

Quorum Sensing System Regulates Virulence and Pathogenicity Genes in *Vibrio harveyi*



A. M. V. N. Prathyusha, G. Triveni, and Pallaval Veera Bramhachari

Abstract Quorum sensing (QS) is intracellular communication among bacteria that perceive population density, regulates the formation of biofilm, virulence factors production and provides resistance to antibiotics through extracellular signal molecules. *Vibrio harveyi*, a marine pathogen, and major cause for loss of productivity in aquaculture hatcheries, farms and for the growth of industry. *V. harveyi* uses multi-channel quorum sensing system, each consisting of an autoinducer-sensor pair that controls the expression of genes required for bioluminescence, virulence, biofilm formation. The multi-channel system is mediated by the *V. harveyi* autoinducer 1 (HAI-1), autoinducer 2 (AI-2), *V. cholerae* autoinducer 1 (CAI-1) which activate or inactivate target gene expressions by a phosphorylation/dephosphorylation signal transduction cascade. The production of extracellular virulence factors are involved in regulation of virulence and pathogenesis of *V. harveyi*. This article focuses on chemical communication mechanism, its regulation of virulence factors and pathogenicity of *Vibrio harveyi*.

Keywords Quorum sensing · Virulence · *Vibrio harveyi* · Pathogenesis · Signal transduction cascade · Gene expression

Introduction

Vibrio harveyi, a gram negative, pathogenic, marine fluorescence emitting bacteria commonly present in gut microflora of aquatic invertebrates viz. crustacean, molluscs and vertebrates viz. fishes. *Vibrio harveyi*, an oligotropic pathogen reported to be associated with Bright red syndrome [33], luminous vibriosis [17], to aquatic invertebrates and skin ulcers, eye lesions, gastro-enteritis [7], vasculitis to vertebrates which impedes the commercial development of aquaculture around the world [3] (Table 1).

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Table 1 Examples of diseases caused by *V.harveyi*

Host	Disease	References
<i>Peneaeuse monodon</i> (Tiger prawn)	Luminescent vibriosis	[17]
<i>Litopenaeus vannamei</i> (White shrimp)	Bacterial white tail disease (BSTD)	[44]
<i>Epinephelus coioides</i> (Estuary cod)	Gastroenteritis	[8]
<i>Rachycentron canadum</i> (Cobia fish)	Gastroenteritis	[21]

Quorum sensing is an intracellular communication among bacteria that enables them to change behaviour in response to variations in cell density. QS includes the species specific synthesis and release of signalling compounds extracellularly named as autoinducers [39]. Accumulation of autoinducer molecules increase as population density of bacteria increases. These changes of autoinducers concentration in surrounding environment was monitored by bacteria to track their cell density and to modify array of gene expression [19]. QS regulates the formation of biofilm, bioluminescence, virulence factor expression, motility, responsible for pathogenicity and virulence of *V.harveyi* [39].

Auto Inducers and Receptors

V. harveyi was first discovered bacterial species to drive communication using chemical signals (auto inducers) and remained the model organism to understand how bacteria process chemical blends. *V. harveyi* synthesizes and process three different auto inducers for communication between intra-genera, intra and inter-species. *V. harveyi* lives in diverse inhabitants probably combat complex mixtures of chemical molecules produced by their own species, their surrounding flora, which act as competitors. *N*-acylated HSL (homoserine lactones) are most common class of auto inducers detected and synthesized by *V. harveyi* for intra-species communication.

AI-1 Autoinducer-I molecules are acyl HSL synthesized by LuxM synthase benefitted for interspecies communication. HAI-1 [*N*-(3-hydroxybutyryl) homoserine lactone] acts as ligands and are produced by LuxM and sensed by LuxN receptor specific to *V.harveyi*. LuxN is a two-component protein comprise of two domains a kinase domain which acts as a sensor and response regulator domain. HAI-1 molecules were constrained to *V. harveyi* and closely related sps *V.parahaemolyticus*, signifying HAI-1 role in intraspecies signalling [5–7].

CAI-1 *V. harveyi* senses (*Z*)-3-aminoundec-2-en-4-one, closely related *V.cholerae* autoinduer molecule known as cholera autoinducer 1 (CAI-1). CAI-1 was first identified in *V.cholerae*. In *V.cholerae* CAI-1 molecule is synthesised by CqsA (CAI-1 autoinducer synthase). CqsA utilises SAM (S-adenosyl methionine) and decanoyl-CoA to synthesise amino-CAI-1. Amino CAI-1 undergo spontaneous hydrolysis and by dehydrogenase to form CAI-1. Interestingly, CAI-1 molecule prevails in

cell-free extracts while, both amino-CAI-1 and CAI-1 are biologically functional molecules. *Vibrio* spp. Synthesis many CAI-1 moieties with different acyl chain lengths and modifications. CAI-1 is detected by CqsS receptor has six transmembrane helices and utilises for intra-species communication. Derivatives of CAI-1 with altered acyl sidechains fail to stimulate CqsS, however autoinducer with extended head group switched the molecule to an antagonist.

AI-2 Autoinducer-2 (AI-2), a furanosyl borate diester one of the few biomolecule having boron and was first identified in *V.harveyi*. AI-2 has a set of interconverting molecules which are derivatives of 4,5-dihydroxy-2,3-pentanedione (DPD). LuxS, (DPD synthase), exists in >500 bacterial species, synthesises AI-2 molecules [9]. AI-2 is the most common bacterial autoinducers known yet. DPD is very reactive and spontaneously cyclizes to form furanone moieties with varied structures. Different bacterial sps respond to diverse forms of DPD. Interestingly, AI-2 molecules has boron in *V. harveyi* sps, while, *E.coli* and *Salmonella* spp., contain non-borated cyclized DPD moiety as AI-2 [34]. As the different DPDs rapidly interconvert, AI-2 provides a means for inter-species communication.

AI-2 signal is sensed and transduced by periplasmic protein LuxP (binding protein). LuxP interacts with LuxQ (hybrid two-component sensor kinase protein) to enable signal transmission [6]. LuxQ transduces signal to its shared histidine phosphotransferase protein (LuxU), which transmits signal to LuxO. LuxO, along with σ_{54} , modulates the expression of target genes [12]. In the absence of AI-2 signal, 2 proteins LuxP and LuxQ complexes to form a symmetric heterotetramer. Binding of AI-2 creates large conformational change that stimulate protomer rotation in periplasmic region and disrupt LuxPQ–LuxPQ tetramer symmetry which inhibits phosphorylation of cytoplasmic domains. Interestingly, binding of AI-2 promotes formation of clusters by LuxPQ–LuxPQ tetramers, which can effect sensitivity of AI-2 and its response dynamics [25].

Quorum Sensing in *Vibrio harveyi*

V. harveyi QS circuit system depends on three cognate transmembrane receptors. However pros and cons of cytoplasmic DNA-binding transcription factors against membrane-bound receptors is yet unidentified. Nonetheless both types of receptors avoid response to autoinducers produced endogenously before reaching ‘a quorum’. Initiation of QS in *Vibrio* sps is decoupled by differential localization of receptors (on membrane) and site of autoinducers synthesis (cytosol) and from recognition in periplasm [26] *V. harveyi* uses CqsS, LuxPQ and LuxN as QS receptors, which binds with CAI-1, AI-2 and HAI-1 signal molecules respectively [19]. At low density of chemical signals, LuxPQ, LuxN, and CqsS acts as kinases and autophosphorylates. Therefore, phosphorylated receptors phosphorylate phosphorelay protein, LuxU which phosphorylates downstream target LuxO (response regulator

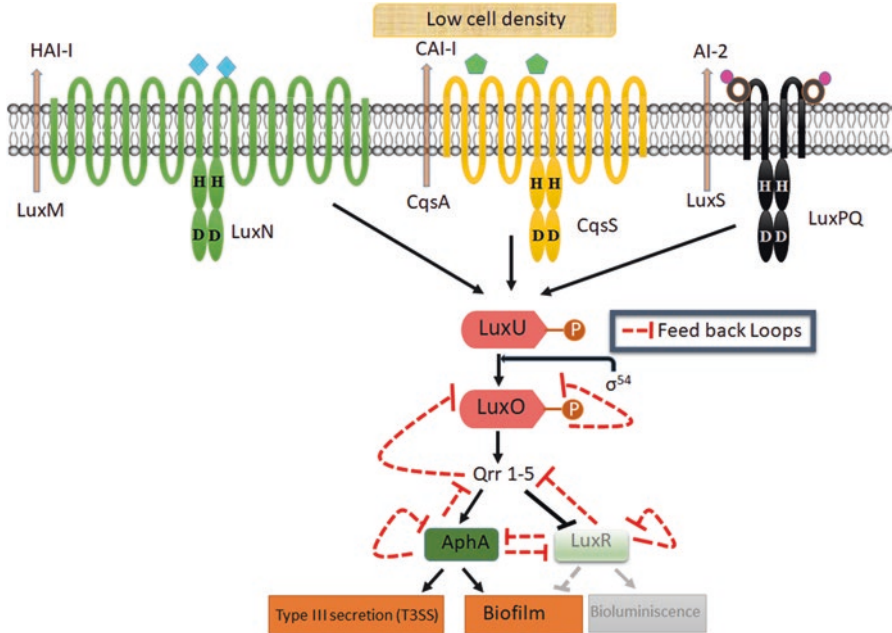


Fig. 1 QS cascade at Low Cell Density. LuxM, CsqA, LuxS synthesises 3 autoinducers that mediate QS in *V.harveyi*. At LCD the receptors acts as kinases and autophosphorylates LuxU and LuxO. Phosphorylated LuxO induces the expression of Qrr sRNAs and degrade/destabilise LuxR a master regulator for LCD. This promotes the expression of T3SS structural genes, biofilm formation, and motility. Feedback loops which plays an important role in Quorum sensing dynamics are represented in red color

protein) [12] LuxO interacts with σ^{54} and activate the transcription of target genes that encode for five homologous quorum regulatory sRNAs (Qrr 1–5) [20, 36]. Qrr sRNAs now modulates the expression of target mRNAs gene through base-pairing which activates/repress translation of 20 mRNAs. Qrr sRNAs stimulate translation of AphA, mRNA at LCD (low cell density master regulator) while limiting translation of LuxR mRNAs (high cell density master regulators) [32] (Fig. 1). At high cell density, binding of autoinducer hinders autophosphorylation, which enables the action of phosphatases. Dephosphorylated LuxO is less active and prevents *qrr* genes expression. In lack of Qrr sRNAs, gene expression of *luxR* is activated while *aphA* is repressed. LuxR is a master transcriptional regulator that activates >70 genes that promote collective QS behaviors [38].

Qrr sRNAs also repress translation of *luxMN* mRNA by coupled degradation. The Qrr sRNAs inhibit *luxR* through catalytic degradation of *luxR* mRNA, suppress translation of *luxO* by sequestration [36], and stimulate *aphA* by revealing of ribosome binding site [10] (Fig. 2). However, Qrr sRNAs mediated catalytic degradation of *luxR* mRNA has no effect on Qrr pool, while sequestration (*luxO*) and coupled degradation (*luxMN*) reduce Qrr sRNAs from system [36]. These regula-

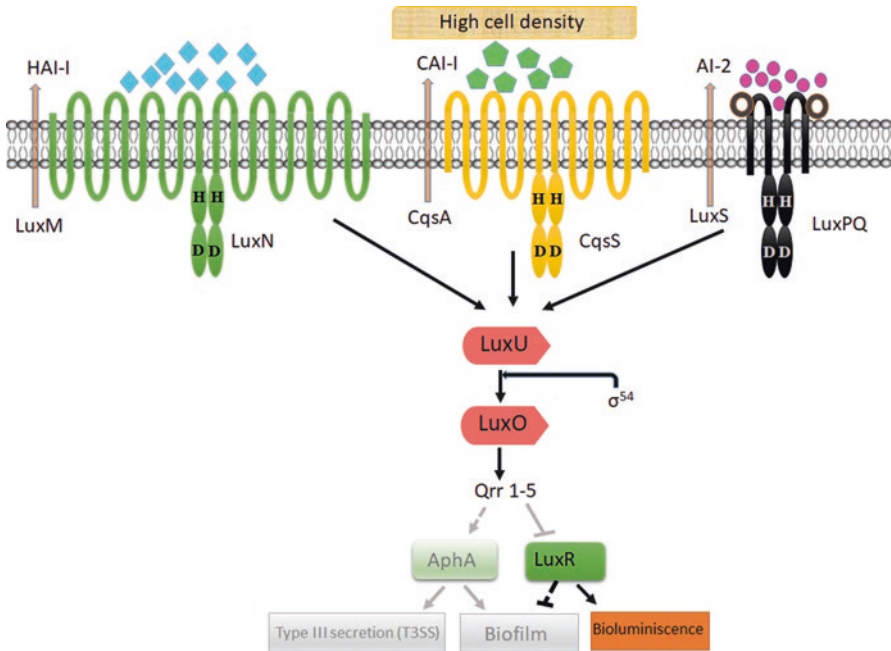


Fig. 2 QS cascade at High Cell Density. At HCD the receptors acts as phosphatases dephosphorylates LuxU and LuxO. LuxO reduces the expression of Qrr sRNAs and promotes synthesis of LuxR and represses synthesis of AphA. LuxR promotes the expression of bioluminescence, biofilm formation while repressing the expression of T3SS genes. H indicates His residues and D indicates Asp residue (phosphorylation targets)

tory pathways are important for the maintenance of defined Qrr pools of system and overall QS dynamics. HAI-1 and AI-2 act synergistically, to mimic HCD conditions [23]. Under LCD conditions, LuxN acts as a kinase in the absence of HAI-1 (AI-2 is present). This function effects the net phosphorylation of protein LuxO which is essential to sense LCD environment. Accumulation of HAI-1, converts LuxN to acts as phosphatase. Now both sensors LuxN and LuxQ are phosphatases and trigger dephosphorylation of total LuxO. This transition senses low- to high-cell-density mode [12].

QS receptors in *V.harveyi* are two component receptors with both kinase and phosphatase activities which phosphorylate/dephosphorylate LuxU. QS system is completely turned on/off unless all the autoinducers are present or absent, respectively. Further QS in *V.harveyi* is controlled by feedback loops and regulatory feedbacks which may fine tune flow of information by chemical signals (Fig. 1).

- (i) LuxO auto represses its own transcription [28, 36]
- (ii) Qrr sRNAs sequester the *luxO* mRNA, which supresses translation of *luxO* gene. In LCD (low cell density) these two loops reduce synthesis of LuxO protein, this reduces protein level below which Qrr sRNAs cannot further represses QS [10, 36].

- (iii) LuxR activates *qrr* genes expression, and the synthesized Qrr sRNAs destabilize *luxR* mRNA. This double loop drives LuxR-mediated QS transitions faster [20, 38].
- (iv) LuxR limits its own transcription, which evades, limited synthesis of protein at HCD, therefore regulating QS output. LuxR family of proteins, the master global transcription factors targets expression of downstream genes in response to alterations in cell density [28].
- (v) AphA and LuxR mutually suppress each other's transcription, which allows maximal expression of AphA protein on LCD and optimal expression of LuxR HCD [22].
- (vi) During LCD, Qrr sRNAs enable degradation of *luxMN* mRNA, results in reduced synthesis of HAI-1. This loop minimises HAI-1 signal at LCD and intensifies HAI-1 sensitivity at HCD [35]. Presumably, all these feedback loops promote fidelity, optimal dynamics between quorum sensing states.

Group Behavior and Co-ordination

Motility

Bacterial motility is one of the important virulence factors in most pathogens. Motility is essential during the early stages of infection for pathogenic bacteria in to weaken repulsive forces between host tissues and bacterial cell. This facilitates bacterial cells attachment to the host. However, regulation of chemotaxis and/or motility is common to *V.harveyi* regulons, LuxR stimulate motility gene expression. *V.harveyi* display maximal motility at HCD. In *V. harveyi*, LuxR positively controls expression of chemotaxis genes, while in *V. parahaemolyticus* and *V. cholerae*, OpaR/HapR negatively control homologous genes. QS positively controls motility by targeting flagellar biosynthesis which significantly affects virulence of *V. harveyi* [41].

Single Cell Heterogeneity

Heterogeneity is essential for bacterial group behaviour to share 'Public goods', viz., substances for ECM (extracellular matrix) or degradative enzymes among the population [1]. Quorum sensing does not frequently effect the homogeneous behaviours of cells instead exhibits phenotypic heterogeneity/diversification of behaviours in clonal populations [16]. QS system induced heterogeneity within the population was reported in *V. harveyi* [1, 2], *Aliivibrio fischeri* [27], *Listeria monocytogenes* [13], *Salmonella enterica* [11] using single cell analysis. Anetzberger et al. [2] reported that some AI-regulated genes (*luxC*, *vscP* and *vhp*) exhibit

functional heterogeneity in a *V. harveyi* in wild type cells. The two genes (*vscP*, *vhp*) exhibit wide intercellular variation in response to AIs at transcriptional levels. AIs regulate expression of *luxC*, *vscP* and *vhp* genes by binding to their promoter regions – that are essential for expression of bioluminescence, type III secretion proteins and exoproteolysis respectively. At HCD (high concentration of autoinducers) *lux* operon and exoprotease gene expressions were induced, while expression of *vscP* is repressed. However *luxS*, an AI-independent gene, is expressed largely in homogeneous manner. AI molecules play a crucial role in the phenotypic diversification of clonal population (heterogeneity). Nonetheless, AIs not only serve as indicators for cell density but also coordinates cooperative behavior to share and synthesize ‘public goods’ and harmonizes QS-regulated processes [1, 2].

Biofilm Formation

Although a plethora of studies reported that LuxR-type proteins regulate biofilm genes expression, the correlation among QS and formation of biofilm in *V. harveyi* is not well established yet [1, 37]. Anetzberger et al. [1] reported that QS positively controls biofilm formation in *V. harveyi*. However, in *V. cholerae*, HapR limits the expression of *VpsR* and *VpsT* genes (activators for biofilm formation), which results in formation of biofilm at LCD.

Virulence and Pathogenicity in V. harveyi

The pathogenic mechanisms responsible for virulence in *V. harveyi* was not yet elucidated completely however, pathogenicity is thought to emerge via adhesion to host cell/surface, colonisation and production of lytic enzymes such as siderophores, hemolysin, proteases, lipases, gelatinase and caseinase [29, 42, 43]. In *vibrio* sps, virulence gene expression can be stimulated by several features of host environment, including low iron, oxygen, phosphate levels, mucin, catecholamines, bile salts and cholesterol [30]. Nonetheless, QS differently regulates different virulence factors viz., metalloprotease, gelatinase and caseinase activities are positively regulated QS, while phospholipase genes, T3SS genes are negatively regulated. In *V. harveyi* hemolysin and lipase activities are independent of QS system [24, 30, 40].

T3SS and T6SS proteins have complex needle like structures that penetrate cellular membranes to deliver effector proteins interfere with various cellular processes to cause cell death [15]. T3SS are usually rupture eukaryotic membranes, whereas T6SS can breach both eukaryotic and prokaryotic membranes [4]. *vscP* and *vhp*, are the two genes essential for pathogenesis of *V. harveyi* encode for a component of type III secretion system and an exoprotease, respectively. Some of the virulence factors generated by pathogenic bacteria translocate to cells exterior by type III

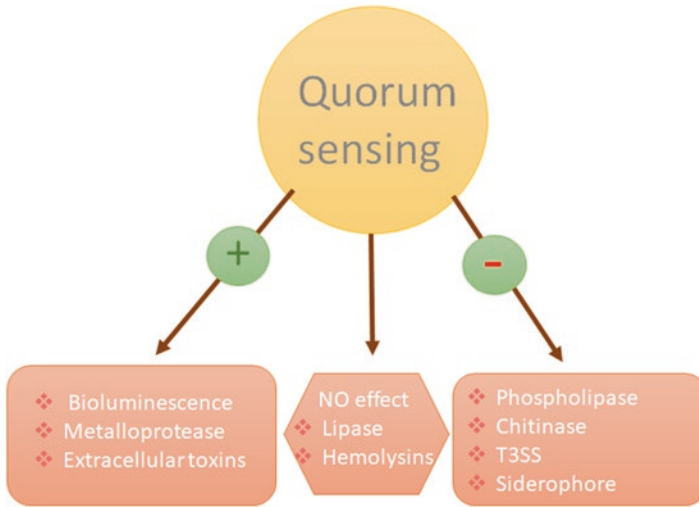


Fig. 3 Regulation of QS and virulence

secretion system (T3SS) [14]. T3SS locus consists of three adjacent operons on chromosome 1 (*vopD*, *vscP*, *vcrD* genes) and one operon located 15 kb apart [30].

In *V.harveyi* expression of T3SS structural genes is activated by ExsA a transcriptional modulators belongs to AraC/XylS family [18]. LuxR suppress expression of T3SS operons, together with genes that encode for structural, effector proteins and transcription factors of T3SS system. Expression of T3SS operon greatly varies between low and high cell density, but the expression is highly enhanced during infection in a QS dependent way. LuxR, a LCD modulator activates expression of two promoters of *exsBA* operon (*exxA*, *exxB*) and promotes production of ExsA. However, deregulated expression of the *exsBA* operon, critical for the QS-mediated control of T3SS genes at HCD [40]. At LCD, AphA represses the expression of >40T3SS genes. Nonetheless repression of T3SS genes during LCD and HCD by AphA and LuxR respectively, results in T3SS genes expression at mid-cell density [4]. Thus, expression of Type III and VI secretion systems (T3SS/T6SS genes) are regulated by QS in various *Vibrio* species [18, 31] (Fig. 3).

Conclusions and Future Perspectives

In this review, we have provided an overview of current knowledge on virulence genes and their regulation in *V. harveyi*. Quorum sensing among *V. harveyi* is central feature for many cellular processes, virulence, heterogeneity, inter and intra species communication, group behaviors. Several complexities of QS networks were disclosed and yielded an important understanding on the role of LuxR type proteins however several questions remain unknown. Does LuxR controls expression of

proteins at transcriptional/translational level? Mechanism through which QS positively regulates biofilm formation. How does environmental factors affect expression of virulence factors? How mellaoproteases promotes virulence in *V. harveyi*. Therefore a better understanding of regulatory mechanisms involved in virulence gene expression of *V. harveyi* (LuxR regulon) hosts several cues in the years to come.

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