Patents on Quorum Quenching: Interfering with bacterial communication as a strategy to fight infections

Running Title: Fighting bacteria with quorum quenching

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Abstract

Numerous bacterial functions, such as virulence and biofilm formation, are controlled by a cell density-dependent communication mechanism known as Quorum Sensing (QS), in which small diffusible molecules are released, allowing bacteria to coordinate their behavior once a minimal effective quorum has been reached. The interference with these signaling systems, also known as Quorum Quenching (QQ), represents a promising strategy to tackle bacterial infections. The growing interest in this approach is reflected by the increasing number of patents within the field (45 up to now), especially in the last few years, as shown by patent applications published since 2009. The fact that biofilm formation is also controlled by QS systems expands the application of QQ to clinically-relevant biofilms such as those responsible for periodontal disease. Moreover, since biofilms increase bacterial resistance to antimicrobials, QQ could represent a new way to fight some of the most recurrent human pathogens, such as nosocomial multiresistant strains, and this deserves further exploration, especially through more proofs of concept. In this article we review the best known QS and QQ systems to date and we describe recent patents on the interference with this type of bacterial communication.

Keywords

Acylase, agonist, AHL, AI-2, antagonist, bacterial communication, biofilm, lactonase, peptide, quorum quenching, quorum sensing, signal, virulence.

1. Quorum sensing: bacteria communicate to infect us

Numerous bacterial species use a coordinated genetic regulation mechanism to monitor their population density and establish a cooperative behavior. This mechanism, known as quorum sensing (QS), consists in the production and release of signal molecules to the medium where they accumulate, triggering the expression of multiple genes once a threshold concentration has been achieved [1]. By means of QS communication, the bacterial populations can be coordinated in order to execute important biological functions, many of them involved in the virulence of pathogens, such as: mobility, swarming, aggregation, luminescence, biosynthesis of antibiotics, virulence factors, symbiosis, transfer of plasmids by conjugation, etc. [2, 3]. Of special significance is the role of QS processes in the formation and differentiation of bacterial biofilms [4, 2], since these organized structures are involved in antibiotic resistance in several important pathogens, and constitute the basis of other important biological problems such as fouling and corrosion, etc. The role of QS in the formation of dental plaque, the most complex and significant biofilm related to human health, has also been demonstrated in oral pathogens related with aggressive periodontitis [5], and in different species of caries related *Streptococcus* [6].

Different types of QS signals, also known as autoinducers (AIs) have been identified, most of them being small organic molecules that either diffuse freely to the media or are secreted by specific membrane transporters [2].

The QS signals most widely studied and known are *N*-acyl-homoserine lactones (AHLs) used by numerous Gram-negative bacteria [2]. AHLs are a family of signal molecules which are based on an homoserine lactone ring (HSL) with a side acyl chain of variable size usually between 4 and 14 carbons, with or without saturation and with or without Oxo- or Hydroxy- substitutions in the third carbon [2]. The bioluminescent bacterium *Vibrio fischeri* constitutes the model of the AHL-based QS system that is composed by two proteins, the signal synthase LuxI responsible for the AHL production, and LuxR, a cell density dependent transcription regulator, which is the receptor of the AHL. This was the first QS mechanism described [7] and became the paradigm for the latter discovered QS systems in Gram-negative bacteria, including numerous animal and plant pathogens.

Gram-positive bacteria mainly use modified oligopeptides of 5-20 amino acids as QS signals [8]. While the AHL receptors are usually cytoplasmic, the oligopeptide receptors in Gram-positive bacteria are membrane bound and the signal transduction occurs through phosphorylation cascades [9]. Examples of peptide-based QS systems are the ComD/ComE system of *Streptococcus pneumoniae* that controls competence development [10], the ComP/ComA system of *Bacillus subtilis* that controls competence and sporulation [11] and the AgrC/AgrA system of *Staphylococcus aureus* that controls pathogenesis and constitutes the paradigm for signal transduction in oligopeptide based QS systems [Reviewed in 12].

Another QS system was discovered in the marine bioluminescent bacterium *Vibrio harveyi*, in which an AHL-mediated quorum sensing is also present. This second QS system is dependent on a diester furanosyl borate called Autoinducer-2 [AI-2, 13]. AI-2 is a collective term for a group of interconvertible furanones derived from dihydroxypentanedione (DPD). AI-2 regulates a wide range of genes with diverse functions, like virulence in enterohaemorrhagic *Escherichia coli* [14] and biofilm formation in *Streptococcus gordonii* [15]. The growing number of Gram-positive and Gram-negative bacteria that

produce AI-2 has led to the suggestion that that AI-2 could be a universal language for interspecies communication [16].

Other specific intercellular communication models have been described, like the Gamma-butyrolactones (GBLs) that control sporulation and antibiotic production in *Streptomyces* [17]. In other cases, as in the phytopathogen *Ralstonia solanaceum*, the expression of virulence factors respond to the concentration of a volatile signal: the methyl-ester of 3-hydroxypalmitic acid (PAME) [18]. In another Gram-negative phytopathogen, *Xanthomonas campestris*, an AI named DSF (diffusible signal factor) with structure similar to fatty acids, is involved in the expression of virulence factors like proteases [19].
In some cases several QS systems act simultaneously or in cascade to control the expression of different genes. In *Pseudomonas aeruginosa*, besides the AHL-based mechanism, that is composed by two different systems that act in cascade, other signals like diketopiperazines (DKPs) and a quinolone signal (PQS) are involved in the detection of population density [20]. In *Vibrio cholerae*, the CAI-1 quorum sensing pathway, mediated through the CqsS receptor, exists in parallel with the AI-2 pathway [21].
Even though the intercellular cense systems have been initially discovered in prokaryotes, several QS mechanisms have been described also in eukaryotes. Processes like mycelial development in *Candida albicans* [22], the growth of the ciliate protozoan *Thetrahymena termophila* [23] and the sexual reproduction or mixis in rotifer (*Brachionus plicatilis*) [24] are controlled by QS signals.

2. Inhibition of bacterial communication: quorum quenching as a novel strategy to fight microbial infections

The development of antibiotic resistance by some pathogenic bacteria is a serious global problem, giving rise to multi-resistance strains wherein treatment is longer and frequently ineffective and forcing the pharmaceutical industry to develop new generations of more potent antibiotics, as well as exploring new strategies to fight bacterial infections. Since many bacterial pathogens use QS to control the production of virulence factors, the interference with this cell density-dependent communication mechanism constitutes a novel and promising strategy to control bacterial infectious diseases [3, 25, 26]. Moreover, QS is reported to be involved in the formation of biofilm [4], a mechanism involved in antibiotic resistance in important pathogens.
The mechanisms causing the inactivation of QS communication systems are generally known as “quorum quenching” (QQ) [26, 27]. Quorum quenching alone or in combination with antibiotics represents an interesting strategy for the treatment of infectious diseases by multiresistant pathogens such as *P. aeruginosa*, in which QS control on virulence mechanisms has been described [20], as well as for other human, animal and plant pathogens. Since the QQ approach does not affect the survival of the pathogen, it could avoid the appearance of resistances, which has been proposed as one of the main advantages of QQ strategies. Nevertheless a recent study has demonstrated that QQ compounds can generate resistance in *P. aeruginosa* [28], therefore a combined strategy of antibiotics and QQ could be more effective [3].

2.1 AHL quorum quenching mechanisms

Although the inactivation of all QS systems is equally interesting, the quenching of AHLs is the most deeply explored to date. The blockage of AHL-mediated QS systems can be achieved by either (a) interfering with the biosynthesis of AHLs, (b) interfering with the signal dissemination or (c) antagonize AHL reception.

a) Even though the inhibition of LuxI homologue, and therefore the blockage of AHL synthesis, will be the most effective communication interception system, only a scarce number of studies have explored this possibility. The inactivation of the *luxI* homologous *lasI* in *P. aeruginosa* resulted in a deficient production of virulence factors [29]. Some analogues of *S*-Adenosylmethionine (SAM), such as *S*-adenosylhomocysteine, *S*-adenosylcysteine or sinefungin, are potent inhibitors of LasI in *P. aeruginosa* [30]. Despite SAM is an intermediary of several metabolic ways in prokaryotic and eukaryotic organisms, the LuxI-mediated AHL synthesis seems to be unique and therefore the SAM analogues described could be used specifically as QS inhibitors. Macrolide group antibiotics have also been described as AHL-based QS when administered in non-lethal concentrations, although the inhibition mechanism on AHL production could not be identified [31].

b) Interference with bacterial communication can also be achieved by decreasing the concentration of active AHL in the media. AHLs suffer spontaneous lactone hydrolysis due to high pH values [32], but this can also occur by enzymatic mechanisms. Several bacteria with the capacity to degrade AHL signals have been described [25], and could therefore be used as QS blockers and their enzymes could have great interest for their biotechnological applications.

c) QS Signal transduction can be inhibited by antagonists able to compete or interfere with the signal and receptor union. The competitive antagonists would be similar enough to AHL to allow their union to the receptor but would not activate later signal transduction; non-competitive antagonists show low or no structural similitude with AHLs and will join to different parts of the receptor.

One of the first AHL antagonism strategies described was the use of AHLs not produced by the bacterium that can block LuxR. Due to the high specificity of most AHL-dependent response regulators, the presence of long acyl chain AHLs (> 10 carbons) can result in inhibition of the response, for this reason long chain AHLs were proposed as inhibitors of short AHLs in some pathogens. However, this use is strongly limited in infection processes because some AHLs are potent modulators of immune system [33] and can even act as virulence factor itself by activating inflammatory processes [34] or apoptotic responses in cell lines [35].

2.3 AHL enzymatic degradation

Mechanisms to avoid bacterial colonization and competence through QS interception have been found in nature. These mechanisms include both, enzymatic degradation of AHL signals and production of antagonists. Regarding enzymatic degradation, the AHL structure implies that four different reactions can be responsible of degradation. Two of them suppose the breakage of the HSL ring and they would be mediated by lactonase or decarboxylase enzymes. The other two reactions separate the HSL ring and the acyl chain and are catalyzed by acylases (amidases) or deaminases (Figure 1A) [25]. Up to now, only lactonase and acylase activities have been described (Figure 1B).

The enzymatic degradation of AHLs was initially described for different species of the genus *Bacillus*, where the gene *aiiA* that codifies for a lactonase has been cloned and characterized [36, 37]. Up to now, numerous bacterial species with enzymatic QQ activity have been identified belonging to the five phyla of the *Bacteria* kingdom: *Actinobacteria*, *Firmicutes*, *Proteobacteria*, *Bacteriodetes* and *Cyanobacteria*, although the activity has not been characterized in many of them [38] (Table 1). The wide distribution of enzymatic QQ activity among bacteria suggests that blocking bacterial communication is important to confer a competitive advantage in bacterial populations.

Besides lactonase and acylase activities, the acyl chain of the AHLs can suffer oxidase or reductase activities that modify them. These reactions do not degrade the signals but the modifications could affect the specificity and therefore the union between the signal and the receptor. This activity was first discovered in *Rhodococcus erythropolis* W2; this strain reduces oxo-substituted AHLs to their hydroxy forms [39]. QQ mechanisms are also present in plants like *Lotus corniculatus* [40]. Paraxonases (PONs) and acylases with QQ activity are present in mammalian cells [41, 42]. An additional mechanism for AHL inactivation is present in the brown alga *Laminaria digitata* that uses haloperoxidases to produce HOBr that reacts with Oxo- substituted AHLs [43].

2.4 AHL inhibition

The best known non-enzymatic QQ system is the inhibition of QS AHL-mediated by the alga *Delisea pulchra*. This alga produces halogenated furanones with structure very similar to AHLs that inhibit the superficial colonization, especially by Gram-negative bacteria. Thirty different furanones are produced by *D. pulchra* that act as antagonists for QS and avoid bacterial processes like swarming and biofilm formation [44]. Several studies demonstrate that furanones antagonize AHLs by displacing them from their receptors and reducing the half-life of the response regulator in the cell [45, 46]. AHL antagonists have been described in other eukaryotes, including the red algae *Ahnfeltiopsis flabelliformes* [47], the briozooan *Flustra foliacea* [48], the marine sponge *Luffareilla variabilis* [49], exudates from the terrestrial plants *Pisum sativum*, *Medicago truncatula* and *Scorzonera sandrasica* [50, 51, 52], and in garlic (*Allium sativum*) that produces three compounds capable of interfering with LasR of *P. aeruginosa* making the biofilms of the bacteria susceptible to antibiotics and detergents [53]. The fungi *Penicillium* spp. produces two inhibitors that enhanced the susceptibility of *P. aeruginosa* to tobramycin [54]. Among bacteria, *Halobacillus salinus* produces phenthylamides, nontoxic metabolites that may act as antagonists of bacterial quorum sensing by competing with *N*-acyl homoserine lactones for receptor binding [55]. Besides the search of inhibitors in extracts from different organisms, several studies have attempted the design of antagonists for LuxR by modifying the structure of AHLs [56]. Another strategy to obtain artificial antagonists is based in the modification of the natural halogenated furanones, which in some cases showed positive effects on *P. aeruginosa* lung infections in mouse [57] or in trout infected with *Vibrio anguillarum* [58].

2.5 Quorum quenching on other signals

Although much less explored, the inhibition of QS signals different from AHLs has also been described. Besides interfering with AHL-mediated QS, halogenated furanones can also block AI-2 communication

system by covalently modifying the AI-2 producing enzyme LuxS [59]. AI-2 based QS systems can also be blocked by cinnamaldehyde analogs, affecting biofilm formation, pigment production and protease synthesis in *Vibrio* spp. [60]. By screening a large number of plant samples, Ren et al. [61] and Lee et al. [62] found that ursolic acid and 7-hydroxyindole can act as biofilm inhibitors in enterohemorrhagic *E. coli* by blocking the AI-2 pathway. Certain fatty acids, such as linoleic acid, oleic acid, palmitic acid, and stearic acid were also identified as inhibitors of AI-2 pathway within a concentration range of 0.1-10 mM [63].

Regarding the inhibition of peptide-type signals in Gram-positive bacteria, the most studied system is the AgrC/AgrA system of *Staphylococcus aureus*, and its signal AIP, due to the enormous clinical relevance of this pathogen. During the studies of structure-activity relationships of AIP, Lyon et al. [64] found one global inhibitor of *S. aureus*: TrAIP-II, a truncated derivative of the AIP thiolactone peptide. The QS inhibitor RIP also inhibits staphylococcal *agr* system [65]. RIP injected systemically into rats has been found to have strong activity in preventing methicillin-resistant *Staphylococcus aureus* graft infections, suggesting that RIP can be used as a therapeutic agent. In addition to using inhibitors of AIP signals, the use of antibodies has been reported, which could effectively inhibit quorum sensing in vitro through the sequestration of the AIP used by *S. aureus* [66].

3. Is Quorum quenching effective in vivo?

The biotechnological applications of the interception of bacterial communication as a promising method to control pathogenic bacteria [25, 26] has attracted attention from numerous researchers, due to the elevated prevalence of QS systems among plant, animal and human pathogens [2]. Several studies have tried to apply QQ to control bacterial infections in plants. Tabaco transgenic plants expressing *N*-oxoctanoyl-L-homoserine lactone (OC8-HSL), a signal used by the plant pathogen *Erwinia carotovora*, showed less susceptibility to infections by this bacterium. This has been explained as an artificial increase of the signal will trigger the expression of bacterial virulence factors before the bacterial population is big enough, which would facilitate plant defenses activation and avoid bacterial colonization [67]. A significant reduction in the production of exoenzymes and a decrease in infection symptoms have been observed in plants infected with *E. carotovora* expressing *aiiA* from *Bacillus* sp. [36]. The same results have been achieved with other important plant pathogens such as *Burkholderia thailandensis* and *Erwinia amylovora* [68, 69].

The heterologous expression of the lactonase AiiA in tobacco and potato plants resulted in plants resistant to *E. carotovora* [27], while the addition of the purified acylase AhlM from *Streptomyces* sp. to the culture media of *P. aeruginosa* reduced the production of virulence factors by the pathogen [70].

Special attention has been paid to the feasibility of the application of QQ strategies to control diseases in aquatic organisms, for which use of antibiotics is highly restricted. In this field, encouraging results have been obtained. Bacterial consortia with AHL degradation activity were demonstrated to be able to protect *Artemia* sp., rotifers and larvae of turbot or prawn from infection [71, 72]. Furanones also inhibit the pathogenicity of *Vibrio harveyi*, *V. campbellii* and *V. parahaemolyticus* against *Artemia* sp. [73]. Tinh et al. [71] also neutralized the negative effect of *V. harveyi* on rotifers with furanones, but unfortunately furanones were toxic for artemias and rotifers, which will limit the use of these natural compounds. The

use of C-30, a synthetic furanone, in no toxic concentrations significantly reduced the pathogenicity of *V. anguillarum* in rainbow trout [58]. Degradation of AHLs by AiiA also increased the survival of carp infected with *Aeromonas hydrophila* [37].

The effectiveness of QQ strategies has been also demonstrated in other infection models. Papaioannou et al. [74] showed that the addition of purified PvdQ acylase to *Caenorhabditis elegans* worms infected with *P. aeruginosa* PAO1 resulted in reduced pathogenicity and increased life span of the nematodes. Similar results were obtained when *P. aeruginosa* PAO1 pulmonary infections were treated with garlic extracts in mouse [75] and when a QS defective, LasR mutant of the same strain was used in burned mouse model [76]. In recent study [77] garlic extracts were also used in the first clinical trial in man of a QS inhibitor. Twenty-six cystic fibrosis patients were administered garlic or olive oil capsules as placebo, although there was no significant effect of garlic compared to placebo, authors report an improvement in lung function, weight and symptom score, with garlic treatment.

Previous examples show that inhibition or AHL degradation could be successfully used for blocking bacterial infections. Moreover, the interest of these mechanisms is that they do not affect directly the survival of the pathogen but the expression of virulence factors, and so they do not exert selective pressure avoiding the appearance of resistances.

4. Patents on quorum quenching

A search carried out in patent databases [78, 79] (Aug 03, 2011) revealed a total of 45 applications related to strategies to interfere with quorum sensing systems as a method to fight microbial infections (Table 2, supplemental data). Following the bias in the literature, the vast majority of the patented technologies based on the inhibition of QS mechanisms target AHL signals, whereas only 5 out of 45 patent cases are based on the inhibition of AI-2 signals and only 4 on the inhibition of peptide-based QS signals from Gram-positives.

4.1 Peptide signals quorum quenching patents

Regarding QQ against peptide-type signals, patent application US2010291093A1 provides a monoclonal antibody against the AP-4 signaling peptide of *S. aureus,* providing evidence that staphylococcal infection in mice is hampered by the use of this antibody [80, 66]. Another patent application, US2008069782A1 [81], is based on peptide QS signals, but the basis for inhibiting the growth of the pathogens consists of giving high doses of QS molecules instead of reducing the amount of QS signals. This work relates to a composition comprising a peptide-based QS signal, the so-called Competence Stimulating Peptide (CSP), which, together with sucrose, reduces the adherence of *Streptococcus mutans* to teeth while enhancing the ability of other non-pathogenic bacteria to more efficiently compete for the bare supergingival pellicle. The invention is based on the hypothesis that the genes encoding glycosyltransferases (*gtf*), which are necessary for efficient colonization of *S. mutans*, are regulated through QS molecules like CSP, and on the fact that high dosages of QS molecules induce cell death in *S. mutans*. A similar mechanism is proposed to reduce the biofilm of *S. aureus* for the activation of the *agr* QS system [82]. Patent application US2003078378A1 [83] provides purified and isolated peptides which inhibit the *agr* system of *S. aureus*. The peptides of the present invention are cyclic, comprise about six to about twelve amino

acids in length, including sequences corresponding to the native peptide from staphylococci bacterium, as well as analogues thereof which contain amino acid substitutions which also result in inhibitor peptides. The purified inhibitor peptides of the present invention may be isolated directly from staphylococci bacterium, recombinantly produced, or synthesized chemically.

4.2 AI-2 quorum quenching patents

Regarding the inhibition of AI-2 signals, only WO2010033236A2 [84] describes the use of an inhibitor of signal synthesis. 5'-Methylthioadenosine/*S*-adenosyl homocysteine nucleosidase (MTAN) inhibitors are applied in order to tackle pathogens like *P. aeruginosa* or *S. pneumoniae*. The underlying mechanism is that MTAN is directly involved in the synthesis of the AHLs and AI-2 autoinducers, so that the blockade of MTAN produces the inhibition of both QS systems. The MTAN inhibitors developed are shown to inhibit AI-2 production in virulent strains of *Vibrio cholerae*, and references are given mentioning that *P. aeruginosa*, *S. pneumoniae*, *Neisseria meningitidis*, *Klebsiella pneumoniae*, *S. aureus* and *Helicobacter pylori* possess MTANs, so that all these pathogens are susceptible of treatment with the MTAN inhibitors. The other patent applications related to AI-2 consist of signal antagonists (Table 2, supplemental data). Patent application US2010137249A1 [85] identifies 42 different compounds, mainly boronic acids, pyrogallol and phenothiazine, amongst others, that are shown to antagonize AI-2 in *V. harveyi*. Other methods are based on the use of furanones that antagonize AI-2 signals and therefore reduce the growth of different pathogens [86, 87]. US2008299153A1 [86] provides different furanones that inhibit growth as well as toxin production in *Bacillus anthracis*, whereas WO2007031826A2 [87] describes an apparatus for inhibiting growth and QS mechanisms in food-borne microorganisms by simultaneously exposing a food product to an antimicrobial gas mixture and a treating agent that inhibits signaling between the microorganisms, the treating agent being a furanone or ascorbic acid. Furanones have also been described in other patent applications [88, 89], in some cases as AHL antagonists [89]. Finally, WO03077844A2 [90] is based on the use of DHCP (4,5-dihydroxy-2-cyclopenten-1-one) in order to regulate the expression of genes involved in QS, with evidence showing that the genes comprising quorum-regulated processes such as virulence, motility and outer membrane functions are down-regulated by DHCP treatment in *E. coli* and suggesting that DHCP regulates the switching on/off of the different QS circuits in this bacterium.

4.3 AHL quorum quenching patents

4.3.1 AHL inhibitors

The majority of patent applications are related to the inhibition of AHL-based QS systems (namely 33 patent applications) (Table 2, supplemental data) and most of them are related to antagonists of the QS signals, while only very few (8) rely on enzymatic degradation of the AHLs (Table 2, supplemental data). That is, most of the applications are based on compounds that somehow imitate the structure of the QS molecule, thereby acting as replacements for naturally occurring bacterial QS ligands and producing an antagonistic effect, or reducing the expression of QS gene expression. The sources of these antagonistic compounds are quite varied. Some are isolated from natural sources, and these include garlic extract (*S*-allyl-cysteine, which has a similar structure to part of the AHL molecule) [91], other bacteria (piericidin

A1, produced by the soil bacteria *Kitasatospora* sp. AR030054, has competitive inhibitory activity on AHLs) [92], red algae (*Rhodophyta* extracts) [93, 47], a marine species of the fungi *Penicillium* (compounds obtained by fermentation of these fungi are shown to inhibit QS mechanisms) [94, 54], *Epimedium* Chinese herbal extracts (flavonoids derived therefrom, such as diphylloside A) [95] and tropical mangrove shrubs like *Conocarpus erectus* (ellagitannins, which are components in some medicinal plants, are shown to interfere with QS mechanisms) [96], amongst others. Interestingly, patent application WO2009114810A2 [96], protecting plant C-glycosidic ellagitannins (castalagin and vescalagin, obtained from the mangrove *C. erectus*) that are shown to affect AHL levels and QS-gene expression in *P. aeruginosa*, is the basis of the technology developed by QuorumEx, a small company based on QS-disruption products, which exploits plant extracts with anti-QS properties from the jungles of Belize. One of their first products to be launched would be "Topic-QS", a topical antibiotic ointment based on quorum sensing disruption technology that relies exclusively on a variety of plant extracts with QQ properties [97].

Additionally, many other antagonists are obtained by chemical synthesis, including examples such as pyrimidinone compounds [98], thiocarbamate structures [99], macrolides [100, 101], isothiocynates and haloacetamides [102], furanone derivatives [86] or other synthetic compounds [103], most of them having in common a chemical structure that reminds that of AHL molecules and differing in the modifications to the head or tail groups. WO2008116029A1 [103] provides synthetic *N*-phenylacetanoyl-L-homoserine lactones which are capable of either inhibiting or, in some cases, strongly inducing QS in *V. fischeri*. Simple structural modifications to these ligands are shown to have remarkable effects on activity. For example, movement of a single constituent on the phenylacetanoyl group transforms QS antagonists into QS agonists. Studies of these structural isomers revealed a synthetic superagonist of QS in *V. fischeri*, [Lambda]/-(3-nitro-phenylacetanoyl)-L-homoserine lactone. Isothiocynates and haloacetamides provided by patent application WO2011001419A1 [102] are covalent inhibitors of QS signals, having an electrophilic group that binds covalently to proteins whose natural ligands are the AHLs, so that they block the action of these QS molecules. More specifically, isothiocynates and haloacetamides target the *P. aeruginosa* QS regulator, LasR, whose native ligand is *N*-oxododecanoyl-L-homoserine lactone (OC12-HSL), so that QS can be inhibited through covalent binding of this protein. In some cases, a great deal of detail is given as for the best modifications in the structure in order to achieve the greatest inhibitory activity. As an example, patent application US2006052425A1 [99] describes the screening of a library with 16.000 synthetic compounds, providing both agonists as well as antagonists of QS signals, especially from *P. aeruginosa*, and especially *N*-oxodecanoyl-L-homoserine lactone (OC10-HSL) and *N*-dodecanoyl-L-homoserine lactone (C12-HSL) signals. Interestingly, they specify that thiocarbamate structures with ester substitutions at the ortho position at the aniline ring provide the greatest activity against OC12-HSL.

It is worth noting that two of these patent applications based on AHL antagonists show promising results suggesting that the inhibition of the QS processes could harbor potential for the treatment of periodontal disease, which is caused by biofilm or plaque formation by bacterial pathogens in the oral cavity. Particularly interesting is patent application GB2472315A [91], which shows results from clinical trials in 83 patients with periodontal disease that had been unsuccessfully treated with conventional treatments. A

composition comprising garlic extract in combination with tetracycline is applied to periodontal pockets on these patients, and 85% of those showed good or excellent response to the treatment, including reduction of gum swelling and bleeding, and a substantial decrease or complete elimination of the inflammatory response. Additionally, patent application WO2010114533A1 [104] provides data showing the activity of different carbonate compounds which are able to avoid the formation of biofilms by *Actinomyces naeslundii*, which causes periodontal disease. However, claims do not include details regarding the type of signal targeted or the possible mechanism of QS inhibition. Finally, patent application US2010256369A1 [98] provides pyrimidinone compounds that are able to inhibit *N*-hexanoyl-L-homoserine lactone (C6-HSL) and *N*-octanoyl-L-homoserine lactone (C8-HSL) synthesis as well as toxin production in *Burkholderia glumae*. *B. glumae* uses QS to regulate the production of a so-called AhpF (alkyl hydroperoxide reductase subunit F) enzyme, which is involved in the aerotolerance mechanism of bacteria. Accordingly, the pyrimidinone compound inhibits the expression of *ahpF* and thereby causes *B. glumae* to self-destruct due to air oxidation. Additionally, they show that the compound is able to prevent the disease caused by *B. glumae* in Korean rice, providing some results of the effects of the compound on biofilm formation by *Burkholderia aeruginosa* and the periodontitis pathogenic bacterium *Porphyromonas gingivalis* in a flow cell system. Although the results show that the pyrimidinone compound can effectively inhibit biofilm formation or remove an already formed biofilm by *B. aeruginosa*, results with *P. gingivalis* only show a certain degree of inhibition on biofilm formation, and at first sight not a significant inhibitory effect.

4.3.2 AHL enzymatic degradation

Only 8 out of 45 patent applications are related to enzymes, mostly lactonases or acylases that degrade AHL signals. Within those, patent application US2009159533A1 [105] protects a membrane bioreactor on which a QQ enzyme is immobilized, in order to avoid the formation of biofilms on wastewater treatment systems. No QQ enzyme is isolated or described in detail; instead, it uses commercially available acylases or lactonases, suggesting that it is preferable to use an acylase because a soluble product of lactonase can be re-synthesized to a signal molecule depending on pH. Two other patent applications are based on different *aiiA* genes and the encoded AHL-degrading enzymes from the genus *Bacillus*, especially effective against plant pathogens such as *Erwinia*. WO02061099A1 [106] protects transgenic plants harboring the *aiiA* gene and expressing functional AiiA protein, which is an AHL lactonase from *Bacillus* sp., since the lactonase protects the plants from bacterial pathogens, especially from Gram-negatives such as *Erwinia*, by degrading the pathogen QS molecules. Patent application WO0216623A1 [107] protects a *Bacillus thuringiensis* gene (and the transformed prokaryotic and eukaryotic cells) encoding a metallohydrolase enzyme, which is postulated to have 35-94% homology to previously described AiiA enzymes. Some *B. thuringiensis* strains and closely related species were found to degrade *N*-oxohexanoyl-L-homoserine lactone (OC6-HSL) and OC8-HSL (produced by the pathogen *Erwinia*), and *aii* genes encoding QQ enzymes and closely related to those of other *Bacillus* species were identified. Additionally, the strains are shown to effectively quench QS activity when co-cultured with QS-producing pathogenic bacteria, suggesting that they can be used as a bio-control tool for the potato soft root disease caused by *Erwinia carotovora*. Patent application WO2010012852A1 [108, 38] is based

on the use of bacteria from the genus *Tenacibaculum* for QQ. A total of 165 strains were isolated from marine environments and a screening for QQ activity was carried out. *Tenacibaculum discolor* 20J was found to be the strain with the greatest QQ activity. In this strain, AHL-degradation activity is found in the cells as well as in the culture medium and in the cell crude extracts. It is worth noting that this strain is able to degrade a wide range of AHLs, namely *N*-butanoyl-L-homoserine lactone (C4-HSL) to *N*-tetradecanoyl-L-homoserine lactone (C14-HSL), and to completely degrade a 30µM solution of C12-HSL in 30 minutes. Additionally, enzymatic QQ activity is different for short- and long-chain AHLs, indicating the presence of both lactonase and acylase activities.

Patent application FR2863168A1 [109] provides an inductor of the Gamma-butyrolactone (GBL) degradation pathway in order to increase the enzymatic degradation of the AHL signals produced by Gram-negatives. It is shown that inductors like GBL are able to promote the degradation of C6-HSL by a bacterial soil suspension, and especially by the species *Agrobacterium tumefaciens*. Patent application WO03075654A2 [110] describes larval excretions from the green bottle fly *Lucilia sericata*, which are able to degrade the AHL signals produced by *P. aeruginosa.* More specifically, degradation of C4 and OC12-HSL, as well as reduction of the biofilms created by *P. aeruginosa* and *S. aureus*, are demonstrated. Lactonase activity together with the enzymatic removal of polysaccharide in the biofilm is considered responsible for the observed anti-biofilm activity.

EP1470221B1 and US2004109852A1 [111, 112] are also based on the use of isolated QQ enzymes. In EP1470221B1, a bacterial biofilm sample was collected from a water treatment system and screened to isolate AHL inactivation bacterial strains. Two *Ralstonia* sp. strains were found to degrade OC8-HSL, with strain XJ12B showing the strongest activity. The *qsbA* gene, isolated from strain XJ12B, was shown to encode an AHL acylase (QsbA protein), which was isolated. QsbA protein was shown to completely inactivate OC8, OC10 and OC12-HSL, and to a lesser extent, C8-HSL and *N*-decanoyl-L-homoserine lactone (C10-HSL), but not OC6-HSL. Finally, US2004109852A1 [112] is based on the use of E.C. 3.5.1 acylases (E.C.3.5.1: hydrolases acting on carbon-nitrogen bonds, other than peptide bonds, in linear amides), that degrade AHLs in order to prevent or remove biofilms on a surface. Examples are provided with porcine kidney acylase I, showing that C4 and C8-HSL are both deacetilated by the acylase. Also shown is the reduction of microbial growth and biofilms in water from a fish tank.

4.3.3 AHL sequestration strategies

Finally, other applications are based on the sequestration of the QS molecules, for instance by using polymers or other trapping structures like cyclodextrins [113]. Patent application WO2008087454A2 [114] provides polymers based on acrylamide, bis-acrylamide and itaconic acid that act by trapping the QS molecules. AHL molecules which contain additional carbonyl group in the acyl chain, in particular in the 3 position, relative to the carbonyl of the acyl group, show a better adsorption to the polymers, so that efficient adsorption of AHLs like OC6-HSL is demonstrated. Patent application JP2011046705A [115] describes the use of antibodies to specifically bind AHLs and their use for treatment of bacterial diseases.

5. Current & Future Developments

An increasing amount of evidence is being accumulated regarding the feasibility of the use of Quorum Quenching strategies to fight bacterial infections. Researchers have focused their attention in the interference with AHL-mediated QS systems, which is reflected in the higher number of patent applications that are related to this strategy (33). Among these, most applications are related to antagonism of the AHL signal (18) and only a few deal with signal enzymatic destruction (8), the inhibition of signal synthesis (3) or signal sequestration (3). The effectiveness of AHL-QQ has been mainly applied to control infections in plants, like the transgenic expression of the lactonase AiiA from *Bacillus* in tobacco and potato plants [27]; and the protection of infections by Gram-negative pathogens in aquaculture, examples are the use of bacterial consortia with AHL degradation activity to protect prawn [71] and the use of the potent antagonists: furanones to significantly reduce the pathogenicity of *V. anguillarum* in rainbow trout [58]. Unfortunately furanones are toxic [71], which will limit the use of these natural compounds. Therefore, a search for non-toxic furanone derivatives by chemical synthesis [85] or alternative antagonist compounds is needed. The screening for antagonists with similar structure to AHLs is particularly interesting, like *S*-allyl-cysteine from garlic extracts [91], with good results in combination with tetracycline in clinical trials in 83 patients with periodontal disease that had been unsuccessfully treated with conventional treatments.

Degradation of AHLs by enzymes also showed positive results in the survival of carp infected with *Aeromonas hydrophila* [37]. The advantage of the enzymatic degradation of AHLs is that a wide range degradation activity will allow the interference with a higher number of signaling systems [108] while antagonists are usually species-specific.

Although less explored, the inhibition of peptide-signaling in the Gram-positive pathogens of the genus *Staphylococcus* and *Streptococcus* is of special clinical significance, and will surely deserve further exploration, since up to now only 4 patent applications have been deposited for this QQ mechanism.

Despite the considerable activity that is being developed in the field and the interest of the strategy for medical and biotechnological applications, more robust proofs of concept and medical trials are surely required before we can see QQ related products in the market.

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Conflict of interest

The authors have no conflict of interest to declare.

References

[1] Fuqua WC, Winans SC, Greenberg EP. Quorum sensing in bacteria: the LuxR-LuxI family of cell density-responsive transcriptional regulators. J Bacteriol 1994; 176: 269-75.

[2] Williams P, Winzer K, Chan WC, Cámara M. Look who's talking: communication and quorum sensing in the bacterial world. Phil Trans R Soc B 2007; 362: 1119-34.

[3] Kalia VC, Purohit HJ. Quenching the quorum sensing system: potential antibacterial drug targets. Crit Rev Microbiol 2011; 37:121-40.

[4] Schembri MA, Givskov M, Klemm P. An attractive surface: Gram-negative bacterial biofilms. Sci STKE 2002; 132: re6.

[5] Shao H, Lamont RJ, Demuth DR. Autoinducer 2 is required for biofilm growth of *Aggregatibacter* (*Actinobacillus*) *actinomycetemcomitans*. Infect Immun 2007; 75: 4211-8.

[6] Petersen FC, Ahmed NA, Naemi A, Scheie AA. LuxS-mediated signalling in *Streptococcus anginosus* and its role in biofilm formation. Antonie van Leeuwenhoek Int J Gen Mol Microbiol 2006; 90: 109-21.

[7] Nealson KH. Autoinduction of bacterial luciferase: occurrence mechanism and significance. Arch Microbiol 1977; 112: 73-9.

[8] Ji G, Beavis RC, Novick RP. Cell density control of staphylococcal virulence mediated by an octapeptide pheromone. Proc Natl Acad Sci USA 1995; 92: 12055-9.

[9] Simon MI, Crane BR, Crane A. Two-component signaling systems. San Diego: Academic Press 2010.

[10] Pestova EV, Havarstein LS, Morrison DA. Regulation of competence for genetic transformation in *Streptococcus pneumoniae* by an auto-induced peptide pheromone and a two-component regulatory system. Mol Microbiol 1996; 21: 853-62.

[11] Magnuson R, Solomon J, Grossman AD. Biochemical and genetic characterization of a competence pheromone from *B. subtilis*. Cell 1994; 77: 207-16.

[12] Novick RP, Geisinger E. Quorum sensing in staphylococci. Annu Rev Genet 2008; 42: 541-64

[13] Bassler BL, Wright M, Silverman MR. Multiple signaling systems controlling expression of luminescence in *Vibrio harveyi*: sequence and function of genes encoding a second sensory pathway. Mol Microbiol 1994; 13: 273-86.

[14] Sperandio V, Mellies JL, Nguyen W, Shin S, Kaper JB. Quorum sensing controls expression of the type III secretion gene transcription and protein secretion in enterohemorrhagic and enteropathogenic *Escherichia coli*. P Natl Acad Sci USA 1999; 96: 15196-201.

[15] McNab R, Ford SK, El-Sabaeny A, Barbieri B, Cook GS, Lamont RJ. LuxS-Based Signaling in *Streptococcus gordonii*: Autoinducer 2 Controls Carbohydrate Metabolism and Biofilm Formation with *Porphyromonas gingivalis*. J Bacteriol 2003; 185: 274-84.

[16] Schauder S, Shokat K, Surette MG, Bassler BL. The LuxS family of bacterial autoinducers: biosynthesis of a novel quorum-sensing signal molecule. Mol Microbiol 2001; 41: 463-76.

[17] Ohnishi Y, Kameyama S, Onaka H, Horinouchi S. The A-factor regulatory cascade leading to streptomycin biosynthesis in *Streptomyces griseus*: identification of a target gene of the A-factor receptor. Mol Microbiol 1999; 34: 102-11.

[18] Flavier AB, Clough SJ, Schell MA, Denny TP. Identification of 3-hydroxypalmitic acid methyl ester as a novel autoregulator controlling virulence in *Ralstonia solanaceum*. Mol Microbiol 1997; 26: 251-9.

[19] Barber CE, Tan JL, Feng JX, *et al*. A novel regulatory system required for pathogenicity of *Xanthomonas campestris* is mediated by a small diffusible signal molecule. Mol Microbiol 1997; 24: 555-66.

[20] Williams P, Cámara M. Quorum sensing and environmental adaptation in *Pseudomonas aeruginosa*: a tale of regulatory networks and multifunctional signal molecules. Curr Opin Microbiol 2009; 12: 182-91.

[21] Wei Y, Perez LJ, Ng WL, Semmelhack MF, Bassler BL. Mechanism of *Vibrio cholerae* autoinducer-1 biosynthesis. ACS Chem Biol 2011; 6: 356-65.

[22] Atkinson S, Williams P. Quorum sensing and social networking in the microbial world. J R Soc Interface 2009; 6: 959-78.

[23] Christensen S, Leick V, Rasmussen L, Wheatley DN. Signaling in unicellular eukaryotes. Int Rev Cytol 1997; 177: 181-253.

[24] Kubanek J, Snell TW. In: Winans SC, Bassler BL, Eds. Chemical communication among bacteria. Washington DC: ASM Press 2008: pp. 453-61.

[25] Dong YH, Zhang LH. Quorum sensing and quorum-quenching enzymes. J Microbiol 2005; 43: 101-9.

[26] Dong YH, Wang LH, Zhang LH. Quorum-quenching microbial infections: mechanisms and implications. Phil Trans R Soc B 2007; 362: 1201-11.

[27] Dong YH, Wang LH, Xu JL, Zhang HB, Zhang XF, Zhang LH. Quenching quorum-sensing-dependent bacterial infection by an N-acyl homoserine lactonase. Nature 2001; 411: 813-7.

[28] Maeda T, García-Contreras R, Pu M, *et al*. Quorum quenching quandary: resistance to antivirulence compounds. ISME J 2011; doi:10.1038/ismej.2011.122.

[29] Shih PC, Huang CT. Effects of quorum-sensing deficiency on *Pseudomonas aeruginosa* biofilm formation and antibiotic resistance. J Antimicrob Chemother 2002; 49: 309-14.

[30] Parsek MR, Val DL, Hanzelka BL, Cronan JEJ, Greenberg EP. Acyl homoserine-lactone quorum-sensing signal generation. Proc Natl Acad Sci USA 1999; 96: 4360-5.

[31] Pechere JC. New perspectives on macrolide antibiotics. Int J Antimicrob Agents 2001; 1: 93-7.

[32] Yates EA, Philipp B, Buckley C, *et al*. 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: 5635-46.

[33] Rice SA, Givskov M, Steinberg P, Kjelleberg S. Bacterial signals and antagonists: the interaction between bacteria and higher organisms. J Mol Microbiol Biotechnol 1999; 1: 23-31.

[34] Smith RA, Iglewski BH. *P. aeruginosa* quorum sensing systems and virulence. Curr Opin Microbiol 2003; 6: 56-60.

[35] Shiner EK, Terentyev D, Bryan A, *et al*. *Pseudomonas aeruginosa* autoinducer modulates host cell responses through calcium signaling. Cell Microbiol 2006; 8: 1601-10.

[36] Dong YH, Xu JL, Li XZ, Zhang LH. AiiA, an enzyme that inactivates acyl homoserine-lactone quorum-sensing signal and attenuates the virulence of *Erwinia carotovora*. Proc Natl Acad Sci USA 2000; 97: 3526-31.

[37] Chen R, Zhou Z, Cao Y, Bai Y, Yao B. High yield expression of an AHL-lactonase from Bacillus sp. B546 in *Pichia pastoris* and its application to reduce *Aeromonas hydrophila* mortality in aquaculture. Microb Cell Fact 2010; 9: 39.

[38] Romero M, Martin-Cuadrado AB, Roca-Rivada A, Cabello AM, Otero A. Quorum quenching in cultivable bacteria from dense marine coastal microbial communities. FEMS Microbiol Ecol 2011; 75: 205-17.

[39] Uroz S, Chhabra SR, Cámara M, Williams P, Oger P, Dessaux Y. N-acylhomoserine lactone quorum-sensing molecules are modified and degraded by *Rhodococcus erythropolis* W2 by both amidolytic and novel oxidoreductase activities. Microbiol 2005; 151: 3313-22.

[40] Delalande L, Faure F, Raffoux A, *et al*. 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: 13-20.

[41] Xu F, Byun T, Deussen HJ, Duke KR. Degradation of N-acylhomoserine lactones, the bacterial quorum-sensing molecules, by acylase. J Biotechnol 2003; 101: 89-96.

[42] Draganov DI, Teiber JF, Speelman A, Osawa Y, Sunahara R, La Du BN. Human paraoxonases (PON1 PON2 and PON3) are lactonases with overlapping and distinct substrate specificities. J Lipid Res 2005; 46: 1239-47.

[43] Borchardt SA, Allain EJ, Michels JJ, Stearns GW, Kelly RF, McCoy WF. Reaction of acylated homoserine lactone bacterial signaling molecules with oxidized halogen antimicrobials. Appl Environ Microbiol 2001; 67: 3174-9.

[44] Givskov M, de Nys R, Manefield M, *et al*. Eukaryotic interference with homoserinelactone-mediated prokaryotic signaling. J Bacteriol 1996; 178: 6618-22.

[45] Zhang LH. Quorum quenching and proactive host defense. Trends Plant Sci 2003; 8: 238-44.

[46] McLean RJ, Pierson LS, Fuqua C. A simple screening protocol for the identification of quorum signal antagonists. J Microbiol Methods 2004; 58: 351-60.

[47] Kim JS, Kim YH, Seo YW, Park S. Quorum sensing inhibitors from the red alga *Ahnfeltiopsis flabelliformis*. Biotechnol Bioprocess Eng 2007; 12: 308-11.

[48] Peters L, König GM, Wright AD, *et al*. Secondary metabolites of *Flustra foliacea* and their influence in bacteria. Appl Environ Microbiol 2003; 69: 3469-75.

[49] Skindersoe ME, Ettinger-Epstein P, Rasmussen TB, Bjarnsholt T, De Nys R, Givskov M. Quorum sensing antagonism from marine organisms. Mar Biotechnol 2008; 10: 56-63.

[50] Teplitski M, Robinson JB, Bauer WD. Plants secrete substances that mimic bacterial N-acyl homoserine lactone signal activities and affect population density-dependent behaviors in associated bacteria. Mol Plant-Microbe Interact 2000; 13: 637-48.

[51] Gao M, Teplitski M, Robinson JB, Bauer WD. Production of substances by *Medicago truncatula* that affect bacterial quorum sensing. Mol Plant-Microbe Interact 2003; 16: 827-34.

[52] Bosgelmez-Tinaz G, Ulusoy S, Ugur A, Ceylan O. Inhibition of quorum sensing-regulated behaviors by *Scorzonera sandrasica*. Curr Microbiol 2007; 55: 114-8.

[53] Persson T, Hansen TH, Rasmussen TB, Skindersø ME, Givskov M, Nielsen J. Rational design and synthesis of new quorum-sensing inhibitors derived from acylated homoserine lactones and natural products from garlic. Org Biomol Chem 2005; 3: 253-62.

[54] Rasmussen TB, Bjarnsholt T, Skindersoe ME, *et al*. Screening for quorum-sensing inhibitors (QSI) by use of a novel genetic system, the QSI selector. J Bacteriol 2005; 187: 1799-814.

[55] Teasdale ME, Liu J, Wallace J, Akhlaghi F, Rowley DC. Secondary metabolites produced by the marine bacterium *Halobacillus salinus* that inhibit quorum sensing-controlled phenotypes in Gram-negative bacteria. Appl Environ Microb 2009; 75: 567-72.

[56] Ni N, Li M, Wang J, Wang B. Inhibitors and antagonists of bacterial quorum sensing. Med Res Rev 2009; 29: 65-124.

[57] Wu H, Song Z, Hentzer M, Andersen JB, 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: 1054-61.

[58] Rasch M, Buch C, Austin B, *et al*. An inhibitor of bacterial quorum sensing reduces mortalities caused by vibriosis in rainbow trout (*Oncorhynchus mykiss* Walbaum). System Appl Microbiol 2004; 27: 350-9.

[59] Zang T, Lee BWK, Cannon LM, *et al*. A naturally occurring brominated furanone covalently modifies and inactivates LuxS. Bioorg Med Chem Lett 2009; 19: 6200-4.

[60] Brackman G, Celen S, Hillaert U, *et al*. Structure-activity relationship of cinnamaldehyde analogs as inhibitors of AI-2 based quorum sensing and their effect on virulence of *Vibrio* spp. PLoS One 2011; 6: e16084.

[61] Ren D, Zuo R, Gonzalez Barrios AF, *et al*. Differential gene expression for investigation of *Escherichia coli* biofilm inhibition by plant extract ursolic acid. Appl Environ Microbiol 2005; 71: 4022-34.

[62] Lee J, Bansal T, Jayaraman A, Bentley WE, Wood TK. Enterohemorrhagic *Escherichia coli* biofilms are inhibited by 7-hydroxyindole and stimulated by isatin. Appl Environ Microbiol 2007; 73: 4100-9.

[63] Lu L, Hume ME, Pillai SD. Autoinducer-2-like activity on vegetable produce and its potential involvement in bacterial biofilm formation on tomatoes. Foodborne Pathog Dis 2005; 2: 242-9.

[64] Lyon GJ, Mayville P, Muir TW, Novick RP. Rational design of a global inhibitor of the virulence response in *Staphylococcus aureus*, based in part on localization of the site of inhibition to the receptor-histidine kinase AgrC. P Natl Acad Sci USA 2000; 97: 13330-5.

[65] Balaban N, Cirioni O, Giacometti A, *et al*. Treatment of *Staphylococcus aureus* biofilm infection by the quorum-sensing inhibitor RIP. Antimicrob Agents Chemother 2007; 51:2226-9.

[66] Park J, Jagasia R, Kaufmann GF, *et al*. Infection control by antibody disruption of bacterial quorum sensing signaling. Chem Biol 2007; 14: 1119-27.

[67] Mäe A, Montesano M, Koiv V, Palva ET. Transgenic plants producing the bacterial pheromone N-acyl-homoserine lactone exhibit enhanced resistance to the bacterial phytopathogen *Erwinia carotovora*. Mol Plant-Microbe Interact 2001; 14: 1035-42.

[68] Ulrich RL. Quorum quenching: enzymatic disruption of Nacylhomoserine lactone-mediated bacterial communication in *Burkholderia thailandensis*. Appl Environ Microbiol 2004; 70: 6173-80.

[69] Molina L, Rezzonico F, Defago G, Duffy B. Autoinduction in *Erwinia amylovora*: evidence of an acyl-homoserine lactone signal in the Fire Blight pathogen. J Bacteriol 2005; 187: 3206-13.

[70] Park SY, Kang HO, Jang HS, Lee JK, Koo BT, Yum DY. Identification of extracellular N-acylhomoserine lactone acylase from a *Streptomyces* sp. and its application to quorum quenching. Appl Environ Microbiol 2005; 71: 2632-41.

[71] Tinh NT, Linh ND, Wood TK, Dierckens K, Sorgeloos P, Bossier P. Interference with the quorum sensing systems in a *Vibrio harveyi* strain alters the growth rate of gnotobiotically cultured rotifer *Brachionus plicatilis*. J Appl Microbiol 2007; 103: 194-203.

[72] Nhan DT, Cam DTV, Wille M, Defoirdt T, Bossier P, Sorgeloos P. Quorum quenching bacteria protect *Macrobrachium rosenbergii* larvae from *Vibrio harveyi* infection. J Appl Microbiol 2010; 109: 1007-16.

[73] Defoirdt T, Crab R, Wood TK, Sorgeloos P, Verstraete W, Bossier P. Quorum sensing-disrupting brominated furanones protect the gnotobiotic brine shrimp *Artemia franciscana* from pathogenic *Vibrio harveyi*, *Vibrio campbellii* and *Vibrio parahaemolyticus* isolates. Appl Environ Microbiol 2006; 72: 6419-23.

[74] Papaioannou E, Wahjudi M, Nadal-Jimenez P, Koch G, Setroikromo R, Quax WJ. Quorum-quenching acylase reduces the virulence of *Pseudomonas aeruginosa* in a *Caenorhabditis elegans* infection model. Antimicrob Agents Ch 2009; 53: 4891-7.

[75] Bjarnsholt T, Jensen PØ, Rasmussen TB, *et al*. Garlic blocks quorum sensing and promotes rapid clearing of pulmonary *Pseudomonas aeruginosa* infections. Microbiol 2005; 151: 3873-80.

[76] Rumbaugh KP, Griswold JA, Iglewski BH, Hamood AN. Contribution of quorum sensing to the virulence of *Pseudomonas aeruginosa* in burn wound infections. Infect Immun 1999; 67: 5854-62.

[77] Smyth AR, Cifelli PM, Ortori CA, *et al.* Garlic as an inhibitor of *Pseudomonas aeruginosa* quorum sensing in cystic fibrosis--a pilot randomized controlled trial. Pediatr Pulmonol 2010; 45:356-62.

[78] Espacenet. Available at: http://worldwide.espacenet.com. (Accessed on: August 3, 2011).

[79] United States Patent and Trademark Office-Patent databases. Available at: http://www.uspto.gov. (Accessed on: August 3, 2011).

[80] Janda KD, Kaufmann GF, Park J. Antibody-mediated disruption of quorum sensing in bacteria. US2010291093A1, 2010.

[81] Goodman SD, Kay O, Shi W, Ql F. Method of inhibiting bacterial growth and biofilm formation with natural QS peptides. US2008069782A1, 2008.

[82] Horswill AR. AGR-mediated inhibition and dispersal of biofilms. WO2009154988A2, 2009.

[83] Novick RP, Ji G, Beavis R. Blocking expression of virulence factors in *S. aureus*. US2003078378A1, 2003.

[84] Schramm VL. Methods and compositions for treating bacterial infections by inhibiting QS. WO2010033236A2, 2010.

[85] Wang B, Ni N, Wang J, Lu CD, Chou HT, Li M, Zheng S, Cheng Y, Peng H. Compositions for regulating or modulating QS in bacteria, methods of using the compounds, and methods of regulating or modulating QS in bacteria. US2010137249A1, 2010.

[86] Jones MB, Blaser MJ, Wood T, Ren D. *Bacillus anthracis* prevention and treatment: mutant *B. anthracis* lacking luxS activity and furanone inhibition of growth, AI-2 QS and toxin production. US2008299153A1, 2008.

[87] Novak JS, Yuan JTC. Method and process of using controlled gas environments to inhibit microbial growth. WO2007031826A2, 2007.
[88] Yoon JY, Kim CJ, Kim JE, Lee CH, Park HY. Antibacterial furanone derivative and method of preventing a biofilm formation. KR100832565B1, 2008.
[89] Villamar DF, Moriarty DJW. Bioactive food complex, method for making bioactive food complex product and method for controlling disease. US2004009160A1, 2004.
[90] Phadtare S, Kato I, Inouye M. Effect of treatment with 4,5-dihydroxy-2-cyclopenten-1-one (DHCP) on gene expression and QS in bacteria. WO03077844A2, 2003.
[91] Steggles RS. Composition comprising garlic extract and an antibiotic and/or antiseptic for use in the treatment of a multispecies bacterial infection. GB2472315A, 2011.
[92] Kim CJ, Lee GS, Lee JK, Yun BS, Lee JC, Park DJ. Piericidin A1 with competitive inhibitory activity of acyl homoserin lactone. KR100743672B1, 2007.
[93] Park SH, Kim JS, Seo YW, Kim YJ, Kim MJ. Antibacterial composition for inhibiting QS. KR100777780B1, 2007.
[94] Qianhong G, Hongbing L, Shouliang Y, Wengong Y, Shanshan Z. Compound separated and extracted from marine *Penicillium* and application thereof. CN101811959A, 2010.
[95] Kangmin D, Yuan W, Yue W. Antibiotic effective ingredient and use thereof. CN101385737A, 2009.
[96] Mathee K, Adonizio A L, Ausubel F, Clardy J, Bennett B, Downum K. Use of ellagitannins as inhibitors of bacterial QS. WO2009114810A2, 2009.
[97] QuorumEx. Available at: http://quorumex.com (Accessed on: September 7, 2011).
[98] Suga H, Igarashi J. QS inhibitor. US2010256369A1, 2010.
[99] Handelsman JE, Borlee BR. Method for modulating microbial QS. US2006052425A1, 2006.
[100] Pechere JC, Van Delden C, Menekse O. Therapeutic process for *P. aeruginosa* infections using macrolide antibiotics. US2004197341A1, 2004.
[101] Menekse O. Method for treatment and prevention of acute and chronic *P. aeruginosa* airway infections with inhalable macrolides. WO2004075874A1, 2004.
[102] Meijler MM, Amara N, Rayo J. Covalent inhibition of bacterial QS. WO2011001419A1, 2011.
[103] Blackwell HE, Geske GD, O'Neill JC. Modulation of bacterial QS with synthetic ligands. WO2008116029A1, 2008.
[104] Trivedi HM, Miksa D, Xu T. Anti-biofilm carbonate compounds for use in oral care compositions. WO2010114533A1, 2010.
[105] Lee CH, Yeon KM. Magnetic carrier for inhibiting biofilm formation on material surface in water system, includes magnetic core, layer for enzyme immobilization, and enzyme for inhibiting biofilm formation immobilized on layer for enzyme immobilization. US2009159533A1, 2009.
[106] Zhang L, Dong Y, Xu J, Zhang X. Control of bacterial infection by quenching QS of plant pathogenic bacteria. WO02061099A1, 2002.
[107] Zhang L, Dong Y, Xu J, Zhang H. Bacterial strains, genes and enzymes for control of bacterial diseases by quenching QS signals. WO0216623A1, 2002.

[108] Otero A, Romero M, Roca-Rivada A. Use of bacteria of the genus *Tenacibaculum* for QQ. WO2010012852A1, 2010.
[109] Faure D, Carlier A, Dessaux Y. Use of inductors of the degradation pathway of gamma-butyrolactone activating a homoserine lactone N-acyl signal. FR2863168A1, 2005.
[110] Pritchard DI. Treatment of surfaces populated by bacteria. WO03075654A2, 2003.
[111] Zhang LH, Lin YH, Xu JL. *Ralstonia* AHL-acylase gene. EP1470221B1, 2004.
[112] Xu F. Methods for eliminating the formation of biofilm. US2004109852A1, 2004.
[113] Ito T, Morohoshi T, Ikeda T, Kato N. Cyclodextrin derivative, method for producing it, and QS inhibition method. JP2009280736A, 2009.
[114] Robinson G, Piletsky S, Primrose S, Piletska O, Karim K, Whitcombe M, Chianella I. Polymer inhibitors of QS. WO2008087454A2, 2008.
[115] Alan CK, Andrew P, Radcliffe J. Method for treating infectious bacterial disease with anti-lactone signal molecule antibody or anti-lactone-derived signal molecule antibody. JP2011046705A, 2011.

719 **Table 1**. Bacteria [as reviewed in 38] and eukaryote organisms with QQ activity against AHL signals.

	Species	QQ activity	Gene	Group
Bacteria	*Agrobacterium tumefaciens*	Lactonase Lactonase	*blcC* *aiiB*	α-Proteobacteria
	Bosea sp.	(n.d.)	(n.d.)	
	Ochrobactrum sp.	(n.d.)	(n.d.)	
	Sphingomonas sp.	(n.d.)	(n.d.)	
	Sphingopyxis sp.	(n.d.)	(n.d.)	
	Stappia sp.	(n.d.)	(n.d.)	
	Hyphomonas sp.	(n.d.)	(n.d.)	
	Phaeobacter sp.	(n.d.)	(n.d.)	
	Roseovarius sp.	(n.d.)	(n.d.)	
	Variovorax paradoxus	Acylase	(n.d.)	β-Proteobacteria
	Ralstonia sp.	Acylase	*aiiD*	
	Comamonas sp.	Acylase	(n.d.)	
	Delftia acidovorans	(n.d.)	(n.d.)	
	Pseudomonas aeruginosa	Acylase Acylase	*pvdQ* *quiP*	γ-Proteobacteria
	Klebsiella pneumoniae	Lactonase	*ahlK*	
	Shewanella sp.	Acylase Lactonase	*aaC* (n.d.)	
	Acinetobacter sp.	(n.d.)	(n.d.)	
	Marinobacterium sp.	(n.d.)	(n.d.)	
	Glaciecola sp.	(n.d.)	(n.d.)	
	Alteromonas sp.	(n.d.)	(n.d.)	
	Halomonas sp.	(n.d.)	(n.d.)	
	Bacillus sp.	Lactonase	*aiiA*	Firmicutes
	Bacillus megaterium	Oxidase	*P450BM-3*	
	Oceanobacillus sp.	(n.d.)	(n.d.)	
	Halobacillus salinus	Antagonists (phenethylamides)	(n.d.)	
	Rhodococcus erythropolis	Lactonase/Acylase/ Oxidoreductase	*qsdA*	Actinobacteria
	Streptomyces sp.	Acylase	*ahlM*	
	Arthrobacter sp.	Lactonase	*ahlD*	
	Tenacibaculum discolor	(n.d.)	(n.d.)	Bacteroidetes
	Anabaena PCC7120	Acylase	*aiiC*	Cyanobacteria
Eukaryotes	Kidney	Acylase	(n.d.)	Mammal
	Respiratory epithelium	Paraxonases	*PONs*	
	Luffariella variabilis	Antagonists (Manoalides)	(n.d.)	Sponge
	Flustra foliacea	Antagonists (Alkaloids)	(n.d.)	Bryozoan
	Scorzonera sandrasica	Antagonists (n.d.)	(n.d.)	Plant
	Medicago truncatula	Antagonists (n.d.)	(n.d.)	
	Pisum sativum	Antagonists (n.d.)	(n.d.)	
	Allium sativum	Antagonists	(n.d.)	
	Lotus corniculatus	(n.d.)	(n.d.)	
	Laminaria digitata	Haloperoxidase	(n.d.)	Alga
	Ahnfeltiopsis flabelliformis	Antagonists (floridoside, betonicine and isethionic acid)	(n.d.)	
	Delisea pulchra	Antagonists (furanones)	(n.d.)	
	Penicillium spp.	Antagonists (penicillic acid and patulin)	(n.d.)	Fungus

720 Enzymatic activity (grey) or antagonist activity is indicated. Not determined (n.d.).

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Figure 1

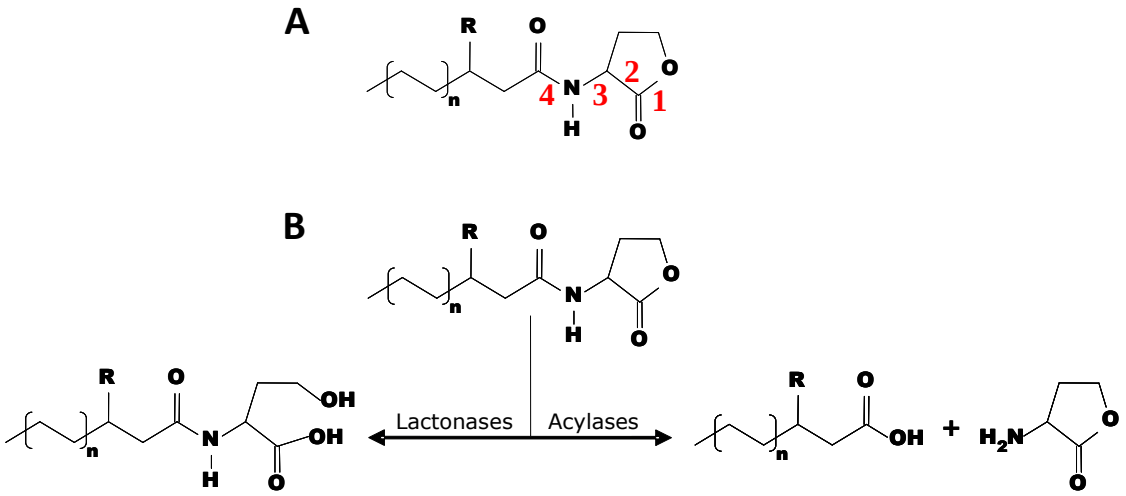

Figure 1. A) Four possible enzyme cleavage sites of an AHL. B) AHL degradation mechanisms of lactonases and acylases. [Modified from 25].

728 **Table 2**. Summary of the patent applications based on technologies that rely on the inhibition of the Quorum Sensing mechanisms.

QS target	QQ mechanism	Publication number	Title	Description	Inventors/Applicants	Publication date
AHL	Enzyme	WO2010012852A1 ES2342807B2	Use of bacteria of the genus *Tenacibaculum* for QQ	Use of bacterial cells of the genus *Tenacibaculum*, its crude cellular extract or its cultivation supernatants, capable of degrading AHLs, for QQ, for treatment of bacterial infections or to inhibit the formation of biofilms. Different bacteria are isolated from marine environments, and in particular, *Tenacibaculum* strain 20J shows the highest activity, being able to effectively degrade AHLs from 4 to 14 carbons in the acyl chain.	Otero A.; Romero M.; Roca-Rivada A. / University of Santiago de Compostela	Feb 2010
AHL	Enzyme	US2009159533A1 US7867392B2 KR2009069086A	Magnetic carrier for inhibiting biofilm formation on material surface in water system includes magnetic core, layer for enzyme immobilization, and enzyme for inhibiting biofilm formation immobilized on layer for enzyme immobilization.	A technique for inhibiting biofouling caused by biofilms grown on a material surface of a water system such as the membrane surface of a membrane bioreactor (MBR) for advanced wastewater treatment. The technique consists of a magnetic carrier comprising an enzyme for quenching QS that inhibits biofilm formation. It includes acylase and lactonase, The enzyme can also be an EPS-decomposing enzyme, such as a protease (aminopeptidase, ellastase) or carbohydrase (cellulose, glucanase), that would work by disintegrating the EPS matrix of the biofilm.	Lee C.-H.; Yeon K.-M. / SNU R&DB Foundation	Jun 2009
AHL	Enzyme	FR2863168A1 WO2005056002A2	Use of inductors of the degradation pathway of gamma-butyrolactone activating a AHL signal.	Inductors of the gamma-butyrolactone degradation pathway in order to increase the enzymatic degradation of AHL signals from Gram-negatives	Faure D.; Carlier A.; Dessaux Y. / Centre National de la Recherche Scientifique (CNRS)	Jun 2005
AHL	Enzyme	EP1470221B1 WO03068951A1 US2005155088A1 US7098014B2 DE60219077T2 AU2002225585A1	*Ralstonia* AHL-acylase gene	It provides the *qsbA* gene, which encodes an acylase useful for inactivating AHLs. This gene was isolated from *Ralstonia* sp., strain XJ12B. The invention also provides the QsbA protein, which possesses AHL inactivating activity.	Zhang L. H.; Lin Y. H.; Xu J. L. / Agency for Science, Technology and Research	Oct 2004
AHL	Enzyme	US2004109852A1 US6777223B2 WO0198214A1 AU6993901A	Methods for eliminating the formation of biofilm	Based on the use of "E.C. 3.5.1 acylases" that degrade AHLs from microorganisms in order to prevent or remove biofilm on a surface.	Xu F. / Novozymes Biotech Inc.	Jun 2004
AHL	Enzyme	WO03075654A2 US2005260183A1 JP2005525849A GB2401788B	Treatment of surfaces populated by bacteria.	Substance having AHL degrading activity which is obtained from the secretions of the larval form of the green bottle fly, *Lucilia sericata*. A synergistic effect is achieved when the larval secretions are used in	Pritchard D. I. / University of Nottingham [GB]	Sept 2003

		EP1485112A2 CN100496514C CA2478401A1 AU2003216995B2		combination with an antibiotic, e.g. tetracycline. It is also useful for avoiding the formation of bacterial biofilms on surfaces, especially those produced by *Pseudomonas aeruginosa* or *Staphylococcus aureus* through the enzymatic removal of exo-polysaccharide.		
AHL	Enzyme	WO02061099A1 EP1358340A1 US2004139495A1 US7205452B2 AR032411A1	Control of bacterial infection by quenching QS of plant pathogenic bacteria	Transgenic plants harboring the *aiiA* gene and expressing functional AiiA protein, which is a lactonase from *Bacillus* sp., are protected from bacterial pathogens, especially from Gram-negatives such as *Erwinia*.	Zhang L.; Dong Y.; Xu J.; Zhang X. / Institute Of Molecular Agrobiology	Aug 2002
AHL	Enzyme	WO0216623A1 US2008182790A1 US788493B2 US7410638B1 EP1325139A1 EP1930440A1 CN1944651A CN1454260A CA2420393A1 AU2000276976B2 AU2000276976B8	Bacterial strains, genes and enzymes for control of bacterial diseases by quenching QS signals	A metalohydrolase enzyme (35-94% peptide homology to previously described AiiA enzymes) and the corresponding gene sequence from *Bacillus thuringiensis* strains , is able to inactivate the AHL signals produced by the pathogen *Erwinia carotovora* SCG1 (causing potato soft root disease)	Zhang L.; Dong Y.; Xu J.; Zhang H. / Institute Of Molecular Agrobiology	Feb 2002
AHL	Antagonist	US2011046195A1	Non-Lactone Carbocyclic and Heterocyclic Antagonists and Agonists of Bacterial QS	Compounds that inhibit and/or activate QS in various bacteria, based on modifications to the AHL head group**.** This work relates to non-AHL-based autoinducer analogs for QS modulation, especially in *P. aeruginosa*.	Blackwell H. E.; Mcinnis C. E./ Wisconsin Alumni Research Foundation	Feb 2011
AHL	Antagonist	GB2472315A WO2011012855A2	Composition comprising garlic extract and an antibiotic and/or antiseptic for use in the treatment of a multispecies bacterial infection	Composition with QS-inhibition activity for use in the treatment of a multi-species bacterial infection, which comprises *S*-allyl cysteine (from garlic extract), and an antibiotic and/or antiseptic (preferably tetracycline).	Steggles R. S.	Feb 2011
AHL	Antagonist	WO2011001419A1	Covalent inhibition of bacterial QS	Covalent inhibitors of QS signals from Gram-negative bacteria, especially from *P. aeruginosa*. These inhibitors are the synthetic compounds isothiocynates and haloacetamides, which have an electrophilic group that binds covalently to proteins whose natural ligands are the AHLs, so that they block the action of these QS molecules.	Meijler M. M.; Amara N.; Rayo J. / National Institute for Biotechnology in the Negev	Jan 2011
AHL	Antagonist	US2010292261A1 WO2008069374A KR20080050844A	QS antagonist, method of preventing a biofilm formation using the quorum sensing	A QS antagonist comprising an homoserine lactone moiety and sulfanylethanoyl group, which by having a similar chemical structure to that of the autoinducer which	Yoon J.-Y.; Kim C.-J.; Kim J.-E.; Park H.-Y. / Seoul National	Nov 2010

		KR100841294B1	antagonist and method of reducing a bacterial contamination using the quorum sensing antagonist	is produced by bacteria as a signal, is able to inhibit the formation of biofilm and reduce the bacterial contamination as well, especially for Gram-negative bacteria.	University Industry Foundation	
AHL	Antagonist	CN101811959A	Compound separated and extracted from marine *Penicillium* and application thereof	A chemical compound (4,6-dimethyl-7-[(1R,2E,4aS,7R,8R,8aR)-1,2.4a,5,6,7,8,8a-octahydro-7-hydroxyl-2-(3-hydroxyl-2-oxygen-propylene)-3,6,8-trimethyl-1-naphthyl]-,(2E,4E,6E)-heptantriene acid) obtained by fermentation of the marine fungus *Penicillium*, which is able to inhibit QS mechanisms in *P. aeruginosa*. The inhibitory effect is dose-depending.	Qianhong G.; Hongbing L.; Shouliang Y.; Wengong Y.; Shanshan Z. / Ocean University of China	Aug 2010
AHL	Antagonist	US2010256369A1 EP2215910A1 WO2009063901A1	QS inhibitor	A specific pyrimidinone compound that has an inhibiting effect on the QS of specific bacteria, especially as a bacterial disease control agent for agricultural and horticultural use. In particular, it is extremely effective for controlling *Burkholderia glumae*.	Suga H.; Igarashi J. / University of Tokyo; Otsuka Chemical CO LTD	Oct 2010
AHL	Antagonist	WO2009148571A1 US2011123586A1	Inhibition of QS-mediated processes in bacteria	Antagonists of the autoinducer AI-1 of *Vibrio harveyi* that bind to *Vibrio harveyi* LuxN at the autoinducer-1 (AI-1) binding site in LuxN, disrupting the AI-1-mediated QS mechanisms, therefore inhibiting QS in Gram-negative bacteria.	Bassler B.; Swem L. / Princeton University	Dec 2009
AHL	Antagonist	EP2100602A1	Method and compositions suitable for treatment of wounds	Compounds capable of inhibiting an AHL-regulated process by inhibiting bacterial signaling, especially for reducing or inhibiting bacterial colonization and infection in areas of compromised skin integrity. The compositions showed to reduce the presence of biofilm and planktonic bacteria such as *P. aeruginosa.* Additionally, the compositions containing silver sulfadiazine demonstrated an ability to synergistically reduce both planktonic bacteria and biofilm formation.	No designation of inventor filed / Quonova Europe GMBH	Sept 2009
AHL	Antagonist	WO2009114810A2 US2011105421A1	Use of ellagitannins as inhibitors of bacterial QS	Plant C-glycosidic ellagitannins (castalagin and vescalagin, from *Conocarpus erectus*), are shown to affect AHL levels and QS-gene expression in *P. aeruginosa*, so that they can be used in order to inhibit QS, and thus to avoid infection and biofilm formation from bacteria.	Mathee K.; Adonizio A. L.; Ausubel F.; Clardy J.; Bennett B.; Downum K. / Florida International University	Sept 2009
AHL	Antagonist/Agonist	WO2008116029A1 US2008312319A1 US7910622B2	Modulation of bacterial QS with synthetic ligands	Compounds that imitate the effect of natural ligands and produce an agonistic or antagonistic effect. The compounds comprise AHLs with a wide range of acyl	Blackwell H. E.; Geske G. D.; O'Neill J. C. / Wisconsin	Sept 2008

		EP2136800A1		groups. *N*-phenylacetanoyl-L-homoserine lactones, in particular, are capable of either inhibiting or, in some cases, strongly inducing QS in the bacterial symbiont *Vibrio fischeri*.	Alumni Research Foundation	
AHL	Antagonist	KR20080049527A1 KR100841289B1	Antibacterial AHL derivatives and method of preventing a biofilm formation	Antibacterial AHL derivative that shows capability as a QS antagonist, thereby effectively blocking formation of a biofilm and inhibiting propagation of Gram-negative bacteria.	Yoon J. Y.; Kim C. J.; Kim J. E. / Seoul National University Industry Foundation	Jun 2008
AHL	Antagonist	KR100777780B1	Antibacterial composition for inhibiting QS	A composition comprising an extract of *Rhodophyta* that inhibits QS, thereby being useful as an antibacterial agent for inhibiting bio-membrane formation. An antibacterial composition for inhibiting QS comprises 0.1-90.0 wt.% of floridoside, 0.1-90.0 wt.% of betonicine, and 0.1-90.0 wt.% of isethionic acid, extracted from *Rhodophyta* such as *Ahnfeltiopsis flabelliformis*.	Park S. H.; Kim J. S.; Seo Y. W.; Kim Y. J.; Kim M. J. / Sum-Jin Est CO LTD	Nov 2007
AHL	Antagonist	KR100743672B1	Piericidin A1 with competitive inhibitory activity of AHL	Piericidin A1, produced by *Kitasatospora* sp. AR030054, and having competitive inhibitory activity of AHL, is provided to block the QS of plant pathogenic bacteria or fungi by inhibiting activity of AHL. Effective against plant diseases caused by infection of *Erwinia carotovora* or *Agrobacterium tumefaciens*.	Kim C. J.; Lee G. S.; Lee J. K.; Yun B. S.; Lee J. C.; Park D. J. / Korea Research Institute Of Bioscience and Biotechnology	Jul 2007
AHL	Antagonist	US2007203128A1 US2007093534A1 US2007184014A1 US2007208012A1 US2007196340A1 US2007197492A1	Modulation of pathogenicity	Based on the use of compounds such as amide, carbazide and hydrazide derivatives as selective inhibitors of bacterial pathogens, in particular by blocking the QS system of Gram-negative bacteria.	Ammendola A.; Aulinger-Fuchs K.; Gotschlich A.; Kramer B.; Lang M.; Saeb W.; Sinks U.; Wuzik A. / Quonova LLC	Apr 2007
AHL	Antagonist/Agonist	US2006052425A1 US2010216835A1	Method for modulating microbial QS	AHLs agonist and antagonist compounds, especially from *P. aeruginosa*, and especially oxoC10 and C12-HSL. Antagonists compete with oxo-C12-HSL. In thiocarbamate structures, ester substitutions at the ortho position at the aniline ring provides the greatest activity against 3-oxo-C12-HSL.	Handelsman J. E.; Borlee B. R. / Wisconsin Alumni Research Foundation	Mar 2006
AHL	Antagonist	EP1475092A1 WO2004099175A2 EP1622890A2 CA2526904A1	Blockers of the QS System of Gram-negative bacteria	Based on the use of compounds such as amide, carbazide and hydrazide derivatives as selective inhibitors of bacterial pathogens and their use for the treatment and prevention of microbial damages and diseases, in particular for Gram-negatives.	Ammendola A.; Aulinger-Fuchs K.; Gotschlich A.; Kramer B.; Lang M.; Saeb W.; Sinks U.;	Nov 2004

					Wuzik A. / 4SC AG	
AHL	Antagonist	US2004009160A1	Bioactive food complex, method for making bioactive food complex product and method for controlling disease	Combined delivery and use of probiotic bacteria and QS inhibitory furanones in a bioactive food complex product for effective disease prevention and control. The bioactive food complex serves to deliver different bioactive components including probiotic bacteria and QS inhibitor molecules to the digestive tract and environment of animals such as shrimp or fish or other livestock raised commercially to effectively control bacterial disease.	Villamar D. F.; Moriarty D. J. W. / Acuabiotec LLC	Jan 2004
AHL	Antagonist	WO03106445A1 AU2003232173A1	Compounds and methods for controlling bacterial virulence	Novel sulfonated homoserine lactones that act as QS inhibitors. The invention relates to a novel class of sulfonamides exerting a QS inhibiting effect on bacteria, such as *P. aeruginosa*.	Nielsen J.; Givskov M. / QSI Pharma AS	Dec 2003
AHL	Sequestration	JP2011046705A	Method for treating infectious bacterial disease with anti-lactone signal molecule antibody or anti-lactone-derived signal molecule antibody	Method for isolation of a bacterial lactone signal molecule it to screen a population of specific binding molecules and administering the specific binding molecule so identified to a patient in need thereof.	Alan C. K.; Andrew P.; Radcliffe J. / Haptogen LTD	Mar 2011
AHL	Sequestration	JP2009280736A	Cyclodextrin derivative, method for producing it, and QS inhibition method	Cyclodextrin derivatives capable of trapping an autoinducer such as an AHL with high selectivity, therefore inhibiting QS mechanisms.	Ito T.; Morohoshi T.; Ikeda T.; Kato N. / University of Utsunomiya	Dec 2009
AHL	Sequestration	WO2008087454A2	Polymer inhibitors of QS	Polymer inhibitors of QS, methods for their preparation and use of such polymers in the prevention and treatment of bacterial infections and in the manufacture of shaped articles having an increased resistance to bacterial infections. QS signaling molecules that may be sequestered (ligand, binding agent, absorbent) by polymers including AHLs. AHL molecules which contain additional carbonyl group in the acyl chain, in particular in the 3 position, relative to the carbonyl of the acyl group, are preferred, especially OC6-HSL. Three polymers based on acrylamide (AA), N, N'-methylene bis acrylamide (MBAA) and itaconic acid (IA) were found to be very efficient for adsorption of OC6-HSL.	Robinson G ; Piletsky S.; Primrose S.; Piletska O.; Karim K.; Whitcombe M.; Chianella I. / University of Kent & Cranfield Centre for Supramolecular Chemistry	Jul 2008

AHL	Inactivates signal receptor LuxR	US2007264715A1 WO2005047514A1 EP1685250A1 EP1685250B1	Proteins involved in signal transduction	Peptide hydrolase that downregulate quorum sensing by preventing the signal receptor LuxR or a homologue of LuxR from activating transcription. This inhibition is used to prevent biofilm formation or to break down established biofilms and may also be used to downregulate the production of virulence determinants by pathogenic bacteria.	Robinson G. K.; Rising H. / University of Kent	Nov 2007
AHL & AI-2	Inhibition of signal synthesis	WO2010033236A2 US2011190265A1 EP2348854A2	Methods and compositions for treating bacterial infections by inhibiting QS	Based on the use of MTAN (5'-Methylthioadenosine/*S*-adenosyl homocysteine nucleosidase) inhibitors, which inhibit QS, especially in *P. aeruginosa*, Streptococcus *pneumoniae*, *Neisseria meningitidis*, *Klebsiella pneumoniae*, *S. aureus* and *Helicobacter pylori*. MTANs are directly involved in the synthesis of autoinducers, so the blockade of MTAN produces inhibition of AI-1 and AI-2 production.	Schramm V. L. / Albert Einstein College of Medicine of Yeshiva University	Mar 2010
AHL	Inhibition of signal synthesis	US2004197341A1 WO03020290A1 JP2005501889A EP1423129A1	Therapeutic process for *P. aeruginosa* infections using macrolide antibiotics	Macrolides, in particular azalides such as azithromycin, for the treatment of nosocomial infections caused by *P. aeruginosa*. The mechanism of action is the inhibition of the QS of *P. aeruginosa*, in particular the impediment of the *las* and *rhl* QS systems synthesis and the impediment of the synthesis of the autoinducers OC12 and C4-HSL.	Pechere J.-C.; Van Delden C.; Menekse O. / Anbics Patents Licenses AG	Oct 2004
AHL	Inhibition of signal synthesis	WO2004075874A1 AU2003205499A1	Method for treatment and prevention of acute and chronic *P. aeruginosa* airway infections with inhalable macrolides	Macrolides, in particular azalides such as azithromycin, for the treatment or prevention of acute or chronic *P. aeruginosa* infections of the airways by inhalation. The mechanism of action is the inhibition of the QS of *P. aeruginosa*, in particular the impediment of the las and rhl QS systems synthesis and the impediment of the synthesis of the autoinducers OC12 and C4-HSL.	Menekse O. / Anbics Patents Licenses AG	Sep 2004
AI-2	Antagonist	US2010137249A1 WO2009029317A2 EP2155705A2	Compositions for regulating or modulating QS in bacteria, methods of using the compounds, and methods of regulating or modulating QS in bacteria.	Antagonists of the AI-2 QS signal. Fourteen commercial databases were screened in order to identify compounds that can bind to LuxP with high affinity and therefore that can function as AI-2 antagonists. 42 were identified and tested for ability to antagonize AI-2 induced QS in *Vibrio harveyi*: boronic acids, pyrogallol and phenothiazine, amongst others.	Wang B.; Ni N.; Wang J.; Lu C.-D.; Chou H.-T.; Li M.; Zheng S.; Cheng Y.; Peng H. / Georgia State University Research Foundation	Jun 2010
AI-2	Antagonist	US2008299153A1 US795596B2 WO2005005598A2 US2005042634A1 US7365184B2	*Bacillus anthracis* prevention and treatment: mutant *B. anthracis* lacking *luxS* activity and furanone inhibition of growth, AI-2 QS and toxin	Method for inhibiting the growth and toxin production of *Bacillus anthracis* by the use of furanones. Furanone treatment of *B. anthracis* inhibits growth by inhibiting the activity of the AI-2 QS molecule, as well as toxin production, specifically the expression of protective	Jones M. B.; Blaser M. J.; Wood T.; Ren D. / New York University & University of	Dec 2008

			production	antigen. Furanones to be used include (5Z)-4-bromo-5-(bromomethylene)-3-butyl-2(5H)-furanone, 3-butyl-5-(dibromomethylene)-2-(5H)-furanone, 5-(bromomethylene)-2-(5H)-furanone, 4-bromo-5-(bromomethylene)-2(5H)-furanone and 5-(dibromomethylene)-2(5H)-furanone.	Connecticut	
AI-2	Antagonist	WO2007031826A2 US2007059414A1	Method and process of using controlled gas environments to inhibit microbial growth	Methods and apparatus for inhibiting growth and QS mechanisms in food-borne microorganisms by simultaneously exposing a food product to an antimicrobial gas mixture and a treating agent adapted to inhibit signaling between the microorganisms. The treating agent is a furanone (2,5-dimethyl-4- hydroxy-3(2H)-furanone (DMHF) or ascorbic acid) or a furanone and a gas mixture (carbon dioxide, nitrogen, hydrogen, nitrous oxide, argon, oxygen, helium, krypton, and combinations thereof that enhance the effect of the furanone).	Novak J. S.; Yuan J. T. C. / L'Air Liquide	Mar 2007
AI-2	Antagonist	WO03077844A2 JP2005519616A EP1482793B1 CN1642418A1 CN1312290C AU2003218013A1	Effect of treatment with 4,5-dihydroxy-2-cyclopenten-1-one (DHCP) on gene expression and QS in bacteria	Based on the use of DHCP (4,5-dihydroxy-2-cyclopenten-1-one) in order to regulate the expression of genes involved in QS. DHCP is shown to inhibit AI-2 activity and the genes comprising quorum-regulated processes such as virulence, motility and outer membrane functions are down-regulated by DHCP treatment in *E. coli*. In addition, *cysK* which is a known QS gene working in an alternate pathway(s) in *E. coli* increases considerably in response to DHCP. These results suggest that DHCP regulates the switching on/off of the different QS circuits in *E. coli*.	Phadtare S.; Kato I.; Inouye M. / Takara BIO INC	Sep 2003
Peptide	Antibody	US2010291093A1 WO2009055054A2 KR20100102100A JP2011500814A EP2211889A2 CN101835484A CA2703133A1 AU2008317331A1	Antibody-mediated disruption of quorum sensing in bacteria	Monoclonal antibody elicited against a rationally-designed hapten that can inhibit QS produced by a Gram-positive bacterium. It is based on the discovery that an antibody specific for the *S. aureus* AP-4 signaling peptide can block QS and prevent staphylococcal infection in mice.	Janda K. D.; Kaufmann G. F.; Park J. / The Scripps Research Institute	Nov 2010
Peptide	Agonist	WO2009154988A2 US2011152176A1	AGR-mediated inhibition and dispersal of biofilms	Based on the use of activators of the "*agr*" (*accessory gene regulator*) QS mechanism from staphylococci (*S. aureus)*, in order to block or inhibit biofilm formation on medical devices, wound surfaces or organs. The strategy	Horswill A. R. / University of Iowa Research Foundation	Dec 2009

				is therefore to activate the QS mechanisms, not to inhibit them, in order to inhibit biofilm formation or biofilm growth, reduce biofilm size or promote detachment of bacteria from a formed biofilm, since pathogens such as *S. aureus* increase the *agr* QS signals in order to avoid biofilm formation. The activator may be an auto-inducing peptide (AIP).		
Peptide	Agonist	US2008069782A1	Method of inhibiting bacterial growth and biofilm formation with natural QS peptides	Inhibition of bacterial growth in a biofilm through the manipulation of QS signals concentration, specifically for treating and protecting against dental caries or infective endocarditis caused by *Streptococcus mutans*. The method targets specifically Gram-positive bacterial species including *Streptococci*, *Staphylococci* and *Bacillus* that have peptide-based QS systems and it is based on increasing the signal concentration to achieve bacterial death or detachment from the biofilm.	Goodman S. D.; Kay O; Shi W.; Ql F. / University of Southern California	Mar 2008
Peptide	Antagonist	US2003078378A1	Blocking expression of virulence factors in *S. aureus*	The present invention provides purified and isolated peptides which inhibit the *agr* system of *S. aureus*. The peptides of the present invention are cyclic, comprise about six to about twelve amino acids in length, and include amino acid number 28 (cysteine) from the AgrD region of a staphylococci bacterium, which is believed to be conserved in the corresponding AgrD regions of various staphylococci bacterium. The inhibitor peptides of the present invention include sequences corresponding to the native peptide from staphylococci bacterium, as well as analogues thereof which contain amino acid substitutions which also result in inhibitor peptides. The purified inhibitor peptides of the present invention may be isolated directly from staphylococci bacterium, recombinantly produced, or synthesized chemically.	Novick R. P.; Ji G.; Beavis R.	April 2003
Not specified	Antagonist	WO2010114533A1	Anti-biofilm carbonate compounds for use in oral care compositions	Carbonate compounds for use in oral care compositions which avoids plaque formation presumably by blocking QS mechanisms produced by bacteria from the oral cavity. Effectiveness shown to avoid the formation of biofilms by *Actinomyces naeslundii*, which causes periodontal disease. The compounds up or down-regulate different periodontal disease metabolites, such as compounds generated by amino acid metabolism, in the urea cycle, in glutathione conversion, in carbohydrate	Trivedi H. M.; Miksa D.; Xu T. / Colgate Palmolive CO	Oct 2010

				metabolism, etc.		
Not specified	Antagonist	US2009192192A1 EP2078713A1 WO2009077844A2	Inhibitors of biofilm formation of Gram-positive and Gram-negative bacteria	Use of synthetic compounds (similar structure to AHL, but modifications in the head or tail, not furanones) as broad spectrum inhibitors of bacterial biofilm formation. Compounds have been developed that can significantly inhibit QS dependent biofilm formation of several bacteria, more preferably selected from the group *P. aeruginosa*, *S. aureus* and *Staphylococcus epidermidis* (inhibitory effect shown), most preferably MRSA strains.	Ammendola A.; Wieber T.; Wuzik A.; Lang M. / Quonova LLC	Jul 2009
Not specified	Antagonist	CN101385737A	Antibiotic effective ingredient and use thereof	Diphylloside A and the derivatives thereof, which belong to components of flavonoids and are derived from Epimedium Chinese herbal medicines, can inhibit the QS system and reduce the pathogenicity of the pathogenic bacteria of the QS system; the diphylloside A can also be used for treating and preventing crop diseases and insect pests as a pesticide.	Kangmin D.; Yuan W.; Yue W. / Northwestern University	Mar 2009
Not specified	Antagonist	KR100832565B1	Antibacterial furanone derivative and method of preventing a biofilm formation	Antibacterial furanone derivative that functions as a QS antagonist, which interrupts communication among bacteria due to a chemical structure similar to the bacterial QS signals, thereby being used for preventing formation of a biofilm. Microorganisms include Gram-negative bacillus.	Yoon J. Y.; Kim C. J.; Kim J. E.; Lee C. H.; Park H. Y. / Seoul National University Industry Foundation	May 2008

Technologies are classified according to the different QQ strategies described in the text, namely, enzymatic degradation of QS signals (acylases, lactonases) and antagonists of the QS signals, which imitate the AHL structure, thereby blocking or destabilizing the AHL receptor (i.e. furanones). Note: when several publication numbers are shown, publication date refers to that of the first application number shown at the top of the list.