid of Agrobacterium tumefaciens Harbors an attM-Paralogous Gene, aiiB, Also Encoding N-Acyl Homoserine Lactonase Activity. *Applied and Environmental Microbiology*, **2003**, *69*, (8), 4989-4993.
[85] Dong, Y.H.; Zhang, L.H., Quorum sensing and quorum-quenching enzymes. *J Microbiol*, **2005**, *43 Spec No*, 101-109.
[86] Afriat, L.; Roodveldt, C.; Manco, G.; Tawfik, D.S., The latent promiscuity of newly identified microbial lactonases is linked to a recently diverged phosphotriesterase. *Biochemistry*, **2006**, *45*, (46), 13677-13686.
[87] Fetzner, S., Quorum quenching enzymes. *J Biotechnol*, **2015**, *201*, 2-14.
[88] Elias, M.; Dupuy, J.; Merone, L.; Mandrich, L.; Porzio, E.; Moniot, S.; Rochu, D.; Lecomte, C.; Rossi, M.; Masson, P.; Manco, G.; Chabriere, E., Structural basis for

natural lactonase and promiscuous phosphotriesterase activities. *J Mol Biol*, **2008**, *379*, (5), 1017-1028.
[89] Uroz, S.; Oger, P.M.; Chapelle, E.; Adeline, M.T.; Faure, D.; Dessaux, Y., A Rhodococcus qsdA-encoded enzyme defines a novel class of large-spectrum quorum-quenching lactonases. *Appl Environ Microbiol*, **2008**, *74*, (5), 1357-1366.
[90] Hiblot, J.; Gotthard, G.; Champion, C.; Chabriere, E.; Elias, M., Crystallization and preliminary X-ray diffraction analysis of the lactonase VmoLac from Vulcanisaeta moutnovskia. *Acta Crystallogr Sect F Struct Biol Cryst Commun*, **2013**, *69*, (Pt 11), 1235-1238.
[91] Chow, J.Y.; Yang, Y.; Tay, S.B.; Chua, K.L.; Yew, W.S., Disruption of biofilm formation by the human pathogen Acinetobacter baumannii using engineered quorum-quenching lactonases. *Antimicrobial agents and chemotherapy*, **2014**, *58*, (3), 1802-1805.
[92] Leadbetter, J.R.; Greenberg, E.P., Metabolism of Acyl-Homoserine Lactone Quorum-Sensing Signals by Variovorax paradoxus. *Journal of Bacteriology*, **2000**, *182*, (24), 6921-6926.
[93] Lin, Y.H.; Xu, J.L.; Hu, J.; Wang, L.H.; Ong, S.L.; Leadbetter, J.R.; Zhang, L.H., Acyl-homoserine lactone acylase from Ralstonia strain XJ12B represents a novel and potent class of quorum-quenching enzymes. *Mol Microbiol*, **2003**, *47*, (3), 849-860.
[94] Leadbetter, J.R.; Greenberg, E.P., Metabolism of acyl-homoserine lactone quorum-sensing signals by Variovorax paradoxus. *J Bacteriol*, **2000**, *182*, (24), 6921-6926.
[95] Huang, J.J.; Han, J.I.; Zhang, L.H.; Leadbetter, J.R., Utilization of Acyl-Homoserine Lactone Quorum Signals for Growth by a Soil Pseudomonad and Pseudomonas aeruginosa PAO1. *Applied and Environmental Microbiology*, **2003**, *69*, (10), 5941-5949.
[96] Chen, C.N.; Chen, C.J.; Liao, C.T.; Lee, C.Y., A probable aculeacin A acylase from the Ralstonia solanacearum GMI1000 is N-acyl-homoserine lactone acylase with quorum-quenching activity. *BMC microbiology*, **2009**, *9*, 89.
[97] Shepherd, R.W.; Lindow, S.E., Two dissimilar N-acyl-homoserine lactone acylases of Pseudomonas syringae influence colony and biofilm morphology. *Appl Environ Microbiol*, **2009**, *75*, (1), 45-53.
[98] Uroz, S.; Oger, P.; Chhabra, S.R.; Camara, M.; Williams, P.; Dessaux, Y., N-acyl homoserine lactones are degraded via an amidolytic activity in Comamonas sp. strain D1. *Archives of microbiology*, **2007**, *187*, (3), 249-256.
[99] Czajkowski, R.; Krzyzanowska, D.; Karczewska, J.; Atkinson, S.; Przysowa, J.; Lojkowska, E.; Williams, P.; Jafra, S., Inactivation of AHLs by Ochrobactrum sp. A44 depends on the activity of a novel class of AHL acylase. *Environmental microbiology reports*, **2011**, *3*, (1), 59-68.
[100] Park, S.Y.; Kang, H.O.; Jang, H.S.; Lee, J.K.; Koo, B.T.; Yum, D.Y., Identification of extracellular N-acylhomoserine lactone acylase from a Streptomyces sp. and its application to quorum quenching. *Appl Environ Microbiol*, **2005**, *71*, (5), 2632-2641.
[101] Bokhove, M.; Nadal Jimenez, P.; Quax, W.J.; Dijkstra, B.W., The quorum-quenching N-acyl homoserine lactone acylase PvdQ is an Ntn-hydrolase with an unusual substrate-binding pocket. *Proc Natl Acad Sci U S A*, **2010**, *107*, (2), 686-691.
[102] Furlong, C.E.; Marsillach, J.; Jarvik, G.P.; Costa, L.G., Paraoxonases-1, -2 and -3: What are their functions? *Chemico-biological interactions*, **2016**.
[103] Chun, C.K.; Ozer, E.A.; Welsh, M.J.; Zabner, J.; Greenberg, E.P., Inactivation of a Pseudomonas aeruginosa quorum-sensing signal by human airway epithelia. *Proc Natl Acad Sci U S A*, **2004**, *101*, (10), 3587-3590.
[104] Yang, F.; Wang, L.H.; Wang, J.; Dong, Y.H.; Hu, J.Y.; Zhang, L.H., Quorum quenching enzyme activity is widely conserved in the sera of mammalian species. *FEBS letters*, **2005**, *579*, (17), 3713-3717.
[105] Draganov, D.I.; La Du, B.N., Pharmacogenetics of paraoxonases: a brief review. *Naunyn-Schmiedeberg's archives of pharmacology*, **2004**, *369*, (1), 78-88.
[106] Lee, H.H.; Molla, M.N.; Cantor, C.R.; Collins, J.J., Bacterial charity work leads to population-wide resistance. *Nature*, **2010**, *467*, (7311), 82-85.
[107] Vega, N.M.; Allison, K.R.; Khalil, A.S.; Collins, J.J., Signaling-mediated bacterial persister formation. *Nature chemical biology*, **2012**, *8*, (5), 431-433.
[108] Lee, J.; Bansal, T.; Jayaraman, A.; Bentley, W.E.; Wood, T.K., Enterohemorrhagic Escherichia coli biofilms are inhibited by 7-hydroxyindole and stimulated by isatin. *Applied and environmental microbiology*, **2007**, *73*, (13), 4100-4109.
[109] Lee, J.-H.; Lee, J., Indole as an intercellular signal in microbial community. *FEMS Microbiol Rev*, **2010**, *34*, 426-444.
[110] Lee, J.H.; Wood, T.K.; Lee, J., Roles of lndole as an Interspecies and Interkingdom Signaling Molecule. *Trends in Microbiology*, **2015**, *23*, (11), 707-718.
[111] Lee, J.; Attila, C.; Cirillo, S.L.G.; Cirillo, J.D.; Wood, T.K., Indole and 7-hydroxyindole diminish *Pseudomonas aeruginosa* virulence. *Microbial Biotech* **2009**, *2*, 75-90.
[112] Kim, J.; Park, W., Indole inhibits bacterial quorum sensing signal transmission by interfering with quorum sensing regulator folding. *Microbiology*, **2013**, *159*, (Pt 12), 2616-2625.
[113] Vega, N.M.; Allison, K.R.; Samuels, A.N.; Klempner, M.S.; Collins, J.J., Salmonella typhimurium intercepts Escherichia coli signaling to enhance antibiotic tolerance. *Proceedings of the National Academy of Sciences of the United States of America*, **2013**, *110*, (35), 14420-14425.
[114] Molina-Santiago, C.; Daddaoua, A.; Fillet, S.; Duque, E.; Ramos, J.L., Interspecies signalling: Pseudomonas putida efflux pump TtgGHI is activated by indole to increase antibiotic resistance. *Environmental microbiology*, **2014**, *16*, (5), 1267-1281.
[115] Lee, J.-H.; Kim, Y.-G.; Baek, K.H.; Cho, M.H.; Lee, J., The multifaceted roles of the interspecies signalling molecule indole in *Agrobacterium tumefaciens*. *Environ Microbiol*, **2015**, *17*, (4), 1234-1244.
[116] Lee, J.-H.; Cho, M.H.; Lee, J., 3-Indolylacetonitrile decreases *Escherichia coli* O157:H7 biofilm formation and *Pseudomonas aeruginosa* virulence. *Environ Microbiol*, **2011**, *13*, 62-73.
[117] Lee, J.-H.; Kim, Y.-G.; Cho, M.H.; Kim, J.-A.; Lee,

J., 7-Fluoroindole as an antivirulence compound against *Pseudomonas aeruginosa*. *FEMS Microbiol Lett,* **2012**, *329*, (1), 36-44.
[118] Bunders, C.; Cavanagh, J.; Melander, C., Flustramine inspired synthesis and biological evaluation of pyrroloindoline triazole amides as novel inhibitors of bacterial biofilms. *Organic & Biomolecular Chemistry,* **2011**, *9*, (15), 5476-5481.
[119] Minvielle, M.J.; Bunders, C.A.; Melander, C., Indole/triazole conjugates are selective inhibitors and inducers of bacterial biofilms. *MedChemComm,* **2013**, *4*, (6), 916-919.
[120] Frei, R.; Breitbach, A.S.; Blackwell, H.E., 2-Aminobenzimidazole Derivatives Strongly Inhibit and Disperse *Pseudomonas aeruginosa* Biofilms. *Angewandte Chemie-International Edition,* **2012**, *51*, (21), 5226-5229.
[121] Bunders, C.A.; Minvielle, M.J.; Worthington, R.J.; Ortiz, M.; Cavanagh, J.; Melander, C., Intercepting bacterial indole signaling with flustramine derivatives. *Journal of the American Chemical Society,* **2011**, *133*, (50), 20160-20163.
[122] Biswas, N.N.; Kutty, S.K.; Barraud, N.; Iskander, G.M.; Griffith, R.; Rice, S.A.; Willcox, M.; Black, D.S.; Kumar, N., Indole-based novel small molecules for the modulation of bacterial signalling pathways. *Organic & biomolecular chemistry,* **2015**, *13*, (3), 925-937.
[123] Oh, S.; Go, G.W.; Mylonakis, E.; Kim, Y., The bacterial signalling molecule indole attenuates the virulence of the fungal pathogen Candida albicans. *Journal of applied microbiology,* **2012**, *113*, (3), 622-628.
[124] Lee, J.-H.; Cho, H.S.; Kim, Y.; Kim, J.-A.; Banskota, S.; Cho, M.H.; Lee, J., Indole and 7-benzyloxyindole attenuate the virulence of *Staphylococcus aureus*. *Applied microbiology and biotechnology,* **2013**, *97*, (10), 4543-4552.
[125] Ueda, A.; Attila, C.; Whiteley, M.; Wood, T.K., Uracil influences quorum sensing and biofilm formation in *Pseudomonas aeruginosa* and fluorouracil is an antagonist. *Microb Biotechnol,* **2009**, *2*, (1), 62-74.
[126] Walz, J.M.; Avelar, R.L.; Longtine, K.J.; Carter, K.L.; Mermel, L.A.; Heard, S.O., Anti-infective external coating of central venous catheters: a randomized, noninferiority trial comparing 5-fluorouracil with chlorhexidine/silver sulfadiazine in preventing catheter colonization. *Crit Care Med,* **2010**, *38*, (11), 2095-2102.
[127] Rangel-Vega, A.; Bernstein, L.R.; Mandujano-Tinoco, E.A.; Garcia-Contreras, S.J.; Garcia-Contreras, R., Drug repurposing as an alternative for the treatment of recalcitrant bacterial infections. *Front Microbiol,* **2015**, *6*, 282.
[128] Lee, J.H.; Kim, Y.G.; Cho, M.H.; Lee, J., ZnO nanoparticles inhibit *Pseudomonas aeruginosa* biofilm formation and virulence factor production. *Microbiol Res,* **2014**.
[129] García-Lara, B.; Saucedo-Mora, M.A.; Roldan-Sanchez, J.A.; Pérez-Eretza, B.; Ramasamy, M.; Lee, J.; Coria-Jimenez, R.; Tapia, M.; Varela-Guerrero, V.; García-Contreras, R., Inhibition of quorum-sensing-dependent virulence factors and biofilm formation of clinical and environmental *Pseudomonas aeruginosa* strains by ZnO nanoparticles. *Letters in applied microbiology,* **2015**, *61*, (3), 299-305.
[130] García-Contreras, R.; Martinez-Vazquez, M.; Velazquez Guadarrama, N.; Villegas Paneda, A.G.; Hashimoto, T.; Maeda, T.; Quezada, H.; Wood, T.K., Resistance to the quorum-quenching compounds brominated furanone C-30 and 5-fluorouracil in *Pseudomonas aeruginosa* clinical isolates. *Pathogens and disease,* **2013**, *68*, (1), 8-11.
[131] García-Contreras, R.; Lira-Silva, E.; Jasso-Chavez, R.; Hernandez-Gonzalez, I.L.; Maeda, T.; Hashimoto, T.; Boogerd, F.C.; Sheng, L.; Wood, T.K.; Moreno-Sanchez, R., Isolation and characterization of gallium resistant *Pseudomonas aeruginosa* mutants. *Int J Med Microbiol,* **2013**, *303*, (8), 574-582.
[132] Kim, H.S.; Lee, S.H.; Byun, Y.; Park, H.D., 6-Gingerol reduces Pseudomonas aeruginosa biofilm formation and virulence via quorum sensing inhibition. *Sci Rep,* **2015**, *5*, 8656.
[133] Deng, Y.; Boon, C.; Chen, S.; Lim, A.; Zhang, L.H., Cis-2-dodecenoic acid signal modulates virulence of Pseudomonas aeruginosa through interference with quorum sensing systems and T3SS. *BMC Microbiol,* **2013**, *13*, 231.
[134] Saroj, S.D.; Rather, P.N., Streptomycin inhibits quorum sensing in Acinetobacter baumannii. *Antimicrobial agents and chemotherapy,* **2013**, *57*, (4), 1926-1929.
[135] Wu, H.; Song, Z.; Hentzer, M.; Andersen, J.B.; Molin, S.; Givskov, M.; Hoiby, N., Synthetic furanones inhibit quorum-sensing and enhance bacterial clearance in *Pseudomonas aeruginosa* lung infection in mice. *J Antimicrob Chemother,* **2004**, *53*, (6), 1054-1061.
[136] Hoffmann, N.; Rasmussen, T.B.; Jensen, P.O.; Stub, C.; Hentzer, M.; Molin, S.; Ciofu, O.; Givskov, M.; Johansen, H.K.; Hoiby, N., Novel mouse model of chronic *Pseudomonas aeruginosa* lung infection mimicking cystic fibrosis. *Infection and immunity,* **2005**, *73*, (4), 2504-2514.
[137] Christensen, L.D.; Moser, C.; Jensen, P.O.; Rasmussen, T.B.; Christophersen, L.; Kjelleberg, S.; Kumar, N.; Hoiby, N.; Givskov, M.; Bjarnsholt, T., Impact of *Pseudomonas aeruginosa* quorum sensing on biofilm persistence in an in vivo intraperitoneal foreign-body infection model. *Microbiology,* **2007**, *153*, (Pt 7), 2312-2320.
[138] Bjarnsholt, T.; van Gennip, M.; Jakobsen, T.H.; Christensen, L.D.; Jensen, P.O.; Givskov, M., In vitro screens for quorum sensing inhibitors and in vivo confirmation of their effect. *Nat Protoc,* **2010**, *5*, (2), 282-293.
[139] Papaioannou, E.; Utari, P.D.; Quax, W.J., Choosing an appropriate infection model to study quorum sensing inhibition in Pseudomonas infections. *International journal of molecular sciences,* **2013**, *14*, (9), 19309-19340.
[140] Harjai, K.; Kumar, R.; Singh, S., Garlic blocks quorum sensing and attenuates the virulence of Pseudomonas aeruginosa. *FEMS immunology and medical microbiology,* **2010**, *58*, (2), 161-168.
[141] Jakobsen, T.H.; van Gennip, M.; Phipps, R.K.; Shanmugham, M.S.; Christensen, L.D.; Alhede, M.; Skindersoe, M.E.; Rasmussen, T.B.; Friedrich, K.; Uthe, F.; Jensen, P.O.; Moser, C.; Nielsen, K.F.; Eberl, L.; Larsen, T.O.; Tanner, D.; Hoiby, N.; Bjarnsholt, T.; Givskov, M., Ajoene, a sulfur-rich molecule from garlic, inhibits genes controlled by quorum sensing. *Antimicrobial agents and chemotherapy,* **2011**, *56*, (5), 2314-2325.
[142] Smyth, A.R.; Cifelli, P.M.; Ortori, C.A.; Righetti,

K.; Lewis, S.; Erskine, P.; Holland, E.D.; Givskov, M.; Williams, P.; Camara, M.; Barrett, D.A.; Knox, A., Garlic as an inhibitor of Pseudomonas aeruginosa quorum sensing in cystic fibrosis--a pilot randomized controlled trial. *Pediatric pulmonology,* **2010**, *45*, (4), 356-362.

[143] Christensen, L.D.; van Gennip, M.; Jakobsen, T.H.; Alhede, M.; Hougen, H.P.; Hoiby, N.; Bjarnsholt, T.; Givskov, M., Synergistic antibacterial efficacy of early combination treatment with tobramycin and quorum-sensing inhibitors against Pseudomonas aeruginosa in an intraperitoneal foreign-body infection mouse model. *J Antimicrob Chemother,* **2012**, *67*, (5), 1198-1206.

[144] Savoia, D., New Antimicrobial Approaches: Reuse of Old Drugs. *Curr Drug Targets,* **2016**, *17*, (6), 731-738.

[145] Tateda, K.; Comte, R.; Pechere, J.C.; Kohler, T.; Yamaguchi, K.; Van Delden, C., Azithromycin inhibits quorum sensing in Pseudomonas aeruginosa. *Antimicrobial agents and chemotherapy,* **2001**, *45*, (6), 1930-1933.

[146] Hoffmann, N.; Lee, B.; Hentzer, M.; Rasmussen, T.B.; Song, Z.; Johansen, H.K.; Givskov, M.; Hoiby, N., Azithromycin blocks quorum sensing and alginate polymer formation and increases the sensitivity to serum and stationary-growth-phase killing of *Pseudomonas aeruginosa* and attenuates chronic *P. aeruginosa* lung infection in Cftr(-/-) mice. *Antimicrobial agents and chemotherapy,* **2007**, *51*, (10), 3677-3687.

[147] Wolter, J.; Seeney, S.; Bell, S.; Bowler, S.; Masel, P.; McCormack, J., Effect of long term treatment with azithromycin on disease parameters in cystic fibrosis: a randomised trial. *Thorax,* **2002**, *57*, (3), 212-216.

[148] Bala, A.; Kumar, R.; Harjai, K., Inhibition of quorum sensing in Pseudomonas aeruginosa by azithromycin and its effectiveness in urinary tract infections. *J Med Microbiol,* **2011**, *60*, (Pt 3), 300-306.

[149] Saini, H.; Chhibber, S.; Harjai, K., Azithromycin and ciprofloxacin: a possible synergistic combination against Pseudomonas aeruginosa biofilm-associated urinary tract infections. *Int J Antimicrob Agents,* **2015**, *45*, (4), 359-367.

[150] Das, M.C.; Sandhu, P.; Gupta, P.; Rudrapaul, P.; De, U.C.; Tribedi, P.; Akhter, Y.; Bhattacharjee, S., Attenuation of Pseudomonas aeruginosa biofilm formation by Vitexin: A combinatorial study with azithromycin and gentamicin. *Sci Rep,* **2016**, *6*, 23347.

[151] Alasil, S.M.; Omar, R.; Ismail, S.; Yusof, M.Y., Inhibition of Quorum Sensing-Controlled Virulence Factors and Biofilm Formation in *Pseudomonas aeruginosa* by Culture Extract from Novel Bacterial Species of Paenibacillus Using a Rat Model of Chronic Lung Infection. *Int J Bacteriol,* **2015**, *2015*, 671562.

[152] Ivanova, K.; Fernandes, M.M.; Mendoza, E.; Tzanov, T., Enzyme multilayer coatings inhibit Pseudomonas aeruginosa biofilm formation on urinary catheters. *Appl Microbiol Biotechnol,* **2015**, *99*, (10), 4373-4385.

[153] Davenport, P.W.; Griffin, J.L.; Welch, M., Quorum Sensing Is Accompanied by Global Metabolic Changes in the Opportunistic Human Pathogen *Pseudomonas aeruginosa*. *Journal of bacteriology,* **2015**, *197*, (12), 2072-2082.

Received: Revised: Accepted:

Figure legend

A: furanone C-30; **B**: furanone C-56; **C**: itc-12; **D**: indole; **E**: 3-indolylacetonitrile; **F**: 7-fluoroindole; **G**: flustramine; **H**: indole/triazole conjugates; **I**: 2-aminobenzimidazole; **J**: desformylflustrabromine; **K**: indole/N-acylated L-homoserine lactone conjugates; **L**: anthranilic acid; **M**: 2-amino-6-fluorobenzoic acid (6FABA); **N**: 2-amino-6-chloro benzoic acid (6CABA); **O**: 2-amino-4 chlorobenzoic acid (4CABA); **P**: 5-fluorouracil; **Q**: 6-gingerol; **R**: cis-2-dodecenoic; **S**: ajoene (ajoene 4,5,9,-trithiadodeca-1,6,11-triene-9-oxide); **T**: azithromycin.