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Quorum sensing: An imperative longevity weapon in bacteria

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Bacterial cells exhibit a complex pattern of co-operative behaviour as shown by their capacity to communicate amongst each other. Quorum sensing (QS) is a generic term used for bacterial cell-to-cell communication which secures survival of its species. Many QS bacteria produce and release autoinducers like acyl-homoserine lactone-signaling molecules to regulate cell population density. Different species of bacteria utilize different QS molecules to regulate its gene expression. A free-living marine bacterium, *Vibrio harveyi,* uses two QS system to control the density-dependent expression of bioluminescence (*lux*), commonly classified as sensor and autoinducer system. In *Pseudomonas aeruginosa*, QS not only controls virulence factor production but also biofilm formation. It is comprised two hierarchically organised systems, each consisting of an autoinducer synthetase (*LasI*/*RhlI*) and a corresponding regulator protein (*LasR*/*RhlR*). Biofilms produced by *Pseudomonas,* under control of QS, are ubiquitous in nature and contribute towards colonizations in patients of cystic fibrosis. Other organisms like *Haemophilus influenzae* and *Streptococcus* also utilize QS mechanism to control virulence in otitis and endocarditic decay. Overall, QS plays a major role in controlling bacterial economy. It is a simple, practical and effective mechanism of production and control. If the concentration of enzyme is critical, bacteria can sense it and perform a prompt activation or repression of certain target genes for controlling its environment. This review focuses on the QS mechanisms and their role in the survival of few important bacterial species.

Key words: Quorum sensing (QS), quorum sensing peptides (QSPs), auto-inducer 1 (AI-1), auto-inducer 2 (AI-2), acyl homoserine lactone (AHL).

INTRODUCTION

Quorum sensing (QS) in bacteria regulates gene expression in response to changes in cell density (Tomasz et al., 1965). The mechanism of QS helps to produce, release and respond to autoinducers and is observed in both gram-positives like *Streptococcus pneumoniae* and gram-negative bacteria e.g. *Vibrio fischeri* and *Vibrio harveyi* (Tomasz et al., 1965; Nealson et al., 1970). Essentially, QS phenomenon gives rise to important phenotypes of biofilm formation, virulence and swarming motility (Wynendaele et al., 2013). In gram positive bacteria, this is driven by quorum sensing peptides (QSPs) while in gram negatives, it is achieved

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by acylated homoserine lactones (AHLs) (Miller et al., 2001). The QSPs can stimulate two-component system by directly binding to the transcription factor, which further stimulates changes in target gene expression (Schauder et al., 2001; Jimenez et al., 2014).

QS in clinically relevant bacteria like *Staphylococcus epidermidis* and *Enterococcus faecalis* is presented by biofilm formation and with expression of pathogenicity-related extracellular protease (Krämer et al., 2009; Nishiguchi et al., 2009). In some species of *Streptococcus,* namely *S. pneumoniae* and *Streptococcus gordonii*, QS is controlled by competence-stimulating peptides (CSPs) (Havarstein et al., 1997). In general, the gram-negative bacteria seem to use AHLs as autoinducers, while the gram-positives use peptide-based signaling systems (Montgomery et al., 2013). QS is also seen in non-clinically relevant organisms like halophiles (eukaryotic algae), acidophiles (*Ferroplasma acidarmanus*), thermophiles (*Thermotoga maritima*), psychrophiles (*Pseudoaltermonas haloplanktis*), piezophiles (*Shewanella benthica*) and archaeas (*Methanothrix*) (Das Sarma et al., 2006). These organisms have been found to contain many genes related to biofilm formation and motility but no *LuxR* or *LuxS* homologues were identified (Baker et al., 2010; Nichols et al., 2009; Medigue et al., 2005; Bodor et al., 2008).

Inhibition of QS mechanism can be important strategy for combating bacterial pathogenicity (Kumar et al., 2013). QS mechanism can be disrupted using small molecules, monoclonal antibodies and receptor antagonists (Thoendel et al., 2010; Dong et al., 2007). Drugs like ambuic acid and RNA III Inhibiting Peptide (RIP) have been seen to inhibit QS mechanism (Nakayama et al., 2009; Nakayama et al., 2007). Some anti-autoinducer monoclonal antibodies have also been found to hinder QS in *Staphylococcus aureus* (Park et al., 2007). In *Escherichia faecalis*, QS was found to be inhibited by Siamycin I (Shojima et al., 2014). Cultivation of *V. fisheri* produces large quantities of luciferase possibly by QS mechanism, which has been beneficial in population's survival by combating environmental threats (Tomasz et al., 1965). This article focuses on QS mechanisms in some important bacteria and its role in their survival. Table 1 compares QS in these important bacterial species.

QS in *V. harveyi*

There are two parallel quorum-sensing systems in *V. harveyi* which can detect either autoinducer 1 (AI-1) or autoinducer 2 (AI-2) signaling molecules (Engebrecht et al., 1983). These QS bacteria produce and release AHL molecules which can affect a signal transduction cascade to change the organism's behavior (Engebrecht et al., 1983). These lactone molecules have various acyl chain lengths, saturation degrees and modifications at third carbon of acyl chain, which upon interaction with a signal, can activate or inhibit *LuxR* homologues, thereby controlling number of biological functions like biofilm formation, bioluminescence and virulence (Engebrecht et al., 1983). The two QS systems in *V. harveyi* control the density-dependent expression of bioluminescence composed of a sensor and a cognate autoinducer (Bassler et al., 1995). Both systems, each with two sensors responding to AI-1 and AI-2, are integrated via a shared regulatory protein to control the light emission (Bassler et al., 1994; Freeman et al., 1999). The AI-1 is identified as hydroxybutanoyl-L-homoserine lactone whose synthesis is depended on *luxL* and *luxM* genes (Cao et al., 1989). The sensor proteins are two component adaptive regulatory proteins which are regulated by a phosphorylation-dephosphorylation mechanism (Bassler et al., 1993, 1994). Surette et al. (????) have done some extensive work in understanding production of AI-2 in *V. harveyi*. In one of their experiments, a library of wild-type *V. harveyi* BB120 genomic DNA was transformed into *E. coli* strain DH5α to understand functions of AI-2 production (Michael et al., 1999). From 2,500 clones, five DH5α clones resulted in a 300-fold stimulation. Furthermore, 962 *E. coli* strains harboring Tn5 insertions in pBB2929 were tested for the loss of the ability to produce AI-2 and four did not produce AI-2. All of the four transposon insertions were found to be in the same 2.6-kb *HindIII V. harveyi* genomic DNA and only one ORF (*LuxSV.h.* gene) was identified to produce AI-2. Addition of culture fluids from the control Tn5 insertion strain induced 780-fold luminescence in the reporter, whereas culture fluid from the *luxSV.h.::Tn5* insertion strain did not induce the expression of luminescence in the reporter concluding that all the null mutants in *luxSV.h.* eliminate AI-2 production. An intricate dependence of *LuxSV.h.* gene and AI-2 production was observed.

QS in *Salmonella typhimurium*

Similar to *V. harveyi, S. typhimurium* LT2 also produces similar AI-2 whose activity is maximal in mid-exponential phase, as detected by autoinducer production assay. This is degraded when the bacteria enter stationary phase (Surette et al., 1998). AI-2 production is influenced by several factors like logarithmic growth, preferred carbon sources, low pH and high osmolarity, and factors like carbon source, neutral pH, and low osmolarity induce degradation (Surette et al., 1999). The signal production and degradation was further found to be depended on the amount of protein synthesis (Surette et al., 1999). The gene responsible for AI-2 production was identified by random mutations in *MudJ* transposon (Maloy et al., 1996). One *MudJ* insertion mutant was identified from 10,000 mutants that lacked detectable AI-2 in culture

Table 1. Comparison of QS in important bacterial species.

Feature	*Vibrio harveyi*	*Salmonella typhimurium*	*Escherichia coli*	*Pseudomonas aeruginosa*	Reference
Molecules	AI-1, AI-2, Acyl-homoserine lactone (AHL)	AI-2, AHLs	AI-2	2-heptyl-3-hydroxy-4-quinolone (PQS), diketopiperazines	Diggle et al. (2007)
Phenotypic effect	Biofilm formation, Bioluminescence and virulence	Virulence	Virulence	Biofilm formation, Virulence	Costerton et al. (1999) and Brown et al. (1988)
Affected genes	*luxR, luxL* and *luxM*	*mudJ, sdiA*	*ygaG, luxS, luxSV.h., luxSS.t.,* and *luxSE.c.*	*lasI, rhli, lasR, qscR* and *rhlR*	Barie et al. (1990), Brown et al. (1988) and Maloy et al. (1996)
External influencing factors	Not applicable	Logarithmic growth, preferred carbon sources, low or neutral pH and high osmolarity, and protein synthesis	Temperature, glucose and carbon	Not applicable	Surette et al. (1999) and Surette et al. (1998)
Anti-QS Products	Furanone compounds	Digoxin, Taxol, Artemisin, Vincristine, Vinblastine, Ginkgo, Flavonoids, Phenols, Stilbenes and non-protein amino acids, Southern Florida seaweeds	Oil extract from mature ripe fruit husk of *Aframomum corrorima*, *Nigella sativa*, *Albiza schimperiana* (ASRM) and *Justica schimperiana*	Extracts of *Conocarpus erectus*, *Chamaesyce hypericifolia*, *Callistemon viminalis*, *Bucida buceras*, *Tetrazygia bicolor*, and *Quercus virginiana*	Huber et al. (2003), Bjarnsholt et al. (2005), Choo et al. (2006), Manefield et al. (1999) and Gao et al. (2003)

fluids at mid-exponential phase (Maloy et al., 1996). PCR amplification and sequencing determined this site mapped to *E. coli MG1655* genome corresponding to an open reading frame (ORF) of unknown function denoted as *ygaG* (Blattner et al., 1997). Further, testing of *E. coli* O157:H7 *ygaG* gene and *V. harveyi luxSV.h.* genes in restoration of AI-2 production via complementation assays revealed that *E. coli* and *S. typhimurium* LT2, respectively produced 1.5 and 1.4 times more AI-2 activity than *V. harveyi* (Blattner et al., 1997). Furthermore, sequence comparison of AI-2 production genes from *V. harveyi, E. coli,* and *S. typhimurium* revealed that the translated protein sequences encoded by the *ygaG* ORFs aligned with the translated LuxS protein sequence from *V. harveyi* and that *E. coli YgaG* proteins were found to be 77% identical to *LuxS* from *V. harveyi* (Blattner et al., 1997). The sequence adjacent to the *MudJ* that inactivated the AI-2-production matched perfectly to the fragment *B_TR7095.85-T7* in the *S. typhimurium.* Moreover, it could be complemented to a full AI-2 production by the introduction of either the *E. coli luxSE.c.* gene or the *V. harveyi luxSV.h.* gene (Michael et al., 1999).

It has been observed that *Salmonella* possesses two QS systems (autoinductor AI-2 and acyl-homoserine-lactones), where AI-2 seems most important in cell to cell communication system by regulating *SdiA*, while a counterpart of *LuxR* which activates the genes of SPI-1 genes is involved in virulence (Janssens et al., 2007).

In *Salmonella*, natural products like digoxin, taxol, artemisin, vincristine, vinblastine, Ginkgo, favonoids, phenols, stilbenes and non-protein amino acids have been shown to have some QS activities (Huber et al.,2003; Bjarnsholt et al., 2005; Choo et al., 2006). Although there are number of quorum-quenching enzymes that can hydrolyse AHLs, only halogenated furanones from the red alga *Delisea pulchra* have been shown to have anti-QS activity (Manefield et al., 1999). Some of the Southern Florida seaweeds and few terrestrial plants have also shown such activities (Gao et al., 2003). Preliminary studies have shown the usage of antibacterial drugs. Further exploration of this property may prove beneficial in

treatment of *S. typhimurium* infection which specifically uses QS as survival strategy (Adonizio et al., 2006).

QS in *Escherichia coli*

Certain strains of *E. coli (DH5α)* do not produce AI-2 but can do so if there is an introduction of *luxS* gene from *E. coli O157:H7* strain (Michael et al., 1999). The genes of QS (*luxSV.h., luxSS.t.,* and *luxSE.c.*) are highly homologous to each other and the *LuxS* genes of *E. coli* have been defined as a new family of autoinducer genes (Michael et al., 1999). Identification of the *ygaG* locus in *E. coli* has been associated with a production defect of AI-2, which may occur because of a premature truncation caused by frameshift mutation resulting from the G/C deletion in *ygaG* (Michael et al., 1999).

Complementation studies demonstrate that the AI-2 production defect in *E. coli DH5α* is recessive to in-trans expression of *ygaG* (Michael et al., 1999). Regulation of AI-2 production differs between pathogenic and nonpathogenic strains, where temperature, glucose and carbon source play important roles (Gilson et al., 1995). Moreover, pathogenic *E. coli* strains have been shown to significantly produce more AI-2 than non-pathogenic (Gilson et al., 1995). AI-2 class of autoinducers are novel as *luxS* genes bear no homology to other genes known to be involved in production of HSL autoinducers (Gilson et al., 1995). LuxS protein of *V. harveyi* has also been detected in other organisms like *Haemophilus influenzae, Helicobacter pylori, Bacillus subtilis, Borrelia burgdorferi, Neisseria meningitidis, Neisseria gonorrhoeae, Yersinia pestis, Campylobacter jejuni, Vibrio cholerae, Deinococcus radiodurans, Mycobacterium tuberculosis, E. faecalis* and *Streptococcus pyogenes* (Bassler et al., 1997). AI-2 is, thus, found to be an important target in regulating the transition from a nonpathogenic existence outside a host to a pathogenic existence inside a host and inducing expression of the Type III secretion system contributing to its virulence (Michael et al., 1999).

QS in *Pseudomonas aeruginosa*

In gram-negative bacteria like *Pseudomonas*, QS molecules like 2-heptyl-3-hydroxy-4-quinolone (PQS) and diketopiperazines have been found to be involved in QS phenomenon (Holden et al., 1999). Biofilms are complex communities of microorganisms embedded in a self-produced matrix which can adhere to surface, either inert or alive (Costerton et al., 1999). Biofilm-associated bacteria on implants or catheters can cause chronic infections like cystic fibrosis by *P. aeruginosa* or the endocarditic decay by *Streptococcus viridans* group (Brown et al., 1988). Biofilm grown cells have been found to be 10 to 1,000-fold more resistant to the effects of antimicrobial agents than their planktonic counterparts (Brown et al., 1988). The QS system in *P. aeruginosa* is formed by autoinducer synthetase (*LasI/RhlI*) and a corresponding regulator protein (*LasR/RhlR*). Each system produces its own AHL synthetase (*LasI* and *Rhli*) and its regulating place (*LasR* and *RhlR*). Regulation of genes encoding the exoproducts depends on a signalling system that encompasses at least two sets of *LuxRI* homologues (*LasI* and *LasR*). The second quorum-sensing system of *P. aeruginosa* is controlled by the *LuxRI* homologues, *RhlRI* which activates expression of *rhlAB*, an operon encoding Rhamnosyltransferase, this leads to reduction of surface tension and thereby allowing *P. aeruginosa* cells to swarm over semi-solid surfaces (Barie et al., 1990). Although, the third regulator, *QscR*, is not seen to participate in the synthesis of AHL, more than 400 genes are affected in *Pseudomonas* which is implicated in virulence (Barie et al., 1990). Biofilms have been shown to have resistance to antibiotics including ampicillin, streptomycin, tetracyclines and gentamicin (Barie et al., 1990). The dosage levels to treat biofilms can reach toxic levels; moreover, *P. aeruginosa* can produce extracellular virulence factors such as proteases, haemolysins, exotoxin A, exoenzyme S and pyocyanin which are controlled by QS thereby contributing to its pathogenesis (Barie et al., 1990).

QS in gram-positive bacteria

In many gram-positive bacteria's, QS autoinducers are diverse in sequence and structure, and are interacted with membrane bound two-component signal transduction systems (Havarstein et al., 1995). The cell membrane in gram-positive bacteria's is impermeable to peptides, and they need some specialized transporters for secretion (Havarstein et al., 1995). All these changes prone autoinducers to do posttranslational modifications (Bouillaut et al., 2008). The sensor kinases of two-component systems auto-phosphorylate and the phosphoryl group is then passed from the histidine to a conserved aspartate on a cytoplasmic protein on binding to autoinducers (Simon et al., 2007). Components of QS in many gram-positive bacteria consist of AI, transporter, histidine kinase receptor, and response regulator on one operon (Peterson et al., 2000). Some of the bacteria's known to operate QS in the aforementioned way are *S. pneumonia*, *Bacillus subtilis*, *S. aureus*, *Listeria monocytogenes*, *E. faecalis*, and *Clostridium perfringens* (Ohtani et al., 2009; Riedel et al., 2009; Thoendel et al., 2011). Couple of important gram-positive bacteria's where QS is studied extensively are stated as the following.

QS in *S. aureus*

S. aureus utilizes a canonical two-component QS system

encoded by the *agr* locus (Thoendel et al., 2011). QS in *S. aureus* has four components which are driven by RNAII expression (Thoendel et al., 2009). AI in *S. aureus* is truncated to a 7-9 residues peptide and coupled with cyclization of a five membered peptide ring, which is bounded to a membrane bound histidine kinase AgrC, the autophosphorylation of which transfers the phosphate group to an aspartate on the regulator AgrA (Thoendel et al., 2009). It is synthesized as a precursor from *agr.* The AgrA then binds to the P2 promoter to autoinduce the *agr* operon, the mature AI is then transported out of the cell via transporter AgrB (Thoendel et al., 2009). Apart from P2 activation, the phosphorylated AgrA can also activate the divergently encoded P3 promoter which controls expression of RNAIII encoding the virulence factor δ-hemolysin, which in turn can activate production of α-toxin and repress the expression of *rot*, fibronectin binding proteins and other surface proteins (Novick et al., 1993). Thus, RNAIII acts as both direct and indirect regulator. The virulence in *S. aureus* is also attributed to its biofilm development.

There has been a functional collision between biofilm development and *agr* system, which may have sought to gain time for establishing a mature biofilm community and when there's a time of virulence dispersion, *S. aureus* terminates biofilm production and decreases surface proteins (Boles et al., 2008). The *agr* regulators in *S. aureus* also can respond to extracellular environmental signals like autoinducers. It has been hypothesized that an unknown regulator of *agr* can control RNAIII levels, which in turn causes direct transcription of surface proteins and pigment production to inhibit expression of secreted toxins following extracellular stress (Lauderdale et al., 2009). This may be needed as some of the stress regulons of sigma ensures that *S. aureus* does not undergo QS under conditions when the bacteria must dedicate resources to decrease stress. Another two-component system, SrrA/SrrB has also been seen to control virulence, where overexpression of SrrA/SrrB has been seen to decrease virulence, likely due to inhibition of *agr* expression (Yarwood et al., 2001). Further, hypervariability amongst *agrD* and *agrB* genes leads to the production of one of four different types of *S. aureus* autoinducers depending on the strain (Dufour et al., 2002). The hypervariability has also been observed in *agrC* gene encoding the sensing domain of the AI receptor (Dufour et al., 2002). The type of AI seems important as it determines the stabilization of an inhibitory confirmation of AgrC; this can halt cell-cell signaling and control the infection (Geisinger et al., 2009).

QS in *B. cereus*

B. cereus is important gram-positive bacteria which is closely related to *B. cereus*, *B. anthrasis*, and *B. thuringiensis* and can cause secretion of a variety of hemolysins and toxins (Bottone et al., 2010). QS in *B. cereus* is controlled by a transcription factor PlcR, which binds intracellular AI derived from the PapR protein which is a 48 amino acids long protein containing an amino-terminal signal peptide (Slamti et al., 2002). Another protein, NprB is a secreted neutral protease B which cleaves the pro-AIP PapR into peptides, which can then activate PlcR activity (Slamti et al., 2002). There is also a sequence diversity in the PapR autoinducers classifying this species into four pherotypes (Slamti et al., 2005). When transported back into the cell, the PapR helps bind AI to the transcription factor PlcR, thereby regulating transcription (Slamti et al., 2005). The PlcR interacting with the PapR AIP can control expression of 45 genes regulating enterotoxins, hemolysins, phospholipases, and proteases (Gohar et al., 2008).

Novel therapeutic techniques to target QS

Quorum sensing peptides (QSPs) drive QS phenomenon in gram-positive bacteria (Miller et al., 2001). Targeting QSPs can be an alternative strategy to combat bacterial pathogenicity (Kumar et al., 2013). Therefore, analysis and prediction of QSPs are of immense importance in gram-positive bacteria. A machine learning tool for identification of novel and effective biofilm inhibitory peptides (BIPs) has recently been proved an efficient method of classification (Akanksha et al., 2015). Furthermore, physicochemical properties like aromaticity, molecular weight and secondary structure have also been observed to differentiate QSPs from non-QSPs (Tian et al., 2009). One study utilizes support vector machine (SVM) to extract physicochemical indices, where QSPs are seen to prefer secondary structure conformations (α-helix, coil and β-sheet) similar to QSPs of *S. mutans* with random coil α-helix conformations (Tian et al., 2009; Syvitski et al., 2007). Biofilms in bacteria are known to resist the environmental stresses like biocidal agents, UV damage, metal toxicity and acid exposure (Hall et al., 2004). They can have a spatiotemporal heterogeneity making them 1000 times more resistant to antibiotics (Costerton et al., 1999). Thus, there seems a significant need to develop antimicrobial peptides (AMPs) as prophylactic and therapeutic agents against drug-resistant bacteria and biofilms (Fox et al., 2013). Studies have been conducted to evaluate action of peptides against multiple bacterial species. Machine learning tools have been used to build six SVM and weka-based models trained on 80 biofilm-active AMPs and 88 QSPs (Arun et al., 2016). The dPABBs web server develops a prediction strategy for the identification and optimisation of such anti-biofilm peptides (Arun et al., 2016). Homology-based prediction has been proven to be extremely successful in identifying antimicrobial peptides (Lynn et al., 2004). Other machine learning prediction

tools based on SVM (Lata et al., 2010; Thomas et al., 2010), hidden markov models (Fjell et al., 2007), sequence alignments and feature selection (Wang et al., 2011) have also been effective. Various techniques have been used for network analysis and visualization of QS data in different organisms. A network of potential anti-quorum sensing agents for *P. aeruginosa* was created with information from biomedical ontologies and curated databases (Martín et al., 2017). Some groups have already applied network approaches to study antibiotic resistance in *P. aeruginosa,* while others have tried to extract information types and apply it to the retrieval and curation of research articles in *P. aeruginosa* QS (Hwang et al., 2016). In *V. fischeri*, LuxI is an important component of QS signaling pathway (Engebrecht et al., 1987). Homology modeling is a good way of predicting docking sites and a three-dimensional structure of LuxI and other QS components (Mihăşan et al., 2010). Homology modeling is a method of structure prediction based on amino acid sequence similarity to closely-related known structures (Mihășan et al., 2010). Groups have tried to utilize such techniques of homology modeling using Phyre2 and GalaxyWEB server (Mohammed et al., 2016). Ultra-high-throughput screening approaches have been utilized for screening around 200,000 compounds for inhibitors of LasR-dependent gene expression (Ute et al., 2006). A theoretical approach has been adopted to build an interactome comprising proteins from *Salmonella* and then analyzing the networks with parameters like centrality and k-core measures (Chandrajit et al., 2014; Chandrajit et al., 2012).

A set of responsible virulent proteins have been identified from published microarray data, which could serve as sensitive predictors and form the foundation for a series of trials in the wet-lab setting. Analysis of protein interaction networks (PINs) has gained importance as one of the promising strategies, where the topology and modularity analysis of the networks have been implied (Pan et al., 2016). Analyses of a PIN starts by determining the number of interacting partners of a particular protein to identify its degree centrality (DC) which correlates with its biological importance. Other important measures like closeness centrality (CC), betweenness centrality (BC) and eigenvector centrality (EC) with a cartographic analysis of identifying the functional modules in the network have been implied to be a useful technique to identify therapeutic targets (Pawar et al., 2017). All these existing and newer *in silico* approaches are promising ways in targeting QS in different bacterial species.

Relationship between QS genes of gram-positive and negative bacteria's

Several groups have tried to explore concept of interspecies communication between gram-positive and negative bacteria. One important and interesting study was recently performed by Rajput et al. (2017) and Akanksha et al. (2017). Here, they have compared gram-positive and negative bacteria group for the presence of putative *LuxI/LuxR* with respect to its conservation in domain, motif, compositions, gene ontology (GO), and taxonomic distribution. A phylogenetic reconstruction of a tree was done to investigate the evolutionary trends in two-component system proteins, *LuxI* and *LuxR* using a Maximum Likelihood (ML) method. As shown in Figure 1, 11 *LuxI* sequences of gram-positive bacteria located with their respective gram-negative bacteria BLAST hits except *Mycobacterium* species *djl-10*. Some of the species used in this study were *Mumia ava*, *Burkholderia*, *Streptomyces purpurogeneiscleroticus*, *Methylobacterium* species *Leaf361*, *Syntrophaceticus* and *Desulfobacterium autotrophicum*. The ML tree for representative *LuxR* sequences of gram-positive bacteria localized with gram-negative bacteria with the exception of two, Streptomycetaceae and Bacillaceae Lactobacillaceae family.

The topological arrangement of six canonical *luxI* and *luxR* genes among these two bacterial species showed that they both are considerably related to each other with fewer differences in amino acids. *Streptomyces purpurogeneiscleroticus* and *Albizia ferruginea* showed similar topology with conserved *LuxI/LuxR* motifs, while the protein of *S. schinkii* was localized in the same clades between two trees. Overall, a significant overlap is seen between these two genes in gram-positive and negative species. Further exploration on likely overlaps in other QS genes and components is very much possible and needs an evaluation.

Conclusion

Bacterial ability to monitor cell density, prior to expressing a phenotype, is due to QS phenomenon. Production and liberation of enzymes by bacteria to reach its adequate concentration is decided by QS which in turn can activate or repress certain target genes. Further, biofilms can help attachment of bacteria to each other and wet surfaces assisted by QS. The knowledge of the molecular mechanisms in QS and biofilms can improve therapeutic approaches. Different bacteria can use QS in different ways. The QS system in *P. aeruginosa* is formed by autoinducer synthetase (*LasI/RhlI*) and a corresponding regulator protein (*LasR/RhlR*). Each system produces its own AHL synthetase (*LasI* and *RhlI*) and its regulating place (*LasR* and *RhlR*). The second quorum-sensing system of *P. aeruginosa* is controlled by the *LuxRI* homologues. In *E. coli*, QS is controlled by *luxSV.h., luxSS.t.,* and *luxSE.c.* genes, while in *V. harveyi* QS is controlled by acyl-homoserine lactone-signaling molecules which can effect a signal transduction cascade

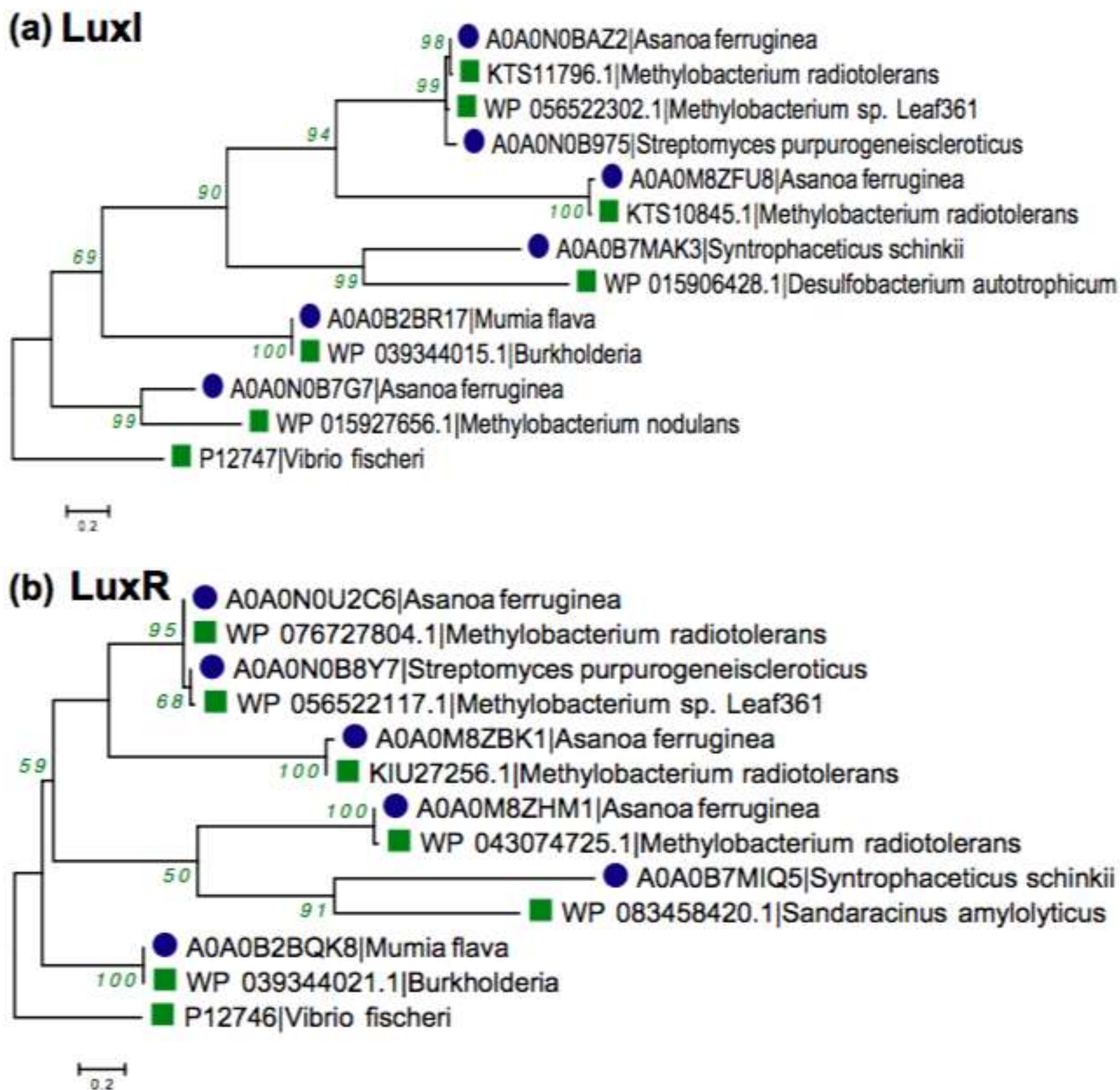

Figure 1. Phylogenetic tree reconstruction using Maximum Likelihood method for gram-positive bacteria and their respective gram-negative BLAST hits (a) *LuxI* containing sequences; (b) *LuxR* containing sequences [Gram-positive bacteria: green colour; Gram-negative bacteria: blue colour].
Source: Reproduced from Rajput et al. (2017).

to change the behavior of organism. In conclusion, a detailed understanding of QS phenomenon can help in manipulation of its behaviour and can shift paradigms of treating bacterial infections.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interest.

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