Quorum Sensing in *Vibrio* and its Relevance to Bacterial Virulence

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Abstract

Quorum sensing is a widespread system of cell to cell communication in bacteria that is stimulated in response to population density and relies on hormone-like chemical molecules to control gene expression. In mutualistic marine organisms like *Vibrio*, this system enables them to express certain processes, like virulence, only when its impact as a group would be maximized. An *N*-acylhomoserine lactone-dependent LuxI/R quorum sensing system has first been exemplified in *Vibrio fischeri* in the 1970s, regulating core bioluminescence genes. Since then, quorum sensing in *Vibrio* has been shown to influence a wide variety of process ranging from virulence factor formation to sporulation and motility. Most quorum sensing pathways produce and detect an autoinducer in a population dependent manner and transmit this information via a phospho-relay system to a core regulator that controls gene expression using certain pivotal elements. With several members of the genus *Vibrio* being known to cause severe foodborne infections, this review aims to present an overview of the quorum sensing systems present in four major *Vibrio* pathogens, *V. fischeri*, *V. harveyi*, *V. cholerae* and *V. vulnificus* and their roles in regulating the virulence of these organisms.

Keywords: Quorum sensing; *Vibrio*; Pathogenicity; Autoinducer

Introduction

The genus *Vibrio* is made up of some of the most ubiquitous bacteria found in the marine ecosystem. Containing 91 different species, these short rod shaped Gram-negative bacteria are found all over the world, in temperate to warm aquatic environments, ranging from off-shore areas and estuaries to coastal waters. Some *Vibrio* species are found as a part of the normal marine flora or on the surface and/or in the gastrointestinal tract of marine organisms like fish, shellfish, coral, sea grass, shrimp, and zooplankton while others are symbionts in light organs or even pathogens causing disease and death [1,2]. The epizootic bacterial disease caused by the genus *Vibrio*, termed vibriosis, brings about grievous economic loss to the worldwide aquaculture industry. It is, thus, essential to understand the virulence mechanisms and the underlying regulatory systems in these pathogens as part of an effort to prevent and control vibriosis.

Bacteria were traditionally considered to be isolated microorganisms living in a complex environment, a notion that has been squashed with the discovery of quorum sensing. Quorum sensing utilizes small "hormone-like" organic compounds referred to as autoinducers (AIs) to control gene expression in a population density-dependent manner. This ensures that energetically expensive processes are not performed till their effect as a collective is maximized. The first example of such a cell to cell communication system was elucidated in the marine bacterium *Vibrio fischeri* [3] controlling genes for bioluminescence. A typical quorum sensing system in Gram-negative bacteria has two types of AIs: acylated homoserine lactones (AI-1) and furanosyl borate diester (AI-2). AI-1 is considered to be an intra-species communication signal molecule due to its distinct structure in various species while, in contrast, AI-2 produced by LuxS is believed to be an inter-species communication autoinducer [4,5]. In addition, there is a unique AI, 3-hydroxytridecan-4-one (CAI-1), found only in *Vibrio* which is supposedly responsible for communication amongst *Vibrio* species [6]. Following intensive investigation of the quorum sensing system in *Vibrio*, it has subsequently been demonstrated that quorum sensing is present universally in both Gram-negative and Gram-positive bacteria and is used to regulate the expression of a vast range of genes based on cell-density, including those for biofilm formation, motility, bioluminescence and virulence [7-10]. It is particularly interesting to see that quorum sensing plays an important role in controlling the virulence of many *Vibrio* pathogens, implying that this pathway could potentially be a target for compounds aimed at preventing and managing vibriosis in the mariculture industry.

In this short review, we concentrated on the different types of quorum sensing in several *Vibrio* species and also discussed their roles in modulating the production of bacterial virulence factors.

Quorum Sensing in *V. fischeri*

Acyl-homoserine lactone (AHL) based quorum sensing was first observed to induce bioluminescence in a cell-population dependent fashion in the association between the marine bacterium *V. fischeri* and its symbiotic host, the Hawaiian bobtail squid [3]. The AHL responsible for this was found to be a species-specific metabolite, N-(3-oxohexanoyl) homoserine lactone (N-3-oxo-HSL), termed as AI-1 [11]. AI-1 molecules are synthesized using S-adenosylmethionine (SAM) and acyl carrier proteins (ACP) as substrates by the LuxI protein [12]. This AI is detected by a transcriptional regulator, LuxR. At low cell densities and hence low AI concentrations, the N-terminal of LuxR folds back onto its HTH domain and thus blocks DNA binding. However, as the bacterial density increases with growth, these AIs accumulate in the growth medium via diffusion from inside the cell. The N-terminal region of LuxR can then specifically bind the AIs with a high affinity, making its HTH domain available for DNA binding and consequently

activating transcription of the bioluminescence genes, *luxICDABE* [13]. The LuxI-LuxR system is found widely and is the paradigm of quorum sensing in many Gram-negative bacteria.

Subsequent research has demonstrated that there are two other quorum sensing systems in *V. fischeri*, AinS-AinR and LuxS-LuxPQ. AinS synthesizes a novel AHL autoinducer, N-octanoyl-L-homoserine (C8-HSL) which then interacts with its cognate receptor kinase AinR [14,15]. LuxS synthesizes the furanosyl borate diester (AI-2) that is responsible for interspecies information dissemination [16]. AI-2 can bind to the periplasmic protein LuxP to form a LuxP-AI-2 complex, which is then sensed by the hybrid sensor kinase LuxQ. At a low cell density, in the absence of the corresponding autoinducers, both AinR and LuxQ function as kinases and first autophosphorylate. The phosphoryl group then transfers in sequence to the shared histine phosphotransferase protein LuxU and then to LuxO. The phosphorylated LuxO cooperates with σ^{54} to activate transcription of the small regulatory RNA, Qrr1, which subsequently silences LitR (homologue to LuxR in *V. harveyi*) expression in the presence of the chaperone protein Hfq. As growth occurs and the cell density increases, the autoinducers accumulate in the cell culture and then bind to their cognate sensor proteins. This converts the sensor proteins into a phosphatase which drains the phosphate group in the reverse direction leaving LuxO in a dephosphorylated state, which is then inactive, resulting in the stable expression of LitR. LitR also positively regulates the transcription of LuxR, causing a modulation of the expression of downstream luminescent genes (Figure 1) [17-19].

Quorum Sensing in *V. harveyi*

Totally, three parallel signal transduction circuits are present in *V. harveyi* that correlate separately to three different autoinducers (Figure 2). A *V. harveyi* specific AI-1(N-3-hydroxybutyryl-L-homoserine lactone) is produced by LuxM, the AHL synthase and homologue of
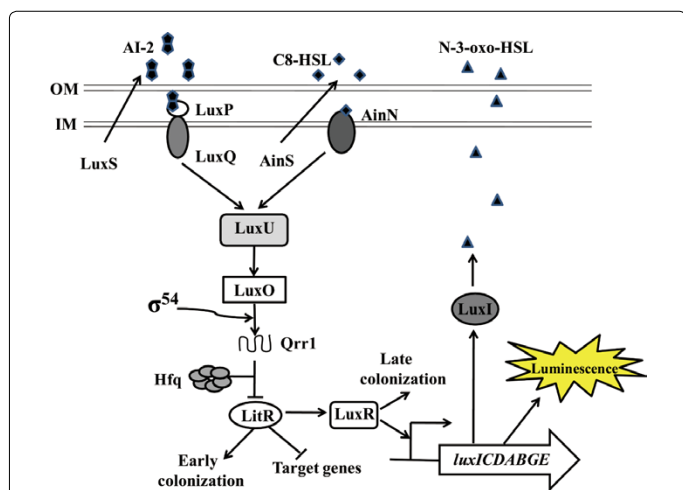

Figure 1: Quorum sensing in *V. fischeri*.
Two AHL autoinducers (N-3-oxo-HSL synthesized by LuxI and C8-HSL synthesized by AinS) and AI-2 are present in *V. fischeri* utilized for bioluminescence regulation in a cell-population-dependent manner. At a high cell concentration, N-3-oxo-HSL impinges on the N-terminal of LuxR, unmasking the HTH domain and resulting in the expression of bioluminescence. The relative kinase/phosphatase activities of the hybrid sensor AinR and LuxQ towards LuxU are dependent on the concentrations of their respective AIs. Phosphorylation of LuxO triggers the transcription of the sRNA, Qrr1 which degrades the *litR* mRNA in the presence of Hfq. LitR takes part in luminescence regulation by direct activation of the expression of LuxR.
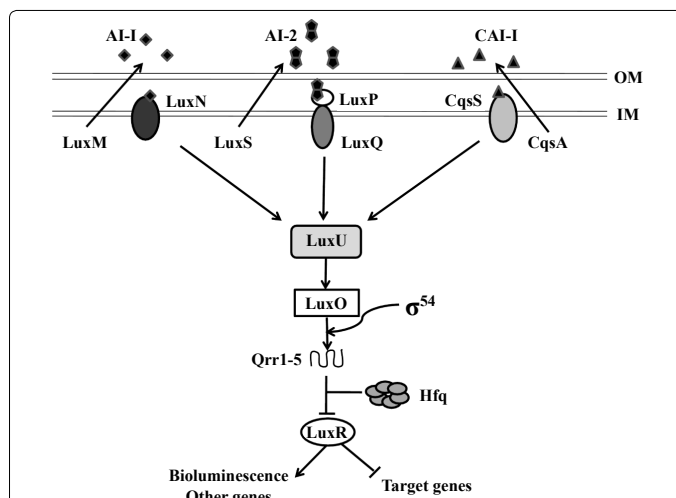

Figure 2: Quorum sensing in *V. harveyi*.
There are three parallel quorum sensing systems, including AI-1/LuxM, AI-2/LuxS, and CAI-1/CqsA. At low cell populations, no signal molecules are detected. While at a high cell population, these three AIs are produced and accumulated to reach a critical concentration to be sensed by their receptors. All the three signal transfer pathways are achieved by a series of hierarchical phosphorylation and dephosphorylation by the same components.

AinS in *V. fischeri*, which is then accumulated and detected only at a high cell density by the inner membrane hybrid sensor, LuxN [4,20]. AI-2 is produced by LuxS, bound by the periplasmic protein LuxP to form an AI-2-LuxP complex. This is subsequently sensed by the two-component sensor kinase, LuxQ, whose periplasmic region is associated with the AI-2-LuxP complex while having a histidine kinase and a response regulator domains in its cytoplasmic region [4,5,21-23]. The third autoinducer present in *V. harveyi* is CAI-1 or 3-hydroxytridecan-4-one, like that in *V. cholerae*, produced by CqsA and detected by CqsS. As of now, CAI-1 is only known to be present in *Vibrio* species and could be considered to be part of a *Vibrio*-specific interspecies language [24,25].

At low cell densities, the AI levels are not high enough to be detected by their cognate sensors and under this condition LuxN, LuxQ, and CqsS function as kinases, autophosphorylate at a conserved histidine residue and then relay this phosphorylation signal to a shared phosphotransferase protein LuxU, which in turn transfers the phosphate to LuxO. The phosphorylated LuxO, together with σ^{54}, activates the transcription of five small noncoding RNAs, Qrr1-5, which then destabilizes the mRNA of the *luxR* gene by incomplete base-pairing in coordination with the chaperone Hfq [26-30]. At a high cell population, the signal transduction pathway is exactly the opposite. The three autoinducers are bound to their cognate receptors, LuxN, LuxQ, or CqsA, inhibiting the kinase activities of these sensors and thus predominantly having them function as phosphatases [31,32]. Accordingly, the phosphate group is removed from LuxO rendering it inactive, leading to the expression of the core regulator, LuxR, which then initiates or inhibits the transcription of the downstream target genes, including those for bioluminescence, production of metalloprotease, exopolysaccharides, and the type III secretion system. In *V. alginolyticus*, three parallel signal transduction pathways, like that in *V. harveyi*, have been characterized [33-36]. Quorum sensing in *V. parahaemolyticus* is poorly characterized, in part because quorum sensing seems to be silenced in many isolates. OpaR, a homologue of LuxR from *V. harveyi*, was identified in this species and was shown to regulate surface sensing and the type III secretion system [37]. It has

been demonstrated that there is an intact quorum sensing cascade, sharing a high similarity to *V. harveyi*, located in the genome of *V. parahaemolyticus*.

Quorum Sensing in *V. cholerae* and *V. vulnificus*

V. cholerae is the causative organism of cholera, a serious diarrheal disease occurring mainly in underdeveloped regions of the world. There are two parallel quorum sensing circuits similar to that in *V. harveyi* primarily depending on two AIs, CAI-1 and AI-2, [25]. Yet, no homologues of *V. harveyi* LuxM/N have been discovered so far. CAI-1, S-3-hydroxytridecan-4-one, is first produced by CqsA and then binds to the sensor protein, CqsS [25,38,39]. The second AI, AI-2, is produced by LuxS and detected by LuxPQ. At low cell densities, the kinase activity of LuxN and LuxQ is predominant and the phosphate is transferred to the response regulator LuxO via LuxU. The transcription of sRNAs, Qrr1-4 is stimulated and these sRNAs base-pair with the *hapR* mRNA (homologue of *luxR* in *V. harveyi*) preventing ribosome binding by overlapping the RBS, and facilitating degradation of *hapR* mRNA. At a high cell density, the dephosphorylated LuxO is unable to initiate the expression of sRNAs and the *hapR* mRNA is available for translation (Figure 3) [30].

V. vulnificus, a Gram-negative marine bacterium, is an opportunistic pathogen causing systemic infections in humans and eels. In this bacterium, the quorum sensing based on AI-2/LuxS system has been the only one characterized and until now no other homologues of AI-1/LuxM, CAI-1/CqsA, or LuxI/LuxR are known to be present in *V. vulnificus*. AI-2 is produced by LuxS and predicted to be sensed by LuxPQ [40]. At low cell densities, phosphorylated LuxO via LuxR activates the Qrr1-4 transcription to block SmcR (homologous to LuxR in *V. harveyi*) expression. At high cell concentrations, the signal molecule is accumulated as cell growth occurs which then binds to its receptor to dephosphorylate LuxO via LuxU. The expression of SmcR is now possible due to the absence of sRNAs. Moreover, another element involved in LuxO-SmcR interaction, LuxT, is found to be activated by LuxO at the exponential stage of growth which afterwards represses SmcR expression at the transcriptional level by direct binding, in *V. vulnificus*. However, this shouldn't be the dominant pathway influencing SmcR expression (Figure 4) [41-43].

Pathogenicity Regulation by Quorum Sensing in *Vibrio* Species

Vibrios are the causative agents of vibriosis, a term used for skin or blood infections caused by *Vibrios*. This is mainly a diarrheal disease in humans. At present, vibriosis has been the dominant threat towards mariculture, worldwide. To effectively prevent the outbreak of this disease and in order to identify better therapeutic targets, it is necessary to figure out the virulence regulation mechanism. Quorum sensing, being ubiquitous and conserved in *Vibrio* species, takes part in various physiological processes and particularly influences the virulence system of many pathogenic bacteria.

Quorum Sensing Controls the Production of Virulence Factors in *Vibrio* Species

Exotoxin plays an important role in the pathogenicity of bacteria by resulting in enzymolysis and pore formation in the host cell, thus, allowing the bacteria to invade and spread to different tissues. In *V. vulnificus*, one of the major virulence factors is an extracellular protease called elastase, encoded by *vvpE*. The expression of VvpE increases with the cell density and is enhanced by LuxS and SmcR [40,42]. SmcR can
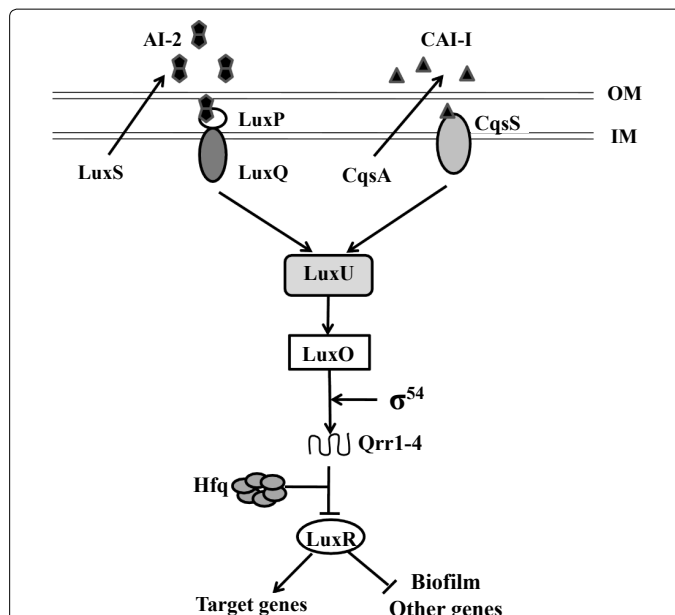

Figure 3: Quorum sensing circuit in *V. cholerae*.

Two kinds of AIs, CAI-1 (synthesized by CqsA) and AI-2 (produced by LuxS), which are detected by their respective receptors, CqsS and LuxPQ, are found in *V. cholerae*. In the absence of AIs at a low cell density, the receptors function as kinases leading to phosphorylation of LuxO via LuxU and activation of transcription of *qrr1-4* genes. The sRNAs, Qrr1-4 together with Hfq, influence the stability of mRNA available for LuxR translation. At a high cell population, AIs bind the receptors inhibiting their kinase activity and converting them to phosphatases. The phosphate group is removed from LuxO, thereby the *qrr* gene expression is stopped and *hapR* expression is increased to initiate or repress the target gene expression.

bind to the stationary-phase promoter of *vvpE* together with CRP to co-activate the expression of VvpE synergistically [44]. In *V. alginolyticus*, its major virulence factor, an extracellular alkaline serine protease, Asp, is closely related to the quorum sensing system and its production is in a cell-density-dependent manner. Given the fact that low level expression of LuxR regulator, a core element of quorum sensing, causes reduced expression of Asp, we speculated that LuxR activates the transcription of *asp* gene by direct binding to its promoter region [45]. In *V. cholerae*, the two main virulence factors are the cholera toxin (CT) and the toxin-coregulated pilus (TCP), encoded respectively by the *ctx* gene and *tcp* gene cluster which are indirectly activated by AphA. At low cell densities, sRNAs are transcribed in the presence of phosphorylated LuxO. sRNAs destabilize the *hapR* mRNA but promote the expression of AphA. At high cell densities, HapR is available for expression and inhibits *aphA* transcription, resulting in the cessation of virulence factor formation [46].

Quorum Sensing Influences the Secretion System in *Vibrios*

Type III secretion system (T3SS) and Type VI secretion system (T6SS) are highly conserved in several *Vibrio* species, such as *V. harveyi, V. cholerae, V. alginolyticus, V. parahaemolyticus*, etc. Both secretion systems utilize an apparatus to form needle-like pores through the membrane of the bacteria and the host cell, and thereby deliver effector proteins which are usually virulence factors directly into their target eukaryotic host cells. They play a pivotal role in the pathogenicity of these organisms. In *V. harveyi*, T3SS genes are activated at lower cell populations and inhibited at high cell concentrations by the quorum sensing core element, LuxR, through the repression of ExsA [47]. In *V.*
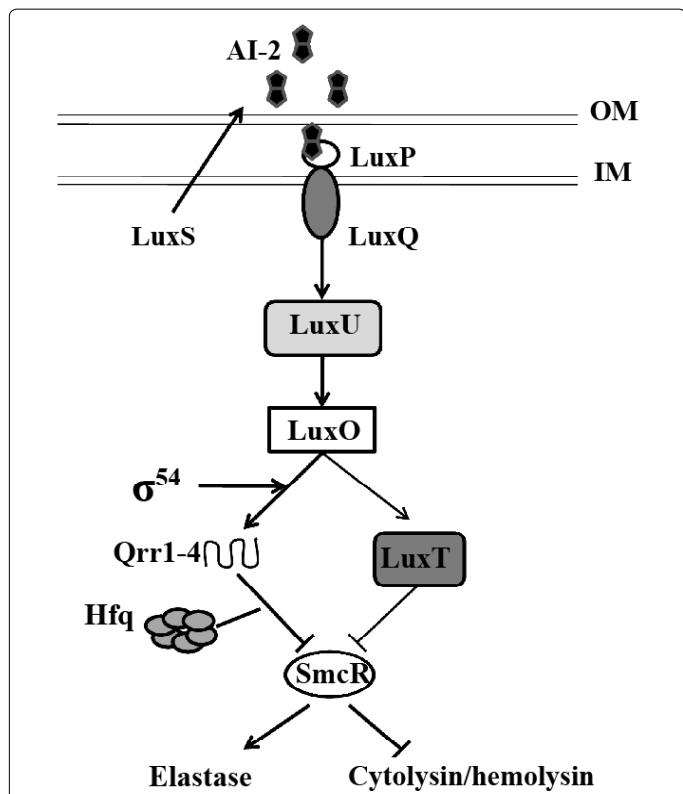
Figure 4: Quorum sensing circuit in *V. vulnificus*.

At low cell densities, expression of SmcR is repressed. At high cell densities, the absence of sRNAs allows the expression of SmcR. Yet, *luxT* transcription is induced by LuxO at exponential stage and then LuxT represses *smcR* transcription by direct binding to the upstream region. But this route is not the dominant one controlling SmcR expression. Then, SmcR is expressed and activates production of elastase.

Bacteria	Process controlled by quorum sensing				References
	Impaired virulence factor production	Impaired motility	Impaired biofilm formation	Reduced virulence	
Vibrio harveyi	-			+	Defoirdt et al.[54]
V. cholerae	-	-	-	-	Zhu et al. [46]
V. parahaemolyticus		-		-	Gode-Potratz and McCarter [37]
V. vulnificus	+	+	+	+	Lee et al. [55]
V. alginolyticus	+	-		+	Rui et al. [35]

(+)/(-) means agree/disagree to the observations; blank represents that phenotype was not tested.

Table 1: Virulence modulation by quorum sensing in *Vibrio*.

parahaemolyticus, T3SS is also repressed by OpaR at high cell densities [48]. In *V. cholerae*, disruption of the quorum sensing response regulator, LuxO, induces expression and secretion of Hcp, the T6SS apparatus and also the translocated substrate, through the downstream regulator HapR which directly binds to the promoter region of the T6SS genes *hcp1* and *hcp2* [49]. In *V. parahaemolyticus*, there are two sets of gene clusters encoding for T6SS in its genome, classified as T6SS1 and T6SS2. It is interesting that the critical quorum sensing regulator OpaR has a different effect on both these clusters ie. OpaR downregualtes the T6SS1 genes but upregulates the T6SS2 genes. Hcp1, the substrate of T6SS1 is transcriptionally repressed to a large extent by OpaR via an unknown factor [50].

Quorum Sensing Affects Various Virulence-Related Phenotypes in *Vibrios*

Quorum sensing in *Vibrio* is also involved in the regulation of many other virulence-related factors, like biofilm formation, motility, iron-sequestering system, and so on. A biofilm is a surface-associated microbial community that is embedded in a 3-dimensional self-produced matrix which is mainly made up of polysaccharides. Bacteria living in biofilms can better protect themselves from an unsuitable environment. In *V. cholerae*, quorum sensing-deficient mutants produce thicker biofilms. CqsA acts through HapR to repress expression of polysaccharide synthesis operons [51]. Motility is closely related to virulence of pathogens. *V. parahaemolyticus* possesses two distinct flagellar systems: the polar system for swimming in liquids and the lateral

(*laf*) system for swarming over solid or viscous surfaces. The swarming motility is demonstrated to be regulated by OpaR, the pivotal element of quorum sensing system, at two levels: on one side, OpaR promotes the expression of *cpsA* locus, encoding the capsular polysaccharide, to make the strain sticky; on the other side, OpaR is found to directly repress the expression of *laf* [52]. In *V. alginolyticus*, the siderophore production is reduced as LuxO expression is interrupted [33,53].

Conclusions

Quorum sensing has been illustrated in a large number of bacteria, both Gram-negative and Gram-positive strains, and is known to participate in numerous biophysical processes, being a pleiotropic regulatory mechanism to control collective traits. *Vibrio* utilizes the quorum sensing system to fine tune bioluminescence, virulence production, biofilm formation, and so on, dependent on the cell population (Table 1). Although the components involved in quorum sensing in different *Vibrio* species share high similarities in their gene sequences, the regulatory mechanisms of quorum sensing are quite versatile. Besides, there are plenty of unknown factors related to quorum sensing system yet to be characterized. It is confounding that there are three parallel signal transduction circuits in *V. harveyi*, *V. alginolyticus*, and *V. fischeri* but only one in *V. vulnificus*. In *V. harveyi*, AI-1 is responsible for intra-species communication, CAI-1 for *Vibrio* genus-level communication, and AI-2 for inter-species communication, meaning that this bacterium can differentiate between self and others in order to respond to alteration of population densities in a timely manner. For *V. vulnificus*, the question remains as to whether there are some other compensatory channels? This demands further studies. Quorum sensing influences the virulence of many pathogens and its components may be targeted towards vaccine development. Some success in this regard has been achieved.

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