Quorum sensing interference in vibrios

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Abstract

Vibrios belonging to the Harveyi clade and the Splendidus clade are important pathogens that cause high economic losses in aquaculture. To control bacterial diseases, antibiotics have been widely applied in aquaculture worldwide for many years, contributing to the development and spread of antibiotic resistance. To further limit the use of antibiotics without affecting the envisaged sustainable growth of the sector, novel therapies to control diseases are urgently needed. As the virulence of many bacterial pathogens is controlled by quorum sensing, quorum sensing interference has been the most intensively studied antivirulence therapy. It aims to disarm rather than to kill the pathogens to prevent them from attacking their host. This strategy is believed to impose less selective pressure upon pathogens for resistance development when compared to antibiotics. In this review, we provide an overview of quorum sensing systems in vibrios belonging to the Harveyi clade and the Splendidus clade, as well as the virulence-related phenotypes controlled by these systems. The major quorum sensing systems in these bacteria include three-channel quorum sensing systems and indole signaling. Furthermore, we discuss different agents that interfere with quorum sensing systems and that protect aquatic animals from disease caused by Harveyi clade and Splendidus clade vibrios. These agents include small molecules (both natural and synthetic) that interfere with three-channel quorum sensing systems or with indole signaling, and signal molecule-degrading bacteria. Finally, we identify knowledge gaps and propose some frontiers for further research in order to move this promising field forward.

Key words: Aquaculture; *Vibrio*; quorum sensing; indole signaling; antivirulence therapy.

1 DISEASES CAUSED BY HARVEYI CLADE AND SPLENDIDUS CLADE VIBRIOS IN AQUACULTURE

Disease outbreaks, including bacterial infections, are playing a prominent role in limiting the further sustainable expansion of aquaculture, especially in the early life stages of the animals.[1,2] Among the groups of pathogenic bacteria, vibrios are a well-known cause of huge losses in the aquaculture industry worldwide.[3-6] Vibriosis, also called *Vibrio* disease, is one of the most prevalent diseases in aquaculture.[7,8] Vibrios are curved rod shaped Gram-negative Gammaproteobacteria that are widespread in the aquatic environment, both free-living and in association with eukaryotes.[3] They are mesophilic and chemoorganotrophic, and have a facultative fermentative metabolism.[9] Some vibrios are non-pathogenic, whereas others are pathogenic, and pathogenicity is a strain- rather than a species-characteristic.[10] Signs of disease caused by pathogenic *Vibrio* strains include lethargy, tissue and appendage necrosis, slow growth, slow metamorphosis, body malformation, bolitas negricans, bioluminescence, muscle opacity and melanization, and erratic movement.[8] In many cases of pathogenic vibrios, they are opportunistic pathogens, i.e. non-obligate and/or non-specialised pathogens of a focal host.[11] Indeed, vibrios are able to reproduce outside of a host and are often able to infect various host types. These characteristics contribute to the widespread problems caused by vibrios as they can reach high densities in the environment surrounding the cultured animals.

The evolutionary history of the genus *Vibrio* has been reconstructed by means of multilocus sequence analysis of nine genes, and 14 different clades were recognized.[12] Among these clades, vibrios belonging to the Harveyi clade (including species such as *V. harveyi*, *V. campbellii* and *V. parahaemolyticus*) and the Splendidus clade (including species such as *V. splendidus*, *V. tasmaniensis* and *V. crassostreae*) are important pathogens of various aquatic organisms, both vertebrates and invertebrates.

Vibrios belonging to the Harveyi clade have resulted in severe losses in shrimp farming, causing up to 100% mortality in postlarvae and juveniles.[13-15] Strains belonging to a number of species, including *V. campbelli*, *V. harveyi*, *V. parahaemolyticus* and *V. alginolyticus*, have been associated with disease outbreaks in shrimp.[16-18] A severe emergent penaeid shrimp bacterial disease, acute hepatopancreatic necrosis disease (AHPND), has been reported during the past decade. It is caused by strains that carry a virulence plasmid encoding the *pirA* and *pirB* toxin genes.[19] Shrimp production in AHPND-affected regions has dropped to 60% when compared to the production before AHPND occurred, and the disease has led to a global loss of USD 43

billion to the shrimp farming industry.[20,21] Harveyi clade vibrios also occur in many fish species like cobia (*Rachycentron canadum),*[22] European sea bass (*Dicentrarchus labrax),*[23] and rainbow trout (*Oncorhynchus mykiss),*[24] with reports of infection by *V. harveyi*, *V. alginolyticus*, and *V. rotiferianus*, respectively. It has been reported that Harveyi clade vibrios are also pathogenic to molluscs, e.g. *V. harveyi*, *V. parahaemolyticus* and *V. alginolyticus* led to mass mortalities in abalone (*Haliotis diversicolor supertexta* and *Haliotis tuberculate).*[25-27]

Splendidus clade vibrios can also infect many aquaculture animals such as oysters, mussels, turbot, urchin, cod and sea cucumber, in which infections of the oyster (*Crassostrea gigas)* and sea cucumber (*Apostichopus japonicus)* are the most extensively studied. For instance, the skin ulcer syndrome (SUS) caused by Splendidus clade pathogens was reported to cause more than 80% mortality and led to 30% economic losses in *A. japonicus* culture.[28-30] Meanwhile, *V. tasmaniensis* LGP32, formerly *V. splendidus*,[31] is a well-known pathogen of *C. gigas* oyster spat in French farming areas and has led to intermediate to high mortality.[32,33]

In addition to affecting aquaculture animals, vibrios can also infect humans and cause skin infections and gastrointestinal disorders.[34,35] For example, human-pathogenic strains of *V. parahaemolyticus* cause mild gastroenteritis to severe debilitating dysentery, making it a notably lethal human pathogen derived from seafood.[36]

2 ANTIVIRULENCE THERAPY – A NOVEL STRATEGY TO CONTROL INFECTIONS

To control infectious diseases, large quantities of veterinary drugs are employed, in which antibiotics often are the only effective agents that farmers have to treat their animals. Antibiotics target vital functions in bacteria and exert their protective effect by killing pathogens or by inhibiting their growth.[37] They have been used in animal medicine since the 1940s[38] and in aquaculture for more than 50 years.[39] Sixty-seven different antibiotic compounds have been used in 11 of the top 15 global aquaculture producing countries between 2008 and 2018, in which oxytetracycline, sulphadiazine and florfenicol were the most frequently used ones.[40] It should be stressed, however, that the use of antibiotics varies widely among producing countries. The use of antibiotics for veterinary use is strictly regulated, for instance, in Europe. As a result of the frequent use of antibiotics, aquaculture has become an important source of antibiotic resistance genes.[41] Resistance genes can often be transferred from one bacterial cell to another, ultimately leading to the emergence of (multiple) antibiotic resistant pathogens.[42] Consequently, antibiotics commonly used in aquaculture are no longer effective against pathogens in some cases.[4] For example, a *V. harveyi* strain, the cause of mass mortality of *Penaeus monodon* larvae, was found to be resistant to cotrimoxazole, chloramphenicol, and streptomycin.[13] Therefore, there is an urgent need for novel strategies to protect aquaculture animals from pathogenic bacteria without the added risk of resistance development.

Bacterial pathogens infect animals through producing different virulence factors, i.e. compounds, cell structures or activities that enable them to infect their host. These factors include motility, adhesion, host tissue degradation, iron acquisition, secretion of toxins, as well as biofilm formation.[43,44] As virulence factors enable pathogens to cause disease, interfering with the expression of these factors can prevent pathogens from attacking their host. This novel strategy for disease control is termed antivirulence therapy, which aims to disarm pathogens rather than to kill them or to inhibit their growth.[45,46] Compared with the use of antibiotics, the advantage of targeting virulence is the lower selective pressure exerted over pathogens,[47] reducing the risk of resistance development and the possibility of transmission of resistance determinants through horizontal gene transfer.[48] Furthermore there are less negative side effects towards the neutral and beneficial bacteria that are associated with the host because –in contrast with antibiotics- antivirulence agents do not kill or inhibit growth of these bacteria.[1]

Antivirulence therapy can work by specifically inhibiting a certain virulence factor or by interfering with the regulation of virulence gene expression to affect several virulence factors at once.[49] Quorum sensing is one of the most intensively studied targets for antivirulence therapy, since it is a cell-to-cell communication system widely used by pathogenic bacteria to regulate the expression of several virulence factors.[44,50] Various types of quorum sensing systems and signals have been identified in different kinds of bacteria. The following paragraphs will give an overview of quorum sensing and quorum sensing interference in Harveyi clade and Splendidus clade vibrios.

3 QUORUM SENSING SYSTEMS IN HARVEYI CLADE AND SPLENDIDUS CLADE VIBRIOS

Bacterial quorum sensing is a cell-to-cell communication system in which bacteria control the expression of certain genes to coordinate their behaviors by producing, detecting and responding to small extracellular signal molecules named autoinducers.[51] Quorum sensing bacteria produce autoinducers intracellularly and the autoinducers then passively diffuse out of the cells or are actively secreted, and the external concentration of autoinducers is proportional to cell density and inversely related to diffusion in the micro-environment surrounding the cell.[52] When the extracellular concentration of signal molecules reaches the threshold level required for detection, they bind to specific receptors, which results in activation or repression of quorum sensing target genes (i.e. some genes are activated at high signal molecule concentrations, whereas others are repressed).[51] Several different signal molecules have been identified in vibrios belonging to the Harveyi clade and the Splendidus clade (Table 1) and these will be discussed in the following paragraphs.

3.1Three-channel quorum sensing systems

Three-channel quorum sensing systems have been firstly and mainly studied in *V. campbellii* strain BB120 (which was previously classified as *V. harveyi*[53]). The three-channel quorum sensing system of this bacterium consists of the LuxM/LuxN, LuxS/LuxPQ and CqsA/CqsS channels (Fig. 1).[54] Harveyi autoinducer 1 (HAI-1) is the N-acylhomoserine lactone (AHL) N-(3-hydroxybutanoyl)-L-homoserine lactone, which is synthesized by LuxM and recognized by LuxN.[55] A variety of other AHLs has been detected and identified in vibrios belonging to the Splendidus clade as *V. tasmaniensis* was found to produce N-decanoyl-L-homoserine lactone, N-3-hydroxy-dodecanoyl-L-homoserine lactone, N-3-oxo-dodecanoyl-L-homoserine lactone and N-tetradecenoyl-L-homoserine lactone by a biosensor-based UHPLC-HRMS/MS method.[56] The gene encoding LuxM has been identified in most *Vibrio* species belonging to the Harveyi clade (such as *V. parahaemolyticus*, *V. campbellii*, *V. alginolyticus*, *V. natriegens* and *V. rotiferianus*), and in some *Vibrio* species belonging to the Splendidus clade (including *V. crassostreae*, *V. tasmaniensis*, *V. splendidus* and *V. lentus*).[57-61] AHL signal molecules have been detected in all of the abovementioned species. Besides this, *V. mytili*, *V. chagasii* and *V. pomeroy* also produce AHL but their AHL systems have not yet been characterized. Finally, no AHL production could be detected by a biosensor-based UHPLC-HRMS/MS method in *V. hemicentroti* and *V. gigantis* although they contain the *luxM* gene.[56] Remarkably, recent

research revealed that *V. campbellii* DS40M4, a strain that is closely related to BB120, does not produce an AHL (it also does not contain LuxM), whereas it contains the LuxN receptor (which, however, does not respond to HAI-1 produced by strain BB120).[62]

The second signal molecule, Autoinducer 2 (AI-2), is a furanosyl borate diester produced by LuxS and is detected by LuxQ via the periplasmic binding protein LuxP.[63] The third signal molecule, Cholerae autoinducer 1 (CAI-1), is (Z)-3-aminoundec-2-en-4-one which is synthesized by CqsA and detected by CqsS.[51,64] The production of each signal molecule differs depending on the bacterial growth phase. HAI-1 and CAI-1 are mainly produced during the late exponential phase, while AI-2 can be detected during the exponential growth phase.[65]

At low concentrations of signal molecules, the receptors LuxN, LuxQ and CqsS autophosphorylate and transfer phosphate to LuxO via LuxU.[66] When phosphorylated, LuxO is active, and in cooperation with the alternative sigma factor σ54, it promotes the transcription of five quorum regulatory small RNAs (Qrr sRNAs).[67] These sRNAs (together with the chaperone Hfq) promote translation of the master regulator AphA and inhibit translation of the master regulator LuxR, respectively.[68] At high concentrations of signal molecules, the receptor proteins switch from kinases to phosphatases, which leads to the dephosphorylation of LuxU and LuxO. Dephosphorylated LuxO is inactive and therefore, the sRNAs are not formed, AphA is not translated and LuxR is translated.[69] Hence, the level of LuxR is proportional to the concentration of the signal molecules, whereas the level of AphA is inversely related to the concentration of the signal molecules. The three channels of the system work synergistically with each other (i.e. the maximal output of the quorum sensing system that can be obtained in the presence of one of the signal molecules is lower than that in the presence of two of the signal molecules and the latter is again lower than the ouput in the presence of all three signal molecules).

In the *V. campbellii* BB120 quorum sensing circuit, AphA and LuxR are the master regulators that coordinate the quorum sensing response and affect the transcription of many target genes. LuxR is considered to be the major quorum sensing regulator, which controls gene expression at both low and high signal molecule concentrations (it is produced at high levels in the presence of high signal molecule concentrations and at low levels in the presence of low signal molecule concentrations).[70] In contrast, AphA is absent in the presence of high signal molecule concentrations and maximally produced in the presence of low signal molecule concentrations.[71] AphA and LuxR can either activate or repress the expression of target genes to different extents. It has been revealed that AphA regulates 167genes, LuxR regulates 625

genes, and they coregulate 77 genes.[72] Interestingly, the quorum sensing regulon of *V. campbellii* strain DS40M4 is considerably smaller than that of BB120 as LuxR only regulates 90 genes in this strain.[62] The three-channel quorum-sensing systems in Harveyi clade vibrios play an important role during infection of a host, because they control the production of different virulence-related phenotypes, including biofilm formation,[73] type III secretion,[66] flagellar motility,[74] production of a siderophore,[67] the Vhp metalloprotease,[75] chitinase A[76] and three phospholipase genes[77]. Furthermore, the activity of this quorum sensing system is proportional to the virulence to a host.[72] Quorum sensing regulating virulence seems to be a general feature in strains belonging to the Harveyi clade since the use of a quorum sensing-disrupting brominated furanone (see lower) could protect gnotobiotic brine shrimp larvae from different strains belonging to the Harveyi clade.[78] Remarkably, the three channels of the *V. campbellii* BB120 quorum sensing system have a different impact on virulence of the bacterium in different hosts. Indeed, AI-2 and CAI-1 are required for full virulence of the bacterium towards brine shrimp (*Artemia franciscana*) larvae, whereas HAI-1 has no effect in this host.[79] On the other hand, in giant river prawn (*Macrobrachium rosenbergii*) larvae and tiger grouper (*Epinephelus fuscoguttatus*) larvae, HAI-1 and AI-2 are required for full virulence, whereas CAI-1 has no effect.[80,81]

A similar three-channel quorum sensing system is found in other vibrios belonging to the Harveyi clade and in vibrios belonging to the Splendidus clade (Table 1). Indeed, the genes encoding different components of the three-channel quorum sensing system are present within the genomes of for instance *V. crassostreae* and *V. tasmaniensis*.[82] In contrast to Harveyi clade vibrios, the three-channel quorum sensing systems of Splendidus clade vibrios have no impact on their virulence. Indeed, quorum sensing mutants of *V. crassostreae* and *V. tasmaniensis*, for instance, did not show a decreased virulence towards blue mussel (*Mytilus edulis*) larvae, and the quorum sensing inhibitor cinnamaldehyde (see lower) also had no effect.[82]

3.2 Indole signaling

Indole is an intercellular, interspecies, and interkingdom signaling molecule produced by various bacteria.[83] It is synthesized from tryptophan by tryptophanase (TnaA) through a reversible reaction, with pyruvate and ammonia as by-products.[84] Indole is produced by more than 85 species of bacteria,[85] including vibrios belonging to the Harveyi clade and the Splendidus clade (Table 1). As a variety of bacterial species can produce large quantities of

indole, indole is widespread in the natural environment and plays an important role in bacterial pathogenesis, both in indole-producing bacteria and in non-indole-producing bacteria.[83] Vibrios belonging to the Harveyi clade and the Splendidus clade have been reported to produce indole at concentrations between 50 and 200 μM, mainly during stationary phase.[86,87]

Many groups have reported that diverse biological functions are controlled by indole, such as spore formation, plasmid stability, drug resistance, biofilm formation, and virulence in different bacteria.[85] Indole signaling has also been studied in vibrios belonging to the Harveyi clade and the Splendidus clade. Indole showed an inhibiting effect on biofilm formation and motility in *V. campbellii*, *V. harveyi*, *V. parahaemolyticus*, *V. crassostreae* and *V. tasmaniensis*.[87,88] The addition of indole affected the expression of a large number of genes, including genes related to metabolism, ABC transporters, flagellar assembly, chemotaxis, and response regulators in *V. crassostreae* and *V. tasmaniensis*.[87] Furthermore, indole decreased bioluminescence and exopolysaccharide levels in *V. campbellii*,[86] and inhibited the expression of the virulence genes *vsm* (metalloprotease) and *vsh* (hemolysin) in *V. splendidus*[89]. These results showed that indole decreases bacterial virulence without affecting bacterial growth in vibrios belonging to the Harveyi clade and the Splendidus clade. As a consequence, indole signaling has been viewed as a valid target for the development of novel therapeutics in order to control infections caused by Harveyi clade and Splendidus clade vibrios in aquaculture. It has been reported that the survival rate of mussel larvae increased 2.4-fold and 1.5-fold when challenged with *V. crassostreae* and *V. tasmaniensis* pretreated with 200 μM indole, respectively.[87] Meanwhile, indole decreased the virulence of Harveyi clade vibrios towards gnotobiotic brine shrimp larvae, and the survival rate of brine shrimp larvae challenged with vibrios pretreated with indole increased 1.3-fold to 1.8-fold.[88] A receptor for indole in vibrios has not yet been identified, although recent work indicated that the transmembrane regulatory protein ToxR is involved in indole sensing in *V. cholerae*.[90]

4 INTERFERENCE WITH QUORUM SENSING IN HARVEYI CLADE AND SPLENDIDUS CLADE VIBRIOS

4.1 Small molecule quorum sensing inhibitors inhibiting three-channel quorum sensing systems

As quorum sensing systems have been shown to control virulence in various bacteria including vibrios, quorum sensing interfering agents are being studied as novel disease control agents.[91,92] To disrupt quorum sensing, three main targets in the signaling mechanisms can be targeted: the signal molecule synthesis, the signal molecules themselves and the signal detection and/or transduction.[93] To date, several compounds that are synthesized or isolated from plants or microorganisms have been described as quorum sensing inhibitors or claimed to be quorum sensing inhibitors in aquaculture pathogens.[94] In the following sections, we summarize the strategies to interfere with quorum sensing with examples targeting aquaculture pathogens belonging to the Harveyi clade. Among these pathogens, *V. harveyi* and *V. campbellii* are the most intensively studied bacteria and a variety of quorum sensing inhibitors has been investigated, including natural compounds and synthetic quorum sensing inhibitors. Several quorum sensing inhibitors have been claimed in literature based on the inhibition of quorum sensing-regulated phenotypes. However, in many cases, controls in which the impact on the same phenotype when not under quorum sensing control is tested, were not included. Hence, it is not clear whether in these cases the claimed quorum sensing inhibitors really are inhibiting quorum sensing or rather have a direct effect on the tested phenotypes (without inhibiting quorum sensing).[95] In the specific case of *V. campbellii*, quorum sensing-regulated bioluminescence is usually used as the phenotype based on which quorum sensing inhibitors are identified (Table 2). However, in these kinds of studies, it is important to verify that the candidate quorum sensing inhibitor has no impact on bioluminescence when it is not controlled by quorum sensing (e.g. in an engineered strain in which bioluminescence is under control of a constitutive promotor instead of its natural, quorum sensing controlled promotor). In this way, false positives can be identified.[46] Unfortunately, in many cases, this control is not included. Another way to confirm quorum sensing inhibition is to identify the molecular target of a candidate quorum sensing inhibitor.

4.1.1 Brominated furanones

Brominated furanones are the most intensively studied quorum sensing inhibitors, and have been reported to disrupt quorum sensing in various Gram-negative bacteria. Both natural and synthetic brominated furanones inhibited quorum sensing-regulated bioluminescence of *V. campbellii* and protected gnotobiotic brine shrimp larvae from *V. harveyi*, *V. campbellii* and *V. parahaemolyticus*.[78] The synthetic furanone (5Z-)-4-bromo-5(bromomethylene)-2(5H)-furanone is slightly more active (but also more toxic) than the natural furanone (5Z)-4-bromo-5-(bromomethylene)-3-butyl-2(5H)-furanone. The synthetic furanone could offer complete protection (no significant difference in survival of challenged and treated larvae when compared to non-challenged larvae) to giant river prawn (*Macrobrachium rosenbergii*) larvae against *V. campbellii* at a concentration of 1 μM, but resulted in complete mortality of the larvae at 10 μM (due to toxicity).[80] The natural furanone was found to block all three channels of the *V. campbellii* quorum sensing system by decreasing the DNA-binding activity of LuxR, the quorum sensing response regulator.[96]

4.1.2 Brominated thiophenones

In order to identify more potent and less toxic quorum sensing inhibitors than brominated furanones, brominated thiophenones have been studied. Thiophenone TF310, (Z)-4-((5-(bromomethylene)-2-oxo-2,5-dihydrothiophen-3-yl)methoxy)-4-oxobutanoic acid, was reported to disrupt quorum sensing of *V. campbellii* by decreasing the ability of the quorum sensing master regulator LuxR to bind to its target promoter DNA.[97] As a quorum sensing inhibitor, thiophenone TF310 increased the survival of challenged brine shrimp larvae when added to the rearing water at 1 μM or more, and offered a complete protection (no significant difference in survival with non-challenged larvae) at a concentration of 2.5 μM, whereas severe toxicity was only observed at 250 μM. In a follow-up study, TF203 ((Z)-5-(bromo(pheny)methylene)thiophen-2(5H)-one), TF319 ((Z)-3-(hydroxy,ethyl)-5-(phenylthio)methylene)thiophen-2(5H)-one), TF339 ((Z)-(5-(bromomethlene)-2-oxo-2,5-dihydrothiophen-3-yl)methy acetate) and TF342 ((Z)-5-(E)-3-bromobut-2-en-1-ylidene)-3-chlorothiophen-2(5H)-one) were the most active thiophenones. All of them inhibited quorum sensing at 0.25 μM.[98] The specific quorum sensing-disrupting activity of the thiophenones (i.e. the ratio between the inhibition of quorum sensing-regulated bioluminescence and quorum sensing-independent bioluminescence) was higher than 10 and was strongly positively correlated with the protection offered to brine shrimp larvae against pathogenic *V. campbellii*.

4.1.3 QStatin: 1-(5-bromothiophene-2-sulfonyl)-1H pyrazole

QStatin (1-(5-bromothiophene-2-sulfonyl)-1H pyrazole) is a novel, potent, and selective *Vibrio* quorum sensing inhibitor, affecting *V. harveyi* homologues of LuxR, the well-conserved master transcriptional regulators of the three-channel quorum sensing systems in *Vibrio* species.[99] QStatin was shown to affect the interaction of SmcR, the LuxR homologue of *V. vulnificus*, with its target promotor DNAs. It was further reported to inhibit the bioluminescence of *V. harveyi*, to affect *V. parahaemolyticus* colony opacity and to improve the survival of brine shrimp larvae challenged with *V. harveyi* and *V. parahaemolyticus* at 20 μM.[99]

4.1.4 Thiazolidinediones and dioxazaborocanes

It has been reported that the structure of dioxazaborocanes resembles oxazaborolidine derivatives which antagonize AI-2 binding to its receptor.[100] Structural resemblances can be found between thiazolidinediones and well-known furanone type quorum sensing inhibitors such as N-acylaminofuranones and/or AHL signaling molecules.[101] Brackman et al. explored the effects of 6 thiazolidinedione compounds and 9 dioxazoborocane derivatives on quorum sensing in *V. campbellii*.[102] Although all compounds blocked quorum sensing (as manifested by an inhibition of the bioluminescence of *V. campbellii*), the thiazolidinediones were the most active AI-2 quorum sensing inhibitors, with EC_{50} values in the low micromolar range. Furthermore, the mechanism of inhibition was elucidated by measuring the effect on bioluminescence in a series of *V. campbellii* quorum sensing mutants and by DNA-binding assays with purified LuxR protein. The results obtained in these experiments indicated that the thiazolidinediones blocked quorum sensing in *V. campbellii* by decreasing the DNA-binding ability of LuxR, while dioxazaborocanes were found to block AI-2 quorum sensing by targeting the AI-2 receptor LuxPQ.[100]

4.1.5 Synthetic cannabinoid HU-210 and cannabigerol

The synthetic cannabinoid HU-210 displays a multiplicity of biochemical, pharmacological, and behavioral effects,[103] and was viewed as potential anti-quorum sensing agent.[104] It has been proven that HU-210 affects the autoinducer-2 (AI-2) pathway, one of three known quorum sensing cascades of *V. campbellii*. The addition of HU-210 (0.02-200 μg/ml) to bacterial medium resulted in an up to 98% decrease in the bioluminescence of *V. campbellii* mutant BB152 (AI-1−, AI-2+), and 85% decrease in the bioluminescence of *V. campbellii* BB170 (Sensor-1−, Sensor-2+). Furthermore, HU-210 inhibited quorum sensing-mediated virulence

factor production without any inhibitory effect on bacterial growth. It significantly reduced biofilm formation at concentrations of 0.2-200 mg/l, decreased swimming motility at concentrations of 2-200 mg/l in the mutant strain *V. campbellii* BB152 . It also altered the expression of several genes, which are regulated by quorum sensing, specifically downregulating the genes of the AI-2 quorum sensing cascade at 2 mg/l.[104] Recently, Aqawi et al. (2020) reported that cannabigerol, a cannabinoid naturally present in Cannabis plants, decreased quorum sensing-regulated phenotypes in *V. campbellii* BB120. It was shown that cannabigerol increased *luxO* mRNA levels, with a concomitant decrease of *luxR* mRNA levels.[105]

4.1.6 Cinnamaldehyde and cinnamaldehyde derivatives

Cinnamaldehyde isolated from cinnamon is a non-toxic flavoring agent that is generally regarded as safe.[94] Similar to brominated thiophenones and furanones, cinnamaldehyde can act as a quorum sensing inhibitor. It was evaluated to assess its potential as a quorum sensing inhibitor using *Chromobacterium violaceum*, *Yersinia enterocolitica*, and *Erwinia carotovora*.[106] Brackman et al (2008) showed that cinnamaldehyde and five cinnamaldehyde derivatives, could interfere with quorum sensing in *V. campbellii* by decreasing the DNA-binding ability of the quorum sensing master regulator LuxR without inhibiting bacterial growth.[107] At 100 µM, cinnamaldehyde and 2-NO_2-cinnamaldehyde were the two most active inhibitors, which inhibited bioluminescence of *V. campbellii* by 65% and 62%, respectively. Furthermore, both of them protected gnotobiotic brine shrimp larvae against *V. campbellii* at a concentration of 100-150 µM.[107] In a further study exploring the structure-activity relationship of cinnamaldehyde analogues, 3,4-dichloro-cinnamaldehyde was found to be the most active compound. It increased the survival of the nematode *Caenorhabditis elegans* infected with *V. campbellii* BB120 at 10 µM, but it was toxic above 25 µM.[108]

4.1.7 Citrus limonoids (isolimonic acid and ichangin)

Five limonoids were purified from sour orange and evaluated for their ability to inhibit cell–to-cell signaling in *V. campbellii*.[109] Among them, ichangin and isolimonic acid significantly inhibited HAI-1-induced bioluminescence in *V. campbellii* BB886 (ΔluxPQ) at 6.25-100 mg/l, reduced AI-2-induced bioluminescence in *V. campbellii* BB170 (ΔluxN) at 25-100 mg/l, and decreased biofilm formation of *V. campbellii* at concentrations of 25-100 mg/l. Meanwhile, isolimonic acid and ichangin treatment resulted in induced expression of the *luxO* gene without any effect on *luxR* promotor activity. Therefore, the authors concluded that the ability of the

limonoids to interfere with *V. campbellii* quorum sensing is a result of the modulation of *luxO* expression. Unfortunately, the impact of these compounds on the virulence of *V. campbellii* was not studied.

4.1.8 Vitamin C (sodium ascorbate)

Sodium ascorbate was viewed to constitute a novel agent for the control of *V. campbellii* infections in aquaculture, as it showed a protective effect on gnotobiotic brine shrimp larvae challenged with *V. campbellii*.[110] Specifically, relatively high concentrations (5 and 10 g/l) of sodium ascorbate significantly decreased swimming motility, biofilm production, and the production of virulence enzymes, such as lipase, caseinase, phospholipase, and hemolysin in *V. campbellii*. Meanwhile, sodium ascorbate improved survival of gnotobiotic brine shrimp larvae by pretreating *V. campbellii* before inoculation into the rearing water. Furthermore, sodium ascorbate inhibited the quorum sensing-regulated bioluminescence of wild type *V. campbellii* while it did not affect the constitutive bioluminescence of strain JAF548 pAKlux1 at 5 and 10 g/l, which suggested that sodium ascorbate could interfere with quorum sensing in *V. campbellii*.[110]

4.2 Indole analogues

Indole analogues are widely present in nature as some bacteria and eukaryotes modify or degrade indole and/or produce indole analogues.[111-113] Although indole has been reported to affect bacterial virulence[83,114] and to show a protective effect on host organisms[87,88], it appeared to be toxic to brine shrimp larvae at a concentration of 200 µM[86]. Therefore, less toxic and more effective indole analogues have been explored, in which indole-3-acetic acid was found to have similar effect as observed for indole.[86] Indole-3-acetic acid is produced by plants, (micro) algae and some bacteria and is found in diverse environments such as marine waters, plants and animal hosts.[115] It has been reported that indole-3-acetic acid inhibited the bioluminescence and the virulence of *V. campbellii* towards shrimp larvae at 50 µM.[86] Furthermore, 30 mg/l indole-3-acetic acid or indole-3-butyric acid decreased biofilm formation, bioluminescence, caseinase and swarming motility of *V. harveyi* and improved survival of infected brine shrimp larvae when combined with 10 mg/l undecanoic acid.[116] Indole-3-acetamide is another similar indole analogue which inhibited bioluminescence, biofilm formation, exopolysaccharide level of *V. campbellii* at 50 µM. The survival of gnotobiotic brine shrimp larvae was significantly improved by pretreating *V. campbellii* with 50 µM indole-3-acetamide.[86] Furthermore, the halogenated indole analogues 4-iodoindole, 7-iodoindole, 4-chloroindole and 7-chloroindole

were found to inhibit biofilm formation, bacterial motility, hydrophobicity, protease activity, and indole production of *V. parahemolyticus* at a concentration of 10-100 mg/l[117] (Table 3).

Recently, we explored the antivirulence properties of 70 indole analogues towards *V. campbellii*, and found 28 indole analogues to have a protective effect on brine shrimp larvae against *V. campbellii* without affecting bacterial growth (Table 3).[118,119] Among them, 17 halogenated indole analogues improved the survival of brine shrimp larvae challenged with *V. campbellii* to over 60% at relatively low concentrations ($\leqslant$ 20μM). More specifically, the most active compounds were 7-bromoindole (increasing the survival of challenged brine shrimp to over 60% at 2 μM or more), 4-fluoroindole, 7-fluoroindole and 5-iodoindole (all at 5 μM). Five of the indoles were able to increase the survival of challenged brine shrimp larvae to over 80% (all at 10 μM): 6-bromoindole, 7-bromoindole, 4-fluoroindole, 5-iodoindole and 7-iodoindole. I*n vitro* work showed that all of the 17 selected halogenated indoles decreased swimming motility at both 10 μM and 100 μM and most of them decreased biofilm formation at a concentration of 100 μM, whereas only a slightly decreased protease activity and no effect on hemolytic activity were observed.[119] Besides halogenated indole analogues, 1-methylindole (100 μM), indene (200 μM), 2,3-benzofuran (200 μM), thianaphthene (200 μM), indole-3-acetonitrile (10 μM), methyl indole-3-carboxylate (20 μM), 3-methylindole (20 μM), and indole-2-carboxaldehyde (20 μM) also exhibited a significant protective effect on brine shrimp larvae against *V. campbellii* infection, resulting in survival rates of challenged brine shrimp above 80%. The highest survival of brine shrimp larvae (98%) was obtained with indole-3-acetonitrile, at a concentration of 20 μM. Meanwhile, all of these 8 indole analogues reduced swimming motility of *V. campbellii*, and 3 of them (1-methylindole, indole-3-acetonitrile, methyl indole-3-carboxylate) decreased biofilm formation at 200 μM.[118]

4.3 Signal molecule-degrading bacteria

Several microorganisms produce quorum quenching enzymes degrading AHL signal molecules, which disrupt the quorum sensing of pathogenic bacteria, thereby preventing the production of their virulence factors.[120] Therefore, the application of signal molecule-degrading bacteria as quorum quenching probiotics may be another particularly useful method to control pathogenic bacteria in aquaculture. Two major types of AHL-degrading enzymes have been reported: acylases and lactonases.[135] AHL acylases cleave the amide bond of AHL molecules resulting in the formation of homoserine lactone and a fatty acid. AHL lactonases, on the other hand, cleave the lactone ring, resulting in the formation of N-acylhomoserines.

It has been reported that the AHL lactonase protein AiiA of *Bacillus thuringiensis* decreased the intensity of bioluminescence of *V. harveyi* by 85% and repressed the pigment synthesis of the quorum sensing reporter strain *C. violaceum*.[121] Later, *B. thuringiensis* QQ1 and *B. cereus* QQ2 were isolated from the intestines of Asian seabass. These strains could degrade AHLs produced by important pathogens belonging to the genus *Vibrio*, such as *V. harveyi* and *V. alginolyticus*.[122] Furthermore, *B. thuringiensis* QQ1 and *B. cereus* QQ2 were reported to significantly improve the cumulative survival of Asian seabass against *V. harveyi*.[123] Specifically, Asian seabass were fed with a basal diet (control groups) or a basal diet containing 1×10^9 CFU/g *B. thuringiensis* QQ1 or *B. cereus* QQ2 for 35 days. The mortality of fish challenged with *V. harveyi* was reduced to 24% and 16% by feeding with QQ1 and QQ2 respectively (72% in fish without probiotics). Meanwhile, the hematocrit (Hct) and respiratory burst activity (RBA) in fish fed with QQ1 or QQ2, globulin in fish fed with QQ2 and total leucocyte count (TLC) in fish fed with QQ1 were significantly increased after infection with *V. harveyi* (day 42), while serum triglycerides, cholesterol, alkaline phosphatase (ALP), alanine aminotransferase (ALAT), aspartate aminotransferase (ASAT) and lactate dehydrogenase (LDH) were significantly decreased in fish fed with QQ1 or QQ2 after infection.

Two different mixtures of AHL degrading enrichment cultures were isolated and viewed as probiotics in aquaculture. One of them, EC5(D), was enriched from intestinal microbiota of European sea bass (*Dicentrarchus labrax*) and the other, EC5(L) from Asian sea bass (*Lates calcarifer*).[124,125] Both of the enrichment cultures were proven to improve the survival of *Macrobrachium rosenbergii* larvae challenged with *V. harveyi*. There were two ways to apply the enrichment cultures: adding them directly into the larval rearing water at 10^6 CFU ml^{-1} or feeding larvae with the enrichment cultures encapsulated in *Artemia* nauplii. Both enrichment cultures had a similar positive effect on larval survival and larval quality.[126]

Pande et al. isolated *Pseudomonas* sp. NFMI-T and *Bacillus* sp. NFMI-C from open cultures of the microalgae *Tetraselmis suecica* and *Chaetoceros muelleri*, respectively.[127] Both of the isolates were able to degrade the AHL N-hexanoyl-L-homoserine lactone, while only *Bacillus* sp. NFMI-C was able to inactivate *N*-hydroxybutanoyl-L-homoserine lactone, the AHL produced by *V. campbellii*. Importantly, *Bacillus* sp. NFMI-C significantly improved the survival of giant river prawn (*Macrobrachium rosenbergii*) larvae challenged with pathogenic *V. campbellii* when added to the rearing water of the larvae at 10^5 CFU ml^{-1}.[127]

Finally, quorum quenching *Bacillus* spp. (*B. subtilis* MFB10, *B. lentus* MFB2, and *B. firmus* MFB7) were isolated from aquaculture ponds and mangrove soil for their high ability to degrade

synthetic AHLs.[128] All of these isolates suppressed the expression of virulence genes encoding protease, lipase, phospholipase, caseinase, chitinase, and gelatinase, and inhibited the biofilm formation of *V. harveyi*. Moreover, *B. subtilis* MFB10, *B. lentus* MFB2, and *B. firmus* MFB7 protected *Penaeus monodon* post-larvae against *V. harveyi* infection when added into the rearing water of the larvae at 10^5, 10^6, and 10^7 CFU ml^{-1}, respectively.[128]

5 SIGNIFICANCE AND FUTURE PERSPECTIVES

The three-channel quorum sensing system regulates the expression of virulence genes in Harveyi clade vibrios.[49] Consequently, a variety of quorum sensing inhibitors has been investigated in these bacteria (including natural and synthetic compounds and signal molecule-degrading bacteria), and these were found to protect aquatic animals from disease. In contrast, no inhibitors of the three-channel quorum sensing system have been documented in Splendidus clade vibrios because this quorum sensing system does not affect the virulence of these vibrios.[82] On the other hand, indole is a signaling molecule which affects the virulence in both Harveyi clade and Splendidus clade vibrios, and indole analogues showed a highly protective effect on aquatic host organisms against these pathogens. All of the data demonstrate that indole and indole analogues have the potential to be novel disease control agents in aquaculture. However, further research will be needed to establish the mode of action of indole and indole analogues as the signal detection and signal transduction cascades are still not known. Moreover, in addition to quorum quenching bacteria discussed higher, bacteria producing indole or indole analogues may also be useful as new probiotics in aquacultural disease control.

The evidence that quorum sensing interfering agents can protect aquatic animals from vibriosis has thus far only been obtained in laboratory experiments, and field experiments have not yet been performed. The major reason for this probably is the fact that these agents either need to be isolated from natural sources or custom synthesised and thus it is not straightforward to obtain sufficiently large amounts of the compounds in order to perform field experiments. Hence, further work will be needed to produce sufficient amounts in a cost effective manner or to identify agents of which sufficient amounts are available at a reasonable price in order to perform field experiments. A notable example of the latter is cinnamaldehyde, which is currently used as an additive in foods and feeds to inhibit the growth of pathogenic bacteria.[129]

There are many factors to be considered and a lot of further work to be done before applying quorum sensing interfering agents to aquaculture. Firstly, it needs to be determined how these

agents will be administered. Thus far, quorum sensing interfering agents (both small molecules and microorganisms) have been added to the rearing water of aquatic organisms and this was found to protect the animals from vibriosis. However, it might be more efficient to add the quorum sensing interfering agents to the feed. It still needs to be established how quorum sensing interfering agents can be added to feed and whether addition to the feed is also effective in protecting animals from vibriosis. Secondly, we know that quorum sensing interfering agents have preventive properties, but in many cases it is still not clear whether they also have curative properties. Indeed, the agents were usually added before the pathogen was added or together with the pathogen. One notable recent exception showed that the indole analogues 1-methylindole, indene, 2,3-benzofuran and thianaphthene have no curative activity as they did not protect brine shrimp from *V. campbellii* when added to the rearing water 1 day after the pathogen.[118] However, in a field situation, these compounds might still be useful to prevent the spread of a disease between animals in a situation where some animals of a group are affected by vibriosis and the others are not yet affected. Third, in several cases (noatbly in the case of indole and indole analogues), the molecular target of the molecules still needs to be identified.

Quorum sensing interfering agents are generally believed to have less side effects towards nontarget organisms and to include a lower risk for resistance development than conventional antibiotics. However, thus far, proof of these assumptions is still lacking .[130] Hence, further research is needed in order to verify that quorum sensing interfering agents have no negative impact on the activity of beneficial bacteria that are present in aquaculture systems (e.g. probiotics or bacteria in biofilters). This is especially true for quorum sensing interfering agents with activity towards a broad spectrum of bacteria. Also, non-pathogenic vibrios can be used as probiotics,[131] and in case such a probiotic is applied together with a quorum sensing interfering agent targeting vibrios, then it will need to be verified that the latter has no negative impact on the beneficial activities of the probiotic. It stands to reason, however, that such an impact will be smaller than that of antibiotics as quorum sensing interfering agents do not kill or inhibit growth of vibrios. Finally, more information with respect to safety of quorum sensing interfering agents for cultured animals is needed. Indeed, in some cases toxicity of the agents for the cultured organisms was tested (and found to be higher than the concentration that protected the animals from infection[97]), whereas for other agents this information is still lacking.

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CONFLICT OF INTEREST

The authors declare no conflict of interest. The funders had no role in the writing of the manuscript, or in the decision to publish it.

AUTHOR CONTRIBUTIONS

Shanshan Zhang: Conceptualization; funding acquisition; visualization; writing – original draft preparation. **Qian Yang:** Supervision; writing – review & editing. **Mieke Eggermont:** Writing – review & editing. **Tom Defoirdt:** Conceptualization; funding acquisition; supervision; writing – review & editing.

DATA AVAILABILITY STATEMENT

Data sharing is not applicable to this article as no datasets were generated or analysed during the current study.

TABLES

Table 1. Quorum sensing signal molecule production in vibrios belonging to the Harveyi clade and the Splendidus clade.

Species	Presence of a three-channel QS system	Production of signal molecules				Reference(s)
		AHL	AI-2	CAI-1	Indole	
Harveyi clade						
Vibrio alginolyticus	+	+	NT	NT	+	54,60,132
Vibrio campbellii	+	+	+	+	+	61,86
Vibrio harveyi	+	+	+	+	+	54,57,78
Vibrio mytili	NT	+	NT	NT	-	56,133
Vibrio natriegens	NT	+/-	-	-	+	61,133,134
Vibrio owensii	NT	+	NT	NT	+	134,135
Vibrio parahaemolyticus	+	+	+	+	+	54,58,78,134
Vibrio rotiferianus	+	+	+	+	+	56,57,136
Splendidus clade						
Vibrio artaborum	NT	NT	NT	NT	-	137
Vibrio atlanticus	NT	-	NT	NT	+	56,137
Vibiro celticus	+	NT	NT	NT	+	137-139
Vibrio chagasii	NT	+	+	-	+	58,61,137
Vibrio crassostreae	+	+	+	+	+	59,87,138
Vibrio cyclitrophicus	+	NT	NT	NT	-	137,138
Vibrio fortis	NT	+/-	NT	NT	+	133,134
Vibrio gigantis	+	+/-	NT	NT	+	56,134,137
Vibrio hemicentroti	+	-	NT	NT	+	56,137
Vibrio kanaloae	+	NT	NT	NT	+	137,138
Vibrio lentus	+	+	NT	NT	+	58,137,138
Vibrio pelagius	NT	NT	NT	NT	-	133
Vibrio pomeroyi	NT	+	+	+	+	61,137
Vibrio splendidus	+	+/-	+	-	+	61,138,140
Vibrio tasmaniensis	+	+	+	+	+	57,59,87
Vibrio toranzoniae	+	NT	NT	NT	+/-	138,141

"NT" means the systems or signals haven't been tested;

"+" indicates the systems or signals have been tested and detected;

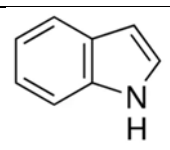
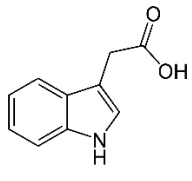
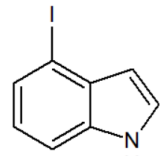
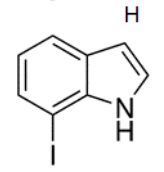
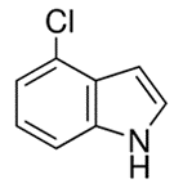
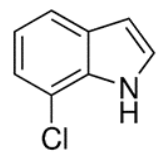
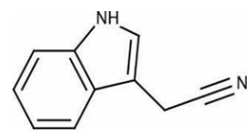
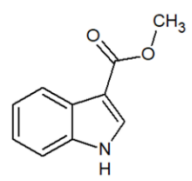
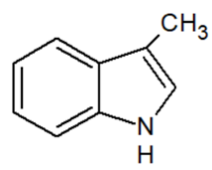
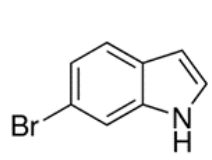
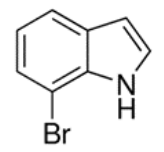
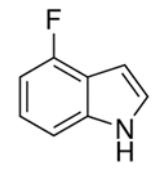
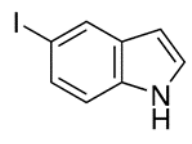
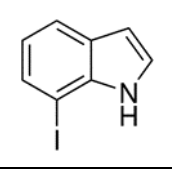
"-" indicates the systems or signals have been tested but not detected;

"+/-" means the systems or signals have been tested but gave variable results in different strains.

Table 2. Representative examples of published data on inhibitors of the three-channel quorum sensing (QS) system in Harveyi clade vibrios.

QSI compounds	Inhibition of signal molecule reporter	Molecular target	Other QS-related assays	Reference(s)
Natural furanone: ((5Z)-4-bromo-5-(bromomethylene)-3-butyl-2(5H)- furanone)	Inhibited bioluminescence of *V. harveyi* BB120 at 1-10 µM.	LuxR	Protected gnotobiotic brine shrimp (*Artemia franciscana*) from *V. harveyi*, *V. campbellii* and *V. parahaemolyticus* infection at 5-20 mg/L of furanone, while high mortality was observed at 50 mg/L of furanone.	[78,96]
Synthetic furanone: (5Z-)-4-bromo-5-(bromomethylene)-2(*5H*)-furanone	Inhibited bioluminescence of *V. harveyi* BB120 at 1-10 µM.	LuxR	A complete protection of giant freshwater prawn larvae from *V. harveyi* infection at 1 µM, but complete mortality at 10 µM.	[78,80]
Sodium ascorbate	Inhibited bioluminescence of wild type *V. campbellii*, and didn't affect mutant type containing plasmid pAKlux1 at 5-10 mg/ml.	LuxR	Decreased swimming motility, biofilm formation and production of virulence enzymes; increased the survival of gnotobiotic brine shrimp larvae at 5-10 mg/ml.	[110]
Brominated thiophenone TF310 (Z)-4-((5-(bromomethylene)-2-oxo-2,5-dihydrothiophen-3-yl)methoxy) -4-oxobutanoic acid	Inhibited bioluminescence of *V. harveyi* BB120 and different QS mutants at 2.5 µM.	LuxR	A complete protection of brine shrimp against *V. harveyi* at 2.5 µM.	[80,97]
Thiophenones TF203, TF319, TF339 and TF342	Inhibited bioluminescence of *V. harveyi* at 0.25 µM.	LuxR	A complete protection of brine shrimp against *V. harveyi* by TF203 (1µM), TF339 (1 µM); over 80% protection by TF319 (1 µM), TF342 (1 µM).	[98]
QStatin: 1-(5-bromothiophene-2-sulfonyl)-1H pyrazole	Inhibited bioluminescence of *V. harveyi* at 20 µM.	LuxR	Improved the survival of brine shrimp larvae challenged with *V. harveyi* and *V. parahaemolyticus* at a concentration of 20 µM.	[99]
Thiazolidinediones and dioxazaborocanes	Inhibited bioluminescence by 50% in *V. harveyi* BB170 (ΔluxN) and 30-90% in *V. harveyi* MM32 (ΔluxN ΔluxS) at 100 µM.	LuxR (Thiazolidinediones); LuxPQ(dioxazaborocanes).	Inhibited *V. harveyi* LuxR DNA binding activity at 10 µM.	[100,102]
Synthetic cannabinoid	Inhibited bioluminescence of *V. harveyi* mutant BB152 (AI-1−, AI-2+) and *V. harveyi* BB170 (Sensor-1−, Sensor-2+) at concentration of 0.02-200 µg/ml.	LuxR	Reduced biofilm formation (0.2-200 µg/ml) and swimming motility (2-200 µg/ml) in *V. harveyi* mutant strain BB152, down-regulated the genes of the AI-2 QS cascade (2 µg/ml).	[104]
Cinnamaldehyde and 2-NO_2-cinnamaldehyde	Inhibited bioluminescence of *V. harveyi* at 100 µM.	LuxR	Protected gnotobiotic *Artemia* shrimp against *V. harveyi* at 100-150 µM.	[80,107]
3,4-dichloro-cinnamaldehyde	Inhibited bioluminescence of *V. harveyi* at 100 µM.	LuxR	Increased the survival of the nematode *Caenorhabditis elegans* infected with *V. harveyi* at 10-100 µM.	[108]
Limonoids (ichangin and isolimonic acid)	Inhibited HAI-1-induced bioluminescence in *V. harveyi* BB886 (ΔluxPQ) at 6.25-100 µg/ml, reduced AI-2-induced bioluminescence in *V. harveyi* BB170 (ΔluxN) at 25-100 µg/ml.	LuxO	Inhibited biofilm formation in *V. harveyi* at a concentration of 25-100 g/ml.	[109,142]

615 **Table 3.** Examples of published data of indole and indole analogues and their impact on pathogens.

Name	Structure	Phenotypic changes affected by indoles	Target pathogens	Reference
Indole		Improved the survival of brine shrimp and giant river prawn larvae and mussel larvae infected with vibrios at 100-200 µM.	*V. campbellii;* *V. harveyi;* *V. parahaemolyticus;* *V. splendidus;* *V. crassostreae;* *V. tasmaniensis*	86-89
Indole-3-acetic acid		Decreased bioluminescence, biofilm formation, exopolysaccharide levels, and the virulence of *V. campbellii* towards brine shrimp larvae at 50 µM; decreased biofilm formation, bioluminescence, caseinase and swarming motility of *V. harveyi* at 30 µg/ml combined with 10 µg/ml undecanoic acid.	*V. campbellii;* *V. harveyi*	86,116,143
4-Iodoindole		Inhibited biofilm formation (20 µg/ml), bacterial motility (50 µg/ml), hydrophobicity (100 µg/ml), protease activity (10 µg/ml), and indole production.	*V. parahaemolyticus*	117
7-Iodoindole		Inhibited biofilm formation (20 µg/ml), bacterial motility (10 µg/ml), hydrophobicity (50 µg/ml), protease activity (10 µg/ml), and indole production.	*V. parahaemolyticus*	117
4-Chloroindole		Inhibited biofilm formation (20 µg/ml), bacterial motility (50 µg/ml), hydrophobicity (100 µg/ml), protease activity (20 µg/ml), and indole production of *V. parahaemolyticus*;	*V. parahaemolyticus*	117,144,145
7-Chloroindole		Inhibited biofilm formation (20 µg/ml), bacterial motility (50 µg/ml), protease activity (10 µg/ml), and indole production.	*V. parahaemolyticus*	117
Indole-3-acetonitrile		Improved the survival of brine shrimp larvae infected with *V. campbellii* at 10 µM; decreased biofilm formation and swimming motility at 200 µM.	*V. campbellii*	118
Methyl indole-3-carboxylate		Improved the survival of brine shrimp larvae infected with *V. campbellii* at 20 µM; decreased biofilm formation and swimming motility at 200 µM.	*V. campbellii*	118
3-Methylindole		Improved the survival of brine shrimp larvae infected with *V. campbellii* at 20 µM; decreased swimming motility at 200 µM.	*V. campbellii*	118
6-Bromoindole		Improved the survival of shrimp larvae infected with *V. campbellii* at 2 µM; decreased swimming motility at 10 µM; decreased protease activity at 100 µM.	*V. campbellii*	119
7-Bromoindole		Improved the survival of shrimp larvae infected with *V. campbellii* at 1 µM; decreased swimming motility and protease activity at 10 µM.	*V. campbellii*	119
4-Fluoroindole		Improved the survival of shrimp larvae infected with *V. campbellii* at 2 µM; decreased swimming motility and protease activity at 10 µM.	*V. campbellii*	119
5-Iodoindole		Improved the survival of shrimp larvae infected with *V. campbellii* at 2 µM; decreased swimming motility and protease activity at 10 µM; decreased biofilm formation at 100 µM.	*V. campbellii*	119
7-Iodoindole		Improved the survival of shrimp larvae infected with *V. campbellii* at 5 µM; decreased swimming motility, protease activity and biofilm formation at 10 µM.	*V. campbellii*	119

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FIGURES

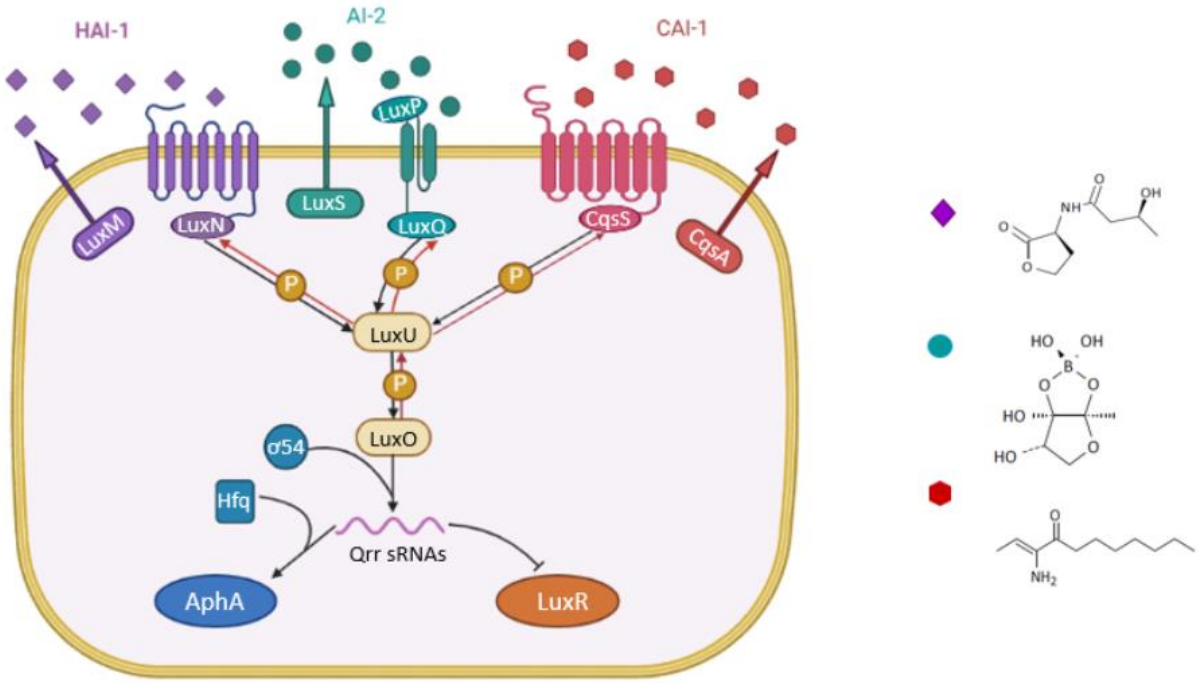

Figure 1. The three-channel quorum sensing system of *Vibrio campbellii*. The three signal molecules HAI-1, AI-2 and CAI-1, are produced by the LuxM, LuxS and CqsA proteins, respectively, and are sensed by the LuxN, LuxPQ and CqsS proteins, respectively. At low signal molecule concentrations, the receptors act as kinases and transfer phosphate to LuxO via LuxU. Phosphorylated LuxO is active and together with the alternative sigma factor σ54, it promotes the production of 5 small RNAs (Qrr sRNAs), which inhibit the production of the master regulator LuxR and promote the production of the master regulator AphA. These master regulators control the expression of many genes. At high signal molecule concentrations, the receptors act as phosphatases that drain away phosphate from LuxO. Dephosphorylated LuxO is inactive and as a consequence, Qrr sRNAs are not produced, LuxR is produced and AphA is not produced. Arrows with "P" denote phosphotransfer; black arrows show the direction of the phosphotransfer at low signal molecule concentrations and red arrows show the direction of the phosphotransfer at high signal molecule concentrations. The chemical structures of the signal molecules are shown on the right.

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