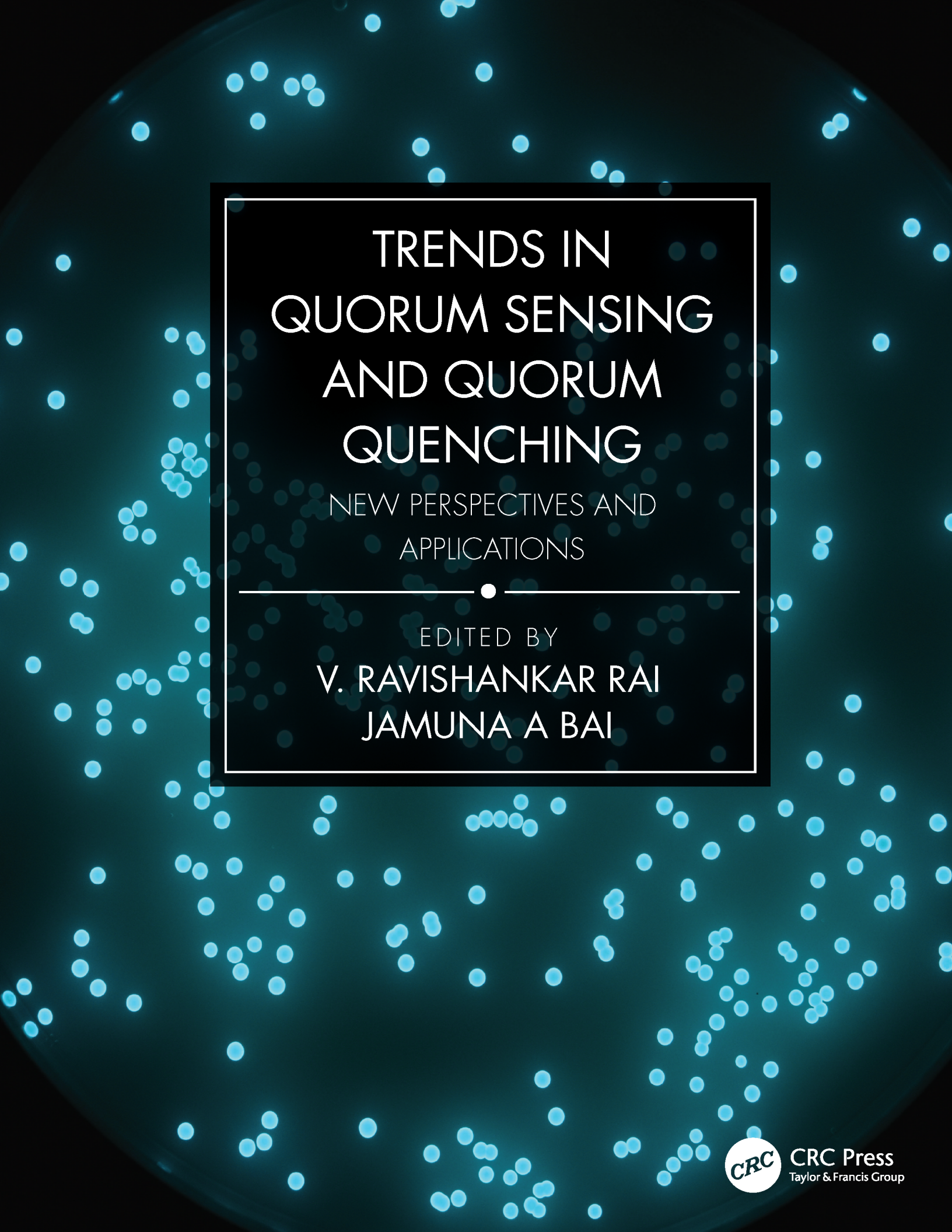

TRENDS IN QUORUM SENSING AND QUORUM QUENCHING

NEW PERSPECTIVES AND APPLICATIONS

EDITED BY
V. RAVISHANKAR RAI
JAMUNA A BAI

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Trends in Quorum Sensing and Quorum Quenching


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New Perspectives and Applications

Edited by
V. Ravishankar Rai
Jamuna A Bai

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Preface

Trends in Quorum Sensing and Quorum Quenching: New Perspectives and Applications focuses on the evolution and our current understanding of quorum sensing mechanisms in bacteria and the potential application of quorum sensing inhibitors in clinical and industrial settings. Discovered in the 1960s and 1970s, quorum sensing has garnered an increasing interest in the research community. Elucidating the quorum sensing mechanism in bacteria has revealed previously unknown coordinated group behavior in bacteria. Studies on cell-cell signaling or intercellular communication started with the understanding of bioluminescence in marine vibrios, fruiting body development in myxobacteria, and competence in pneumococci. Research on quorum sensing further advanced when it was realized that it had a central and crucial role in regulation of bacterial pathogenicity. The discovery of quorum sensing inhibitory compound furanones from red seaweed *Delisea pulchra* and the characterization of quorum quenching enzyme, the AiiA lactonase from *Bacillus*, indicated the novel strategy that could be used to combat and control bacterial infections.

This book has two major sections with key topics. Section one deals with advances and perspectives on molecular mechanism of QS in bacteria. The topics covered include influence of quorum sensing on bacterial central metabolism; novel quorum sensing signaling molecules and detection techniques; molecular insights in the role of QS in clinical pathogens, foodborne bacteria, agriculturally important bacteria, industrial relevant bacteria and its application in metabolic engineering; the evolution and role of QS in biofilm formation and development; and QS in regulating morphology and metabolic pathways in eukaryotic microbes (fungi).

The second section focuses on trends in the development and application of quorum sensing inhibitors. The emphasis is on the mechanism and types of QS inhibitors; evolution of quorum quenching in bacteria; application of metagenomics tools for the identification of novel quorum quenching genes and enzymes; bioprospecting of bacteria, fungi, actinomycetes and endophytes from rhizospheres and marine ecosystems for novel QS inhibitors; design of QS inhibitors based on nanotechnology; etc. The potential application of QS inhibitors as—anti-infectives and therapeutics (quorum quenching monoclonal antibodies and AHL acylase nanoparticles), novel intervention techniques in the food industry (sanitizers for food contact surfaces and as preservatives), anti-biofouling agents with commercial and industrial applications, infection control strategies in aquaculture, and as biocontrol agents for plant pathogens is discussed.

The book is comprehensive and detailed in nature, covering all the important aspects and highly relevant topics in quorum sensing and quorum quenching in bacteria. Special focus is given on exploring quorum sensing inhibitors from microbes and flora inhabiting biodiversity rich regions including tropical rain forests, various tropical soils, and oceans. Graduate students, researchers and academicians from the field of Medical Microbiology, Pharmaceutical Biology, Genetics and Food Biotechnology will find the book an invaluable tool.


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Editors

Prof. V. Ravishankar Rai received his MSc (1980) and PhD (1989) from the University of Mysore, India. Currently, he is working at the Department of Studies in Microbiology, University of Mysore, Mysore. His current research and publications in food microbiology, microbial quorum sensing, microbial influenced corrosion, and nanotechnology has been well received by the international scientific committee. His series of edited books with reputed publishers such as CRC Press and Wiley Publications—*Biotechnology: Concepts and Applications* (2009), *Microbial Food Safety and Preservation Techniques* (2014), *Beneficial Microbes in Fermented and Functional Foods* (2014), *Advances in Food Biotechnology* (2015), *Food Safety and Protection* (2016), and *Nanotechnology Applications in the Food Industry* (2018)—are comprehensive in nature and have contributions from international experts in the field. Prof. Rai has received awards from UNESCO Biotechnology Action Council Programme (Visiting Fellow, 1996), UGC Indo-Israel Culture Exchange Programme (1998), DBT Overseas Fellowship (2008), Indo-Hungarian Educational Exchange Programme fellowship (2011), INSA—bilateral exchange fellowship (2015), Incoming Fellowship (2017) from Cardiff University, UK, and an invitation from Mauritius Research Council, Mauritius (2018) to conduct collaborative research with renowned scientists from international universities. He has been awarded the Bilateral Exchange Fellowship by the Indian National Academy of Sciences to visit Germany (2020) and Cambridge-Hamied Visiting Lecture Scheme (2020) to visit University of Cambridge, UK.

Dr. Jamuna A Bai has completed her MSc and PhD in Microbiology from the University of Mysore, India. She is working as an Assistant Professor in JSS Academy of Higher Education and Research, Mysore. She has previously worked as a Researcher in UGC sponsored University with the Potential Excellence Project, University of Mysore, India and as ICMR Senior Research Fellow. She has carried research work on food safety, role of quorum sensing, and biofilms in food-related bacteria and developing quorum-sensing inhibitors. Her research interests also include antimicrobial application of functionalized nanomaterials and peptides against pathogenic bacteria.


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Expanding Roles and Regulatory Networks of LadS/RetS in Pseudomonas aeruginosa

Chuanmin Zhou, Maryam Dadashi, and Min Wu

CONTENTS

1.1 The Two-Component Systems

Gram-negative opportunistic pathogen *Pseudomonas aeruginosa* is a severe host pathogen, found widely in nature, exposing in dynamic environmental conditions. Of note, the two-component system (TCS) is important for sensing those environmental challenges which in turn modulate a number of gene expressions (Stock, Robinson, and Goudreau 2000). Typically, TCS is coupled with a sensor histidine protein kinase and a response regulator protein. Histidine protein kinase is responsible for detecting extracellular signals, regulating the downstream effectors in response to the stimuli through phosphorylated response regulator protein (Stock, Robinson, and Goudreau 2000). To date, over 100 TCS genes have been found in *P. aeruginosa* (Rodrigue et al. 2000, Stover et al. 2000).

1.2 Discovery of RetS and LadS

Hybrid sensor kinase RetS (regulator of exopolysaccharide and Type III secretion) was first described in *P. aeruginosa* in 2004, which encoded 942 amino acids. This protein not only contains N-terminal cleaved signal sequences, a large periplasmic domain, and seven transmembrane domains (associated with environmental signal transduction), but also possesses TCS-like histidine kinase and response regulator domains in tandem, revealing that other TCS regulators may exist (Laskowski, Osborn, and Kazmierczak 2004, Goodman et al. 2004). RetS orthologs were also found in *Pseudomonas putida*, *Pseudomonas fluorescens*, *Pseudomonas syringae*, and *Azotobacter vinelandii* (Goodman et al. 2004). In 2005, another hybrid sensor kinase named LadS (lost adherence sensor) was noticed in *P. aeruginosa* PAO1 genome, which showed an opposite role of RetS, promoting biofilm formation and inhibiting T3SS activation (Ventre et al. 2006).

Domain analysis of LadS amino acids showed that LadS contained 795 amino acids with similar domains seen in RetS, including N-terminal cleaved signal sequences, a large periplasmic domain, seven transmembrane domains, as well as a histidine kinase and a response regulator domain (Ventre et al. 2006). These transmembrane domains in LadS and RetS were also observed in a number of other carbohydrate binding proteins (Ventre et al. 2006, Anantharaman and Aravind 2003). In particular, these domains exhibited 35% sequence identity, suggesting that this periplasmic sensor may respond to similar but not 100% identical environmental signals through its unique transmembrane domains (Ventre et al. 2006) (Figures 1.1 and 1.2).

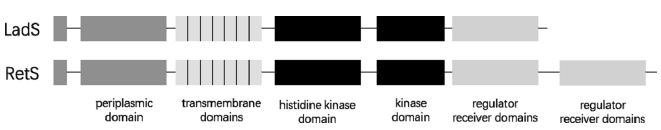

FIGURE 1.1 Domain organization of LadS and RetS hybrid sensors.

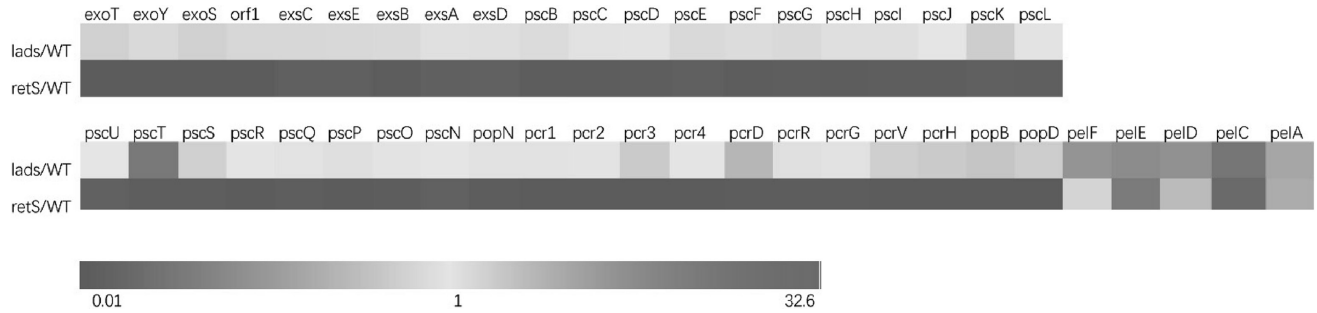

FIGURE 1.2 Pel and TTSS expression levels in Δ*ladS* and Δ*retS* and WT *P. aeruginosa*. (From Goodman, A. L. et al., *Dev. Cell*., 7, 745–754, 2004; Ventre, I. et al., *Proc. Natl. Acad. Sci. USA*, 103, 171–176, 2006.)

1.3 Opposing Roles of RetS and LadS

RetS was characterized as a global pleiotropic regulatory protein, and the expression levels of almost 400 genes were significantly altered in a *retS* mutant strain (Goodman et al. 2004). RetS was necessary for the transcription of the T3SS operons under low calcium and host cell contact conditions. Deletion of *retS* significantly prohibited the activation of T3SS (Laskowski, Osborn, and Kazmierczak 2004). However, no DNA binding motifs were identified in RetS protein domains, indicating that it might modulate T3SS function indirectly (Laskowski, Osborn, and Kazmierczak 2004). The *retS* deletion strain exhibited robust biofilm formation by promoting the expression of biofilm related genes *pel* and *psl* (Goodman et al. 2004). A mutant with a deletion of *ladS* behaved in an opposite manner (Ventre et al. 2006). Transcriptome analysis of *ladS* mutant strain compared to WT strain showed that 79 genes were significantly affected, including that *pel* and *psl* gene expression were repressed and T3SS were activated in *ladS* mutant strain (Ventre et al. 2006). Compared to transcriptome analysis in *retS* mutant strain, 49% were oppositely regulated in *ladS* mutant strain, indicating that LadS and RetS signaling transduction pathways are antagonistic (Ventre et al. 2006).

RetS promotes the formation of heterodimers with GacS, reducing the GacS autophosphorylation. Gene screening of transposon insertions showed that RetS may modulate GacS/GacA (Goodman et al. 2004), whereas LadS showed an opposite activity by promoting the activation of GacS/GacA pathway (Ventre et al. 2006). Interestingly, LadS did not interact with GacS and hence may upregulate the GacS/GacA pathway through a phosphor-relay mechanism, resulting in phosphotransfer to the HPT domain of GacS, which in turn promoted chronic infection (Chambonnier et al. 2016). Furthermore, another histidine kinase PA1611 showed a similar role of GacS by interacting with RetS to modulate the GacS/GacA pathway (Kong et al. 2013). The phenotype of *retS* mutant strain was completely blocked in *gacS* mutant strain, indicating that RetS works through the GacS/GacA pathway (Ventre et al. 2006). Phosphorylated GacA in turn directly modulated the expression of small noncoding regulatory RNA (sRNA) *rsmY/rsmZ*. RsmA was a global post-transcriptional regulator, influencing expression of over 500 genes by binding to targeted mRNAs, which was inhibited by sRNA *rsmY/rsmZ*. Different pathogens including *P. aeruginosa*, *Escherichia coli*, *Legionella pneumophila*, *Vibrio cholera*, and *Salmonella typhimurium*, are found expressing *rsmA*. Finally, deletion of *rsmA* also showed similar phenotypes to *retS* deletion mutant strain, exhibiting activation of biofilm and T3SS, which in turn contributes to acute infection (Coggan and Wolfgang 2012).

Although the *retS* deletion mutant exhibited increased attachment to host cells, it showed less cytotoxicity in eukaryotes and less virulence in a pneumonia mouse model, indicating that the *retS* mutant strain was unable to respond to environmental signals (Laskowski, Osborn, and Kazmierczak 2004, Goodman et al. 2004). Deletion of *ladS* showed hyper cytotoxicity compared to the WT strain, and the phenotype of *ladS/retS* double mutant strain was similar to *retS* mutant, showing no cytotoxicity, indicating that LadS may function at the upstream of RetS in response to input signals (Ventre et al. 2006) (Figure 1.3).

The stimuli triggering RetS and LadS activity remain largely uncharacterized. Recent research revealed that deletion of *ladS* causes *P. aeruginosa* calcium-blind through genetic, biochemical, and proteomic study (Broder, Jaeger, and Jenal 2016). LadS detected calcium, while did not respond to other divalent cations (Mg^{2+}, Fe^{2+}, Zn^{2+}, Mn^{2+} or Cu^{2+}), through its DISMED2 domain, promoting chronic infection. The presence of an additional helix inhibited the binding of carbohydrate, which in turn promoted the binding of calcium. In addition, deletion of *gacA*, *gacS*, and *rsmA* also showed calcium unresponsive, whereas *rsmY* or *rsmZ* single deletion remained calcium sensitive. These studies suggest that calcium signaling plays a key role in host-*P. aeruginosa* interaction by facilitating acute-to-chronic infection transformation.

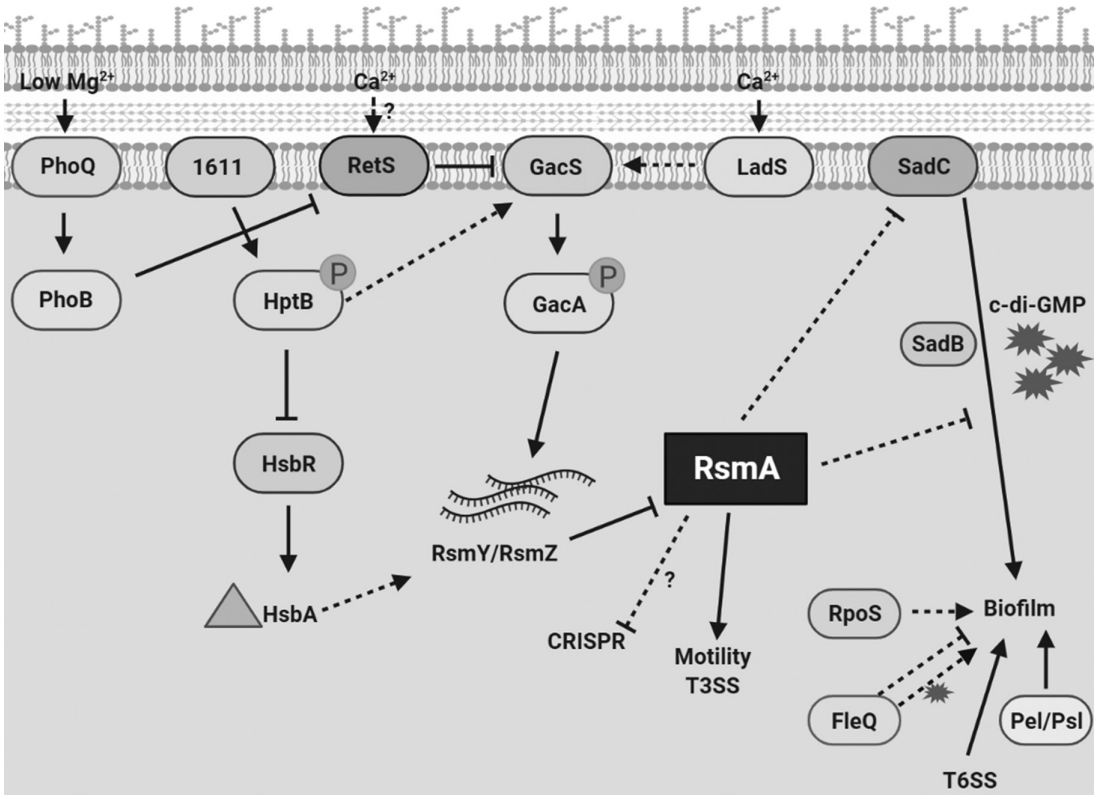

FIGURE 1.3 Schematic representation of RsmA signaling in *P. aeruginosa*.

1.4 Function of LadS/RetS Is Dependent on Small RNAs

Researchers have studied the mechanism of QS systems for decades. To date, LasI/LasR, RhlI/RhlR QS systems are found important for encoding AHLs, and more than 10% of *P. aeruginosa* genes are affected by AHLs. It is noticed that production of *las* and *rhl* dependent AHLs are positively modulated by two sRNAs, *rsmY* and *rsmZ*, and negatively by RsmA. Most QS-dependent genes are repressed by post-transcriptional RsmA effector by bind to its mRNA motif. Considering that LadS/RetS are in the upstream of RsmA, LadS and RetS also showed important roles in influencing QS. Importantly, QS showed important roles associated with acute and chronic infections, indicating that QS is also necessary for acute-to-chronic infection transformation. In addition, RetS contributes in transcriptional repression of sigma factor RpoS, Pel, Psl, and FleQ. FleQ is a repressor of Pel in the absence of c-di-GMP. When c-di-GMP is available, FleQ activates the Pel operon. It was shown that the two-component system PhoQ/B directly interact with RetS. TCS PhoQ/B is a Mg^{2+} sensing system. When exogenous Mg^{2+} content is low, the PhoQ/B represses RetS and promotes biofilm formation.

1.5 Other Hybrid Sensor Kinases in Gac/Rsm Pathway

When planktonic cells hit a proper surface, they form biofilms stepwise. Several factors participate in establishment of *Pseudomonas* biofilms. Below we looked into some role players of biofilm formation. c-di-GMP is a secondary messenger, and its abundance in the cell decides the transition between motility and sessility of the bacterial cells. Biofilms of *P. aeruginosa* have 75–110 pmol mg^{-1} c-di-GMP in total cell extracts compared to planktonic cells, which bear merely 30 pmol mg^{-1} (Basu Roy and Sauer 2014). High levels of c-di-GMP are the hallmark of a biofilm forming lifestyle, which is modulated by diguanylate cyclases like SadC and phosphodiesterases (Merritt et al. 2007).

Recent study indicated that another hybrid sensor kinase, PA1611, modulated genes of acute and chronic infection, which played an important role in downregulation of T3SS and upregulation of biofilm formation (Kong et al. 2013). PA1611 showed similar function to LadS. However, PA1611 did not show a Lads dependent manner. In addition, PA1611 associated with RetS which was similar to GacS showing phosphorelay independent, causing PA1611 shared similar protein domains with GacS

(Kong et al. 2013). PA1611 is capable of influencing the Gac/Rsm pathway by promoting the phosphorylation of HptB (Kong et al. 2013). Phosphorylated HptB phosphorylates HsbR, which further phosphorylates HsbA. HsbA is an anti-anti-sigma factor. HsbA indirectly and positively modulates the expression of RsmY (Bordi et al. 2010), resulting in modulation of RsmA function. Further, two other hybrid sensor kinases, PA1976 and PA2824, are also involved in phosphorylating HptB like PA1611 (Lin et al. 2006, Hsu et al. 2008). Additional research showed that deletion of *hptB* and *retS* led to similar phenotypes. However, HptB signaling only controlled the expression of *rsmY*, which is dependent on the σ^{28} dependent genes (Bordi et al. 2010).

1.6 Other sRNAs as Regulators

1.6.1 P27 sRNA

Polynucleotide phosphorylase (PNPase) is an RNA processing enzyme. It modulates several virulence factors by destabilizing RsmY and RsmZ (Chen et al. 2019). PNPase mutant cells have an increased level of rsmY/Z (Chen et al. 2016). Also, rhamnolipid production is defective in this mutant, leading to lower biofilm formation. Rhamnolipids are regulated by RhlI-RhlR QS system, and PNPase modulates the translation of RhlI by sRNA P27. P27 sRNA directly binds to 5′-untranslated region (UTR) of RhlI mRNA by recruiting Hfq and represses RhlI translation. Mutations in P27 or 5′UTR of RhlI result in unpairing of these two RNAs and RhlI expression restoration (Chen et al. 2019). These results indicate that P27 sRNA may play critical roles in RhlI QS through Hfq-mediated signaling.

1.6.2 PhrS sRNA

In addition to Las and Rhl QS molecules, *Pseudomonas* quinolone signal (PQS), a member of 4-hydroxy-2-alkylquinolines compounds, links the Las and Rhl systems. PQS controls its own expression by inducing *pqsABCDE* operon when it binds to PqsR responsive regulator (Sonnleitner et al. 2011). *pqsABCDE* encodes for intermediate molecules called HHQ, which converts to PQS by LasR-dependent PqsH. PqsR also regulates PqsE and aids RhlR to respond to (C4-HSL) molecules (Farrow et al. 2008).

ANR is an oxygen-responsive regulator. When the oxygen content of the cell is low, ANR induces oxygen limiting-dependent sRNA PhrS. PhrS is shown to activate the PqsR and found to be the first sRNA, linking the QS system with oxygen availability (Sonnleitner et al. 2011).

1.6.3 RsmV and RsmW sRNAs

In *Pseudomonas*, sRNAs play a pivotal role in cellular response to signal molecules (Jakobsen et al. 2017). When RsmY and RsmZ are abundant in the cell, RsmA is sequestered and the production of AHL-based molecules is increased. The sRNA RsmV, which shares sequence similarity to RsmY and RsmZ, is capable of sequestering RsmA and is under control of RhlR. A predicated RhlR binding site is found upstream of RsmV (Janssen et al. 2018).

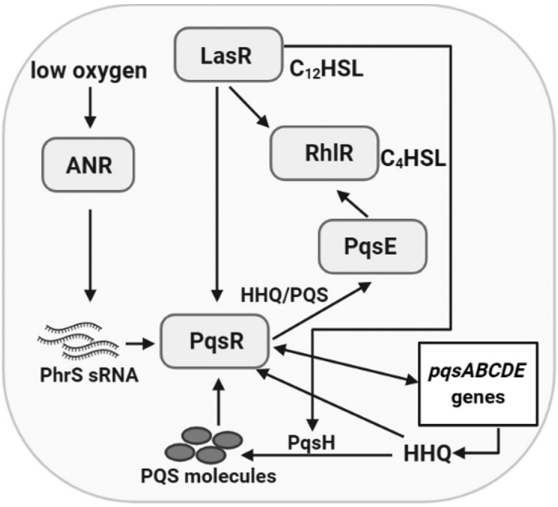

FIGURE 1.4 Schematic representation of sRNA and QS axis.

RsmW indeed is an additional sRNA whose expression is increased during stationary phase in minimal media resembling a biofilm forming environment (Janssen et al. 2018). RsmW sequesters RsmA in a lower efficacy than RsmY and RsmZ. RsmV and RsmW are not under positive regulation of GacA/S system. The transcription regulation of RsmV and RsmW has remained to be elucidated (Janssen et al. 2018) (Figure 1.4).

1.7 Is RetS a Calcium Sensitive Histidine Kinase?

It should be mentioned that RetS also contains a DISMED2 domain, but the RetS input signals are still elusive except that the activation signal was noticed in lyzed kin cells (LeRoux et al. 2015). It remains elusive whether calcium binds to the DISMED2 domain of RetS to activate its kinase activity. Although transcription of *retS* was not modulated in response to calcium, RetS might be activated under low calcium conditions (Laskowski, Osborn, and Kazmierczak 2004). Broder et al., reported that deletion of *retS* led to a calcium blind (Broder, Jaeger, and Jenal 2016). Also, *P. aeruginosa* challenged with lower to higher calcium concentrations promoted the transformation of acute infection to chronic status, indicating that low calcium might activate RetS dependent acute infection. Also, deletion of *ladS* showed lower chronic infection levels compared to the WT counterpart in response to calcium treatment. Hence, this raises the possibility that RetS also acts as a calcium responsive kinase and interacts with LadS. The histidine kinase RetS/LadS are responsible for lower or higher calcium concentration stimulation. Nevertheless, it is unknown whether LadS/RetS play a role in detecting other unknown exogenous signals, which is worth further investigating.

1.8 Can RetS and LadS Interact with CRISPR-Cas Systems?

Bacteria possess multiple defense systems against the invading bacteriophages (Mohanraju et al. 2016, Koonin, Makarova, and Wolf 2017, Forsberg and Malik 2018). Among these, clustered

regularly interspaced short palindromic repeats (CRISPR), first described in 1987 (Ishino et al. 1987), was found as a heritable immunity system in 2007 (Barrangou et al. 2007). To date, 2 classes and 6 types CRISPR-CRISPR-associated (Cas) systems, based on the characteristic of Cas proteins, have been identified in various bacteria and archaea (Makarova et al. 2015, Koonin, Makarova, and Zhang 2017). Class 1 CRISPR-Cas systems rely on multiple CRISPR-Cas protein effector complexes, while Class 2 CRISPR-Cas systems are dependent on a single CRISPR-Cas effector protein. CRISPR and their Cas proteins function as prokaryotic adaptive immunity by targeting acquired mobile genetic elements (MGEs) against invasion of bacteriophages or plasmids (Marraffini 2015, Makarova et al. 2015, Mohanraju et al. 2016). Approximately 45% bacteria and 84% archaea were found containing CRISPR-Cas systems (Grissa, Vergnaud, and Pourcel 2007). Generally, CRISPR-Cas adaptive immunity processes function through three sequential phases. First, acquiring short DNA sequences occurs in CRISPR arrays upon the bacteriophage invasion (Levy et al. 2015, Yosef, Goren, and Qimron 2012, Makarova et al. 2015). Then, the integrated CRISPR arrays containing the recently acquired foreign genetic substance are transcribed and processed into crRNAs (Deltcheva et al. 2011, Haurwitz et al. 2010). Finally, Cas proteins and crRNAs are assembled together to degrade the invading complementary nucleic acids (Brouns et al. 2008).

Most CRISPR-Cas regulators were found to target *cas* promoters (Patterson, Yevstigneyeva, and Fineran 2017). Quorum sensing (QS) was found to modulate different CRISPR-Cas systems (I-E, I-F and III-A) in *Serratia* sp. ATCC39006 and *P. aeruginosa* by influencing the expression of *cas* promoters (Patterson et al. 2016, Hoyland-Kroghsbo et al. 2017). Additionally, cAMP receptor protein (CRP) and histone-like nucleoid structuring proteins (H-NS) play pleiotropic roles in regulating CRISPR-Cas systems (Agari et al. 2010, Shinkai et al. 2007, Patterson et al. 2015, Pul et al. 2010, Westra et al. 2010, Medina-Aparicio et al. 2011).

We showed that CRISPR-Cas targets endogenous RNA to regulate the master QS molecule, LasR and impacting the host response in a TLR4-mediated manner (Li et al. 2016). Because the regulation is reciprocal, we recently identified some QS regulating signals, such as CdpR, can also modulate the activity of CRISPR-Cas (Lin et al. 2019). This line of research is recently broadened to small RNAs that regulated CRISPR-Cas (Lin et al., manuscript under revision). Due to the necessity for precise regulation of CRISPR-Cas activity to respond to foreign invasion but avoiding potential autoimmunity or toxicity, the CRISPR-Cas is tightly controlled by a complex network in the prokaryotes (Figure 1.5).

The *Pseudomonas* species has a well-characterized type I CRISPR-Cas system, which lead us to speculate that LadS/RetS may interact with the adaptive immune system to coordinate virulence. Thus far, it remains unclear whether calcium and LadS/RetS are capable of modulating CRISPR-Cas systems. RsmA, the downstream regulator of LadS/RetS, is a global post-transcriptional regulator through targeting 5' untranslated regions (UTR) or early coding regions of targeting mRNA motif (ANGGA). CRISPR mRNA blast in *P. aeruginosa* showed that a conserved RsmA binding site exists in the *cas1* mRNA 5' UTR, which may be worthwhile to study in the future.

Despite the function of CRISPR-Cas systems in adaptive immunity and its biotechnological application being well studied

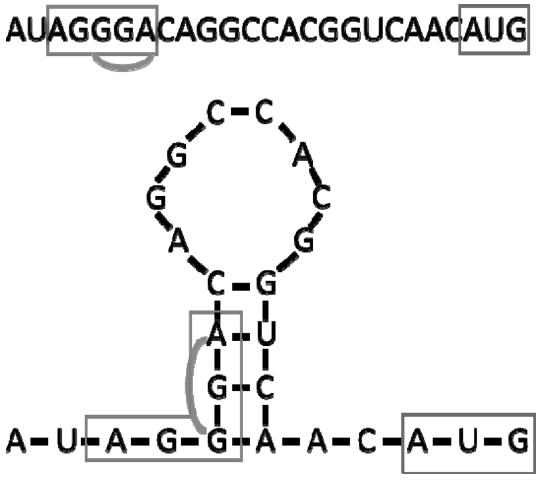

FIGURE 1.5 Predicted RsmA binding sites using RNA fold for *cas* promoter. GGA motif; long box, start codon; short box, predicted RBS (proteins primarily bind to the sequence motif A(N)GGA in single-stranded mRNA regions).

(Barrangou et al. 2007, Barrangou and Doudna 2016, Hsu, Lander, and Zhang 2014), limited research has discussed the regulation of CRISPR-Cas systems in bacteria (Patterson, Yevstigneyeva, and Fineran 2017). It is still unknown whether RsmA takes part in endogenous regulation of CRISPR. Furthermore, it remains to be discovered whether CRISPR function is influenced by environmental signals and calcium through a LadS/RetS/Gac/Rsm cascade. We speculate that the LadS/RetS/Gac/Rsm axis may also interact with or regulate CRISPR-Cas immunity.

1.9 Drug Targets

Disruption of small molecules (i.e., QS molecules) is considered as an antimicrobial strategy (Jakobsen et al. 2017). QS inhibitors are found in many herbal extracts such as Ajoene presenting in garlic. It is shown that, ajoene could block the production of rhamnolipid, which is regulated by QS. Ajoene helped polymorphonuclear neutrophils (PMNs) phagocytize biofilms more efficiently. Moreover, the biofilms were more susceptible to antibiotics, like tobramycin, showed milder pulmonary infection in mice treated with ajoene. Ajoene exerts its effect through modulation of RsmY and RsmZ (Jakobsen et al. 2017).

Cells in biofilm state are 1,000 times more resistant to antimicrobial therapy. By manipulating the c-di-GMP content of the cell and switching the bacteria to planktonic lifestyle, the susceptibility of the bacterial cells to antibiotics will increase (Valentini and Filloux 2016). Therefore, either direct regulating QS signaling or modulating biofilm may affect the clinical control of bacterial infection.

1.10 Concluding Remarks

Although it is known that LadS/RetS are essential for the regulation of *P. aeruginosa* infection status, its roles in sensing other exogenous signals and interacting with other endogenous signal pathways are still elusive. We are just a beginning to unravel the

detailed mechanisms of LadS/RetS. Several questions remain to be discovered: Is RetS responsible for detecting exogenous environmental signals? What is the structure mechanism of LadS/RetS in detecting calcium and signal transduction? Are there any other new co-factors that interact with LadS/RetS signal transduction? Are LadS/RetS capable of being served as novel drug targets for infection modulation? Are LadS/RetS taking part in modulation of CRSIPR system? If so, how do LadS/RetS and CRSIPR systems interact with each other? Are there other new exogenous signals involved in modulating CRSIPR system? Discovery of homologous calcium sensors in other bacteria as well as answering and dissecting those possible interconnections would enhance our understanding of the functioning mechanisms of this unique immunity system and bacterial virulence, meanwhile providing better tools to enable accurate gene-editing or transcription regulation.

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2

Autoinducer-1 Quorum Sensing Communication Mechanism in Gram-Negative Bacteria

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CONTENTS

2.1 Introduction

Bacteria have extraordinary ability to survive and grow in basically every niche in the environment. Microbial life strategies should consider stress tolerance such as high or low pH, temperature, osmolarity, nutrient availability, antimicrobials, and population density that lead to competitive relationships. In order to respond to changes in their immediate environment, bacterial cells must be able to alter the cellular pathways to survival or resume growth. The response and adaptation to diverse environmental conditions are related to reception and processing signals present outside their borders. This adaptation process is mainly mediated by a striking combination of transcriptional regulatory networks, which allow bacteria to sense and convert physical or chemical extracellular stimuli into a specific response that results in altered gene expression and enzyme activities. Whereas some of these alterations are reversible and disappear when the stress is over, others are maintained and can even be passed on to surviving bacteria.

The bacterial response could be related to individual cells, but in a community, bacteria are able to interact and regulate, in a coordinated way, their response to environmental changes through the sophisticated mechanism of cellular communication called quorum sensing (QS) (Bassler and Losick 2006; Fuqua, Winans, and Greenberg 1994). This signaling process allows communication between cells leading to differential gene expression in response to changes in population density and allows bacteria to act as a group.

The ability of bacteria to communicate and to present social interactions like a multi-cellular organism has provided significant benefits to bacterial populations in host colonization, formation of biofilm, defense against competitors, and adaptation to changing environments (Li and Tian 2012). Bacteria are not limited to communication within their own species but are capable of "listening in" and "broadcasting to" unrelated species to intercept messages and coerce cohabitants into behavioral modifications, either for the good of the population or for the benefit of one species over another (Atkinson and Williams 2009). The perception that bacteria are social organisms has produced new insights into bacterial physiology and gene regulation from the point of view of population and evolutionary biology (Goo et al. 2015).

The mechanism of QS is mediated by diffusible signaling molecules called autoinducers (AIs) synthesized throughout the growth of the bacteria and released into the surrounding medium. At low population densities, the production and secretion of QS signal molecules proceed at a basal level. As population density increases, the signal molecules accumulate above the threshold in the external environment and bind to and activate receptors inside bacterial cells, and collectively induce the expression of specific target genes to activate behaviors that are beneficial under the particular condition encountered. Therefore, one of the common and often observed consequences of the QS is gene regulation in the increased synthesis of the proteins

involved in the signaling molecule production. The higher the concentration of signaling molecules, the greater the signaling protein synthesis, which leads to positive feedback loop. This is the reason for the term autoinducers; the signaling molecule initiates the synthesis of the protein responsible for its own production (Geethanjali et al. 2019).

Over the years, since its introduction as a cell density dependent mechanism, the use of the term quorum sensing has evolved to become the general description of the signaling production process and response at the level of gene expression. However, many biotic and abiotic environmental factors can influence the chemical gradients of these signaling molecules. These factors include the spatial distribution of signaling molecule-producing cells, the rate at which the signaling molecule is produced and diffused, and the stability of the signaling molecule. This has led to the proposition of new terms, including diffusion sensing, confinement-induced QS, and efficiency sensing, to describe these genetic and biochemical processes (Platt and Fuqua 2010). These new names emphasize different adaptive functions of regulation by the QS mechanism, related to a specific subset of factors that influence the concentration of signal molecules in the environment. However, the use of a different term for each adaptive function may complicate the understanding of the QS process rather than clarify it. Thus, it is important to remember that the ecological context of QS regulation, as the process itself, is complex and influenced by multiple aspects of natural environments (Platt and Fuqua 2010).

The QS signaling molecules, also known as AIs, are chemically diverse, and many bacteria synthesize and utilize multiple signaling molecules from the same or different classes that constitute a regulatory hierarchy. Most signaling molecules are small organic molecules (<1000 Da) or small peptides with five to 20 amino acids in length (Williams 2007). Multiple QS signals have been identified in bacteria, and the most common ones are *N*-acyl-homoserine lactones (AHLs, AI-1) in Gram-negative bacteria, oligopeptides (AIPs) in Gram-positive bacteria, and furanosyl borate diester (AI-2) in both Gram-negative and Gram-positive bacteria. Other signals, such as auto-inducer-3 (AI-3), 2-heptyl-3-hydroxy-4(1*H*)-quinolone (*Pseudomonas* quinolone signal [PQS]) and its precursor 2-heptyl-4(1*H*)-hydroxyquinoline (HHQ), *cis*-11-methyl-2-dodecenoic acid (diffusible signal factor [DSF]) (LaSarre and Federle 2013), 3-hydroxypalmitic acid methyl ester (3-OH PAME), indole (Lee et al. 2015), diketopiperazines (DKP), and others have been detected in a limited number of bacteria or suggested as a signal molecule for bacterial communication. A number of other extracellular bacterial metabolites, including compounds with antibiotic activity, have the potential to function as signal molecules. However, it is important to differentiate between a true signal molecule involved in cell-to-cell communication and other metabolites.

AHLs are the most studied QS signaling molecules, also known as AI-1, and are produced by Gram-negative bacteria. The AHLs are neutral lipid molecules normally produced by proteins homologous to LuxI from the lactone fraction of S-adenosyl methionine (SAM) and, in most cases, the acyl chain is obtained from intermediates of the fatty acid biosynthesis pathway (Papenfort and Bassler 2016). The length of the acyl chain can range from four to 18 carbons and contains possibly a 3-oxo or 3-hydroxy function (Churchill and Chen 2011; Galloway et al. 2011; Yajima 2014). Short side-chain AHLs are directly released out of the cell upon synthesis while long side-chain AHLs are actively secreted to the environment (Liu et al. 2018). This diversity in the AHLs is recognized by different and compatible LuxR proteins promoting specificity to intraspecies-specific cell-cell communication in bacteria (Husain et al. 2019).

Although all known QS mechanisms differ in the regulatory components and molecular mechanisms, they are dependent on three basic principles: first, secretion of signaling molecules (AIs); second, detection of AIs by the receptors existing in the cytoplasm or in the membrane; and third, activation of gene expression necessary for cooperative behaviors (Figure 2.1).

Several bacterial phenotypes have been described as being controlled by the QS, among them bioluminescence, competence, biofilm, metabolism, cell differentiation, sporulation, surface motility, toxin production, expression of virulence genes, and others (Table 2.1). Therefore, the mechanism of communication by QS plays a critical role for survival and colonization both in symbiotic and pathogenic host-bacterial interactions. The use of QS to regulate processes associated with virulence increases the pathogen's prospects for survival, because the coordinated attack against the host will only be done when the bacterial population reaches high population density, increasing the probability of successfully overcoming host defenses.

While it is advantageous that the regulation of some phenotypes is quorum-dependent, the communication incurs a cost in terms of signal production; therefore, the communication has only been maintained throughout the evolution because this transfer of information gives benefits to both parties, signaling and receiving bacteria (Diggle et al. 2007; Keller and Surette 2006). The QS regulators LuxI and LuxR arose early in the evolution of the Proteobacteria and subsequently diverged within each group of organisms (Gray and Garey 2001). The construction of phylogenetic trees indicates that duplication and horizontal gene transfer have played an important role in the distribution of the system across bacterial species (Lerat and Moran 2004). The inducer/receptor elements in the LuxI/R systems evolved together and maintained their paired functional relationship, but loss and exchange of elements occurred in several γ-Proteobacteria lineages (Lerat and Moran 2004). In a systematic survey for LuxR QS-domains in sequenced bacterial genomes included in the InterPro database, it was identified that 40%–70% have a complete QS system depending on taxa, while the remaining species have only LuxR solos or orphans (Subramoni, Florez Salcedo, and Suarez-Moreno 2015). It is believed that in some bacteria belonging to the Enterobacteriaceae family, such as *Escherichia coli* and *Salmonella*, a deletion event has removed the *luxI* homolog after their divergence from *Pantoea* and *Erwinia* genera, leaving only the LuxR homolog known as SdiA (Sabag-Daigle and Ahmer 2012).

The mechanism of QS was first described in the regulation of bioluminescence in *Vibrio fischeri* (Nealson, Platt, and Hastings 1970), now *Aliivibrio fischeri*, a marine bacterium found in a Hawaiian squid known for its striking bioluminescence. The luciferase operon in *A. fischeri* is regulated by two proteins, LuxI, responsible for the production of the AHL, and LuxR protein, which is activated by this auto-inducer to increase the transcription of the luciferase operon (Engebrecht and Silverman 1984).

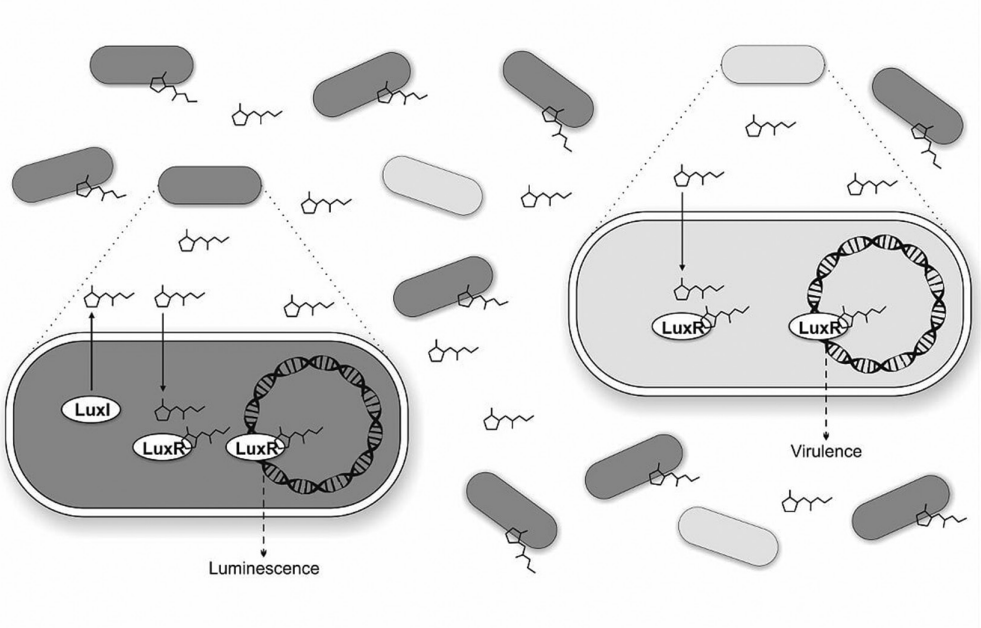

FIGURE 2.1 LuxIR signaling circuit. The LuxIR QS circuit, represented in dark gray cells, is composed of an AHL synthase LuxI that synthesizes the AIs which are exported to the exterior of the cell. When these AIs reach a threshold concentration, they are internalized, bind to the response regulatory protein LuxR and bind to the DNA regulating the expression of target genes. Some bacteria (light gray) lack the LuxI protein and do not synthesize AHL. However, they possess the LuxR homologue and are able to recognize and respond to molecules produced by other bacteria.

TABLE 2.1

Phenotypes Regulated by Auto-Inducer-1 Quorum Sensing Mechanisms

Bacteria	Phenotype	Regulation[a]	References
Bacteria with complete auto-inducer-1 quorum sensing mechanisms			
A. fischeri MAV	Bioluminescence	+	Nealson et al. (1970)
A. fischeri B-61	Bioluminescence	+	Eberhard et al. (1981)
A. fischeri MJ-1	Bioluminescence	+	Engebrecht and Silverman (1984)
A. fischeri ES114	Bioluminescence	+	Lupp et al. (2003)
	Persistence in squid	+	
	Motility	−	Lupp and Ruby (2005)
A. salmonicida LFI1238	Biofilm formation in polystyrene	−	Hansen et al. (2015)
V. cholerae O1	Resuscitation of cell in the viable but non-cultivable state	+	Bari et al. (2013)
V. harveyi BB120	Bioluminescence	+	Henke and Bassler (2004)
	Type III secretion system	+	
	Metalloprotease production	+	
P. aeruginosa PAO1	Elastase production	+	Jones et al. (1993)
	Elastase production	+	Brint and Ohman (1995)
	Pyocyanin production	+	
	Protease production	+	
	Rhamnolipid production	+	
	Rhamnolipid production	+	Ochsner and Reiser (1995)
	Lung infection	+	Wu et al. (2001)
P. fluorescens B52	Biofilm formation in glass	+	Allison et al. (1998)
P. fluorescens NCIMB 10586	Mupirocin biosynthesis	+	El-sayed, Hothersall, and Thomas (2001)
P. fluorescens 395	Protease production	+	Liu, Wang, and Griffiths (2007)

(*Continued*)

TABLE 2.1 (*Continued*)

Phenotypes Regulated by Auto-Inducer-1 Quorum Sensing Mechanisms

Bacteria	Phenotype	Regulation[a]	References
P. fluorescens 07A	Growth	Ø	Pinto et al. (2010)
	Proteolytic activity	Ø	
P. putida IsoF	Biofilm formation	+/Ø	Steidle et al. (2002)
P. syringae B728a	Epiphytic fitness	+	Quiñones, Pujol, and Lindow (2004)
B. cepacia H111	Swarming motility	+	Huber et al. (2001)
	Biofilm formation	+	
B. glumae BGR1	Production of excreted oxalate	+	Goo et al. (2012)
B. thailandensis E264	Production of excreted oxalate	+	Goo et al. (2012)
A. hydrophila SSU	Type VI secretion system	+	Khajanchi et al. (2009)
	Metalloprotease production	+	
	Biofilm formation	+	
A. hydrophila ATCC 7966	Proteolytic activity on casein	+	Ponce-Rossi et al. (2016)
	Proteolytic activity on gelatin	+	
	Amylolytic activity	+	
	Lipolytic activity	+	
	β-hemolytic activity	+	
	Biofilm formation on stainless steel	+	
C. violaceum ATCC 31532	Violacein production	+/–	McClean et al. (1997), Chen et al. (2011)
	Chitinase production	+	Chernin et al. (1998)
	Swarming motility	+	Oca-Mejía et al. (2014)
	Cell aggregation	+	
	Biofilm formation in glass	+	
	Oxidative stress resistance	+	
	Exoprotease production	+	
C. violaceum ATCC 12472	Violacein production	+/–	Morohoshi et al. (2008), Chen et al. (2011)
	Chitinase production	+	Liu et al. (2013)
	Biofilm formation in glass	+	
S. liquefaciens MG1	Surfactant production	+	Lindum et al. (1998)
	Growth	Ø	Givskov et al. (1998)
	Cell elongation	Ø	
	Cell flagellation	Ø	
	Swarming motility	+	
S. plymuthica RVH1	Nuclease production	+	Van Houdt, Givskov, and Michiels (2007)
	Chitinase production	+	
	Protease production	+	
	Butanediol fermentation	+	
S. proteamaculans B5a	Lipolytic activity	+	Christensen et al. (2003)
	Proteolytic activity	+	
	Chitinolytic activity	+	
Serratia sp. ATCC 39006	Carbapenem antibiotic production	+	Thomsonet al. (2000)
	Prodigiosin pigment production	+	
A. tumefaciens	Ti plasmid copy number	+	Cho, Pinto, and Winans (2009)
	Ti plasmid conjugation	+	
	Tumorigenesis in plants	+	

(*Continued*)

TABLE 2.1 (*Continued*)

Phenotypes Regulated by Auto-Inducer-1 Quorum Sensing Mechanisms

Bacteria	Phenotype	Regulation[a]	References
P. carotovorum SCRI193	Elastase production	+	Jones et al. (1993)
P. carotovorum EC153	PCWDE production	+	Chatterjee et al. (2005)
	Virulence	+	
Bacteria with incomplete auto-inducer-1 quorum sensing mechanisms			
E. coli	Cell division	+	Ahmer et al. (1998)
E. coli MG1655	Growth	Ø	Van Houdt et al. (2006)
	Acid resistance	+	
E. coli K-12	Biofilm formation in polystyrene	–	Lee et al. (2007)
	Acid resistance	+	
	Quinolones resistance	+/Ø	Dyszel et al. (2010)
	Acid resistance	+/Ø	
	Cell division	+/Ø	
EHEC	Calve fitness	+	Dziva et al. (2004), Hughes et al. (2010)
	Quinolones resistance	+/Ø	Dyszel et al. (2010)
	Acid resistance	+/Ø	
	Cell division	+/Ø	
	Adherence to HEp-2 cells *in vitro*	–	Sharma and Bearson (2013)
aEPEC ONT:H25	Biofilm formation	–	Culler et al. (2018)
	Motility	–	Culler et al. (2018)
Salmonella Typhimurium ATCC 14028	Cell division	Ø	Ahmer et al. (1998)
	Invasion to HEp-2 cells *in vitro*	+	Nesse et al. (2011)
Salmonella Enteritidis PT4 578	Growth	Ø	Campos-Galvão et al. (2016)
	Biofilm formation in polystyrene	+	
	Growth	Ø	Almeida et al. (2017b)
	Initial adhesion in polystyrene	Ø	
	Biofilm formation in polystyrene	+	
	Swarming motility	Ø	
	Twitching motility	Ø	
	Level of thiol	+	Almeida et al. (2018)
Salmonella Typhi ST_8	Adherence to HeLa cells *in vitro*	+	Liu et al. (2014)
	Biofilm formation in polystyrene	+	
	Survival in rabbit serum *in vitro*	+	
	Survival in guinea pig serum *in vitro*	+	

[a] Positive = +, Negative = –, no effect = Ø.

PCWDE = plant cell wall-degrading enzymes.

Different homologues of the LuxI-LuxR mechanism have been identified in other bacteria and, in all these mechanisms, its components have been observed to be performing the same functions such that the AHLs are the AIs that bind to the LuxR-type proteins that regulate different phenotypic characteristics. Although this mechanism of communication was considered exclusive to some marine vibrios for many years, the presence of homologues of a complete QS (*luxI*/*luxR*) mechanism has also been demonstrated in many Gram-negative bacteria capable of producing specific AHLs, including *Agrobacterium*, *Aeromonas*, *Acinetobacter*, *Brucella*, *Burkholderia*, *Chromobacterium*, *Citrobacter*, *Enterobacter*, *Erwinia*, *Hafnia*, *Nitrosomonas*,

Obesumbacterium, *Pantoea*, *Pseudomonas*, *Rahnella*, *Ralstonia*, *Rhodobacter*, *Rhizobium*, *Serratia*, *Vibrio*, and *Yersinia* (Smith and Iglewski 2003).

2.2 Bacteria with Complete Auto-Inducer-1 Quorum Sensing Mechanism

2.2.1 QS in *Aliivibrio* and *Vibrio*

Aliivibrio and *Vibrio* are moderately halophilic bacteria, inhabitants of marine and estuarine environments, and several species are related to infections in humans and in animals reared in aquaculture (Urbanczyk et al. 2007). The cell signaling system dependent of AI-1 in marine *Aliivibrio* and *Vibrio* comprises a LuxIR-type, although they received different denominations, such as LuxMN in *V. harveyi* (Cao and Meighen 1989), LuxIR in *A. fischeri* (Liu et al. 2018), and VanIR in *Vibrio anguillarum* (Milton et al. 2001). Different AHLs have been noted among the various strains of the same species. Based on current literature, a total of 32 AHLs-producing marine *Aliivibrio* and *Vibrio* species have already been identified and 23 different AHLs were definitely classified, including 10 short side-chain and 13 long side-chain AHLs (Liu et al. 2018). Marine species of *Aliivibrio* and *Vibrio* produce many types of long side-chain AHLs, such as C14-HSL (Girard et al. 2017) and, different from those found in terrestrial bacteria, AHLs such as C7-HSL, 3-OH-C9-HSL, 3-oxo-C9-HSL, 3-OH-C11-HSL, and 3-oxo-C11-HSL are also detected (Rasmussen et al. 2014).

The mechanism of cell communication based on the LuxIR system of *A. fischeri* is the paradigm of Gram-negative QS systems; however, it is not found in all vibrios. In luminescent *V. harveyi*, QS positively regulates phenotypes such as bioluminescence (Freeman and Bassler 1999), metalloprotease, siderophore, and exopolysaccharide production (Henke and Bassler 2004). In *V. anguillarum*, AHLs regulate biofilm formation, metalloprotease, and siderophore production (Milton 2006). AHLs also participate in the regulation of marine *Vibrio* pathogenicity via other virulence-related proteins. For example, ToxR, a classic *Vibrio* virulence factor encoded by the virulence-related gene *toxR*, is directly regulated by AHLs. ToxR was first discovered in *V. cholerae*, and subsequent studies showed that homologous genes of *toxR* also exist in many pathogenic *Vibrio* species such as *V. parahaemolyticus, V. vulnificus*, and *V. alginolyticus* (Liu et al. 2018).

In 2004, the regulation in QS system by sRNAsQrr (quorum regulatory RNAs) was identified in *Vibrio* and was the first demonstration of a role for sRNAs in QS in Gram-negative bacteria (Lenz et al., 2004). The sRNAsQrr are classified as *trans*-sRNAs and act to activate or repress translation of target mRNAs via unique base-pairing to the 5′ UTR in conjunction with the RNA chaperone Hfq (Shao and Bassler, 2012).

In low population density, LuxO is phosphorylated and together with the σ^{54} activates the expression of sRNAsQrr (Lilley and Bassler 2000; Waters and Bassler 2006). Five QrrsRNAs were identified and characterized in *V. harveyi* and, with Hfq, they strongly repress translation of the master QS regulator LuxR by occupying the ribosome binding site or mediating degradation of the *luxR* mRNA (Bejerano-Sagie and Xavier 2007; Feng et al. 2015; Tu and Bassler 2007). Without the bound LuxR protein, the *lux* operon is not expressed, resulting in inhibition of luminescence (Miyamoto et al. 1996). The regulation by sRNA is important because it provides a fine-tuning to the bioluminescence mechanism due to the highly dynamic nature of sRNAs (Rutherford et al. 2015). The regulation of AHL-QS by sRNAs has also been described in *Sinorhizobium meliloti* and *P. aeruginosa* (Gao et al. 2015; Malgaonkar and Nair 2019).

2.2.2 QS in *Pseudomonas*

Members of the family Pseudomonaceae are ubiquitous Gram-negative bacteria, comprising many genera and several hundred species. Most species have great metabolic and physiological versatility, which explains their presence in several environmental niches, including soil and fresh water, and have the ability to undergo transitions to become important and dangerous pathogens. Some species cause disease in plants, like *Pseudomonas syringae*, and a few cause serious diseases in humans, as in the case of *Pseudomonas aeruginosa* (Venturi 2006).

Several *Pseudomonas* produce AIs such as AHL by QS system, which control important functions including pathogenicity, biofilm formation, and production of a variety of extracellular metabolites and enzymes. The most extensive studies on QS have been performed on *P. aeruginosa*, making this bacterium a well-known study model. Additionally, many AHL QS systems, as well the involved genes, have been reported in other species such as *P. aureofaciens*, *P. chlororaphis*, *P. putida*, *P. fluorescens*, and *P. syringae*, demonstrating the comprehensiveness of QS in the genus (Chen et al. 2019; Venturi 2006; Martins et al. 2014; Pang et al. 2019; Barbarossa et al. 2010).

Pseudomonas aeruginosa is a nonspore-forming bacterium that presents motility through a single polar flagellum, and it has a bacillar shape. It is an opportunist pathogen that causes severe infections and diseases in both plants and animals, and is a problematic human pathogen since it causes serious infections mainly in hospitalized and immunocompromised individuals, such as those with cancer or AIDS (Azam and Khan 2019; Schütz and Empting 2018; Lee and Zhang 2014). Some infections caused by *P. aeruginosa* are hospital-acquired infections (HAIs), hospital-acquired pneumonia (HAP), and gastrointestinal, bone, and skin infections, besides representing a major cause of morbidity and mortality in burn patients and those with cystic fibrosis (CF) (Azam and Khan 2019; Schütz and Empting 2018; Pang et al. 2019). The bacterial genome is relatively large, which provides metabolic versatility and high adaptability to environmental changes (Pang et al. 2019). This may explain the variety of virulence mechanisms employed during *P. aeruginosa* infections, like the ability to form a biofilm matrix, motility, iron scavenging, and cytotoxicity capabilities, and many of these virulence factors are regulated by QS (Lee and Zhang 2014; Schütz and Empting 2018). In addition, the infections may be aggravated by the intrinsic and acquired antibiotic resistance of this pathogen, including formation of multidrug-tolerant persister cells in biofilm settings which generate chronic diseases that cannot be eradicated with antibiotic treatment (D'Angelo et al. 2018; Pang et al. 2019). *P. aeruginosa* became the main model in anti-virulence strategy studies for multiple reasons: first, the

TABLE 2.2

QS Mechanisms in *P. aeruginosa*

Autoinducer (AI)	AI Abbreviation	AI Synthesis	AI Receptor Protein	References
N-(3-oxododecanoyl)-homoserine lactone	OdDHL (3-oxo C12-HSL)	LasI[a]	LasR[b]	Pearson et al. (1994)
N-butyrylhomoserine lactone	BHL (C4-HSL)	RhlI[1]	RhlR[b]	Ochsner and Reiser (1995), Pearson et al. (1995)
2-heptyl-3-hydroxy-4-quinolone/2-heptyl-4-hydroxyquinoline	PQS/HHQ	PqsABCDE	MvfR (PqsR)[c]	Pesci et al. (1999), D'Angelo et al. (2018)
2-(2-hydroxyphenyl)-thiazole-4-carbaldehyde	IQS	AmbBCDE	IqsR	Lee et al. (2013)

[a] LuxI homologue.
[b] LuxR homologue, based in LuxI-LuxR QS mechanism of *A. fisheri*. Source: Lee and Zhang (2014).
[c] MvfR "Multiple virulence factor Regulator" and PqsR are the same protein that historically have been named differently by several research groups.

QS network is well characterized; second, QS regulates the expression of multiple virulence-related factors; and third, this bacterium is important to the medical community due to antibiotic resistance (LaSarre and Federle 2013).

In *P. aeruginosa*, a complex QS network consisting of four interconnected systems, i.e., *las*, *rhl*, *pqs*, and *iqs* is found (Table 2.2). These systems collectively control group behaviors and the expression of virulence determinants, such as proteolytic and lipolytic enzymes, swarming motility, toxin production, tolerance to stress, and biofilm formation (D'Angelo et al. 2018; Turan et al. 2017; Choudhary and Schmidt-Dannert 2010; Azam and Khan 2019; Quecan et al. 2019).

Two different QS LuxI-LuxR mechanisms exist in *P. aeruginosa*: LasI-LasR and RhlI-RhR (Table 2.2). LasI is a synthase that produces an extracellularly diffusible AHL signal molecule called *N*-(3-oxododecanoyl)-L-homoserine lactone (3-oxo-C12-HSL), which is recognized by the transcriptional regulator LasR that directs the expression of various genes. Likewise, RhlI produces the *N*-butyryl-L-homoserine lactone (C4-HSL) signaling molecule that can bind to its Rh1R cognate transcription regulator. The transcriptional regulators LasR and RhlR are activated when sufficient levels of 3-oxo-C12-HSL and C4-HSL are present as a result of the high population density, and regulate the production of multiple virulence factors (Smith and Iglewski 2003).

The QS cascade in *P. aeruginosa* is organized in a hierarchical way, starting with the *las* QS system. More recently, a circular model, in which multiple feedback loops control QS gene expression, has been proposed by the Rahme's group (Maura et al. 2016). Activation of the *las*QS system (LasR-OdDHL) stimulates the transcription of the other QS systems, *rhl*, *pqs*, and *iqs* (Lee and Zhang 2014). The *rhl* system is under control of *las* and *pqs*, and several virulence factors regulated by QS are predominantly activated by the RhlR-BHL complex, demonstrating its importance for the bacterium. On the other hand, RhlR represses PQS signal production by interfering with *mvfR* (*pqsR*) and *pqsABCDE* expression. Thus, a reduced activity of *pqs* QS system may be due to a negative effect of *rhl* QS system (D'Angelo et al. 2018). MvfR autoinducers, PQS, and its precursor HHQ can bind and activate the transcription of the MvfR (PqsR) regulon (Schütz and Empting 2018). However, PQS has additional functions, such as iron chelation, and it is 100 times more potent than HHQ. The *iqs*QS system has been recently identified (Lee et al. 2013) as strictly dependent on LasI-LasR under rich media conditions, and disruption of LasI or LasR completely abolishes *ambBCDE* expression and the IQS production (Lee and Zhang 2014; D'Angelo et al. 2018; Schütz and Empting 2018).

The necessity to develop new strategies to combat infections caused by *P. aeruginosa* is urgent. This concern was highlighted in a recent World Health Organization report in which this pathogen was classified into the most critical group (priority 1) for which new antibiotics are urgently needed, due to antibiotic resistance (D'Angelo et al. 2018; WHO 2017). Thus, the understanding of the QS inhibition mechanism by gene expression may be the key to develop this new treatment generation.

Differently from *P. aeruginosa*, *P. fluorescens* is not generally known as a pathogen in humans. It is especially considered for its role in soil and the rhizosphere as well as in food spoilage, especially refrigerated raw products. However, it does possess functional traits that provide it with the capability to grow in mammalian hosts (Scales et al. 2014).

P. fluorescens is reported to have a significant ability to form biofilms and is one of the most important spoilage bacteria of refrigerated foods. Both traits can be regulated by QS (Zhang et al. 2019). In addition, biofilm formation is a serious problem for the food industry. The structure formation can be accelerated by the bacterium's ability to use swarming motility to colonize nutrient-rich environments, facilitating colony spreading. There is little information about *P. fluorescens* biofilm formation on mammalian surfaces, but, whether in humans or plant cells, this structure is very important for successful long-term colonization (Scales et al. 2014; Martins et al. 2014).

Two QS systems have been described for some strains of this species. First, a LuxI-LuxR homologue pair was discovered in *P. fluorescens* NCIMB 10586 and was termed *mupI-mupR* system due to its regulation of mupirocin (pseudomonic acid) biosynthesis, an important and potent polyketide antibiotic (El-sayed, Hothersall, and Thomas 2001; Scales et al. 2014). Second, the *hdtS* system, a new class of AHL synthase, was discovered in *P. fluorescens* F113. The HdtS enzyme synthesizes at least three signaling molecules: (i) *N*-(3-hydroxy-7-*cis*-tetradecenoyl) homoserine lactone (3-OH-C14:1-HSL), (ii) *N*-decanoylhomoserine lactone (C10-HSL), and (iii) *N*-hexanoylhomoserine lactone (C6-HSL). HdtS is not a member of the LuxI family and received this name since it directs the synthesis of AHLs with acyl side

chains of six (hexa-), ten (deca-) and fourteen (tetradeca-) carbons in length (Laue et al. 2000).

Among Gram-negative psychotrophic bacteria, *P. fluorescens* constitutes the major raw milk deteriorative species due to proteolysis and lipolysis. There is some speculation whether the secretion of these enzymes is regulated by AHLs. There have been studies showing that protease and lipase production by milk isolates of *P. fluorescens* is not regulated by AI-1 type QS system (Pinto et al. 2010; Martins et al. 2014). On the other hand, other works have shown AI-1 QS regulation in *P. fluorescens* strains isolated from milk and fish (Tang Rong et al. 2019; Liu, Wang, and Griffiths 2007). Considering the genetic versatility of the species, it is likely that there are different regulatory systems controlling the production of these enzymes, depending on the strain.

Pseudomonas putida has importance at the ecological level, since it promotes the growth of plants, as well as inhibits plant pathogens and contributes to the degradation of toxic organic compounds (Barbarossa et al. 2010). In this microorganism, QS is mediated by AHL autoinducer molecules. Given that a large proportion of root-colonizing bacteria produces AIs, these interactions appear to be important in controlling many populations within the rhizosphere community, *P. putida* being a highly attractive candidate for agricultural and environmental uses (Steidle et al. 2002). Examples of QS systems homologous to the LuxI-LuxR are present in this bacterium (Barbarossa et al. 2010). This is the case for PhzR-PhzI, which utilizes *N*-hexanoyl-homoserine lactone (C6-HSL) to control the synthesis of phenazine antibiotics (Wood et al. 1997), and the PpuI-PpuR in *P. putida* IsoF, which uses at least four kinds of AHLs to influence biofilm structural development (Steidle et al. 2002).

Pseudomonas syringae is a bacterium studied mainly for its role as a plant pathogen, and it has the capability to control virulence through the QS signaling system (Pérez-Velázquez et al. 2015; Venturi 2006). This microorganism possesses a LuxI-LuxR homolog pair, called AhlI-AhlR. The genes *ahlI* and *ahlR* (Quiñones, Pujol, and Lindow 2004), also called *psyI* and *psyR* (Nakatsu et al. 2019; Ichinose et al. 2016), are responsible for encoding AHL protein synthase and the AHL transcriptional factor. The molecules *N*-(3-oxo-hexanoly)-L-homoserine lactone (OHHL) and *N*-hexanoly-L-homoserine lactone (HHL) are the main AIs detected in this bacterium (Nakatsu et al. 2019; Quiñones, Pujol, and Lindow 2004). For instance, *P. syringae* pv. *syringae* strain B728a is a plant pathogen that causes brown spots in beans and produces and responds to 3-oxo-C6-HSL in a cell-density dependent manner (Quiñones, Pujol, and Lindow 2004; Venturi 2006). However, this system is not generalized in *P. syringae*, since some isolates such as *P. syringae* pv. *tomato* DV3000 do not produce AHLs (Nakatsu et al. 2019). The authors suggest that ancestors of *P. syringae* had produced AHL, but the production might have become inconvenient for successful infection, and then, most strains have lost the signal production through mutations in *psyI* or *psyR* genes (Nakatsu et al. 2019).

2.2.3 QS in *Chromobacterium violaceum*

Another example of a Gram-negative bacteria with a complete QS mechanism is *C. violaceum*, an opportunistic human pathogen that can cause fatal sepsis, skin lesions, and liver and lung abscesses in immunocompromised individuals (De Lamo Marin et al. 2007; Jitmuang 2008; Yang and Li 2011). The *C. violaceum* QS consists of the LuxI/LuxR homologues called CviI/CviR, which produce and respond to AHLs of different acyl lengths (McClean et al. 1997). This bacterium produces violacein, a water-insoluble purple pigment with antibacterial activity which is synthesized from tryptophan by the products of the *vioABCD* operon (August et al. 2000). The production of violacein is regulated by QS and is the most well-studied phenotype in *C. violaceum* (McClean et al. 1997; Stauff and Bessler 2011). For this reason, and because it is an easily observable phenotype, *C. violaceum* wild type and mutants, with interruptions in the QS mechanism, have been used as tools in several studies related to bacterial communication mechanisms as well as inhibition of the mechanism of QS (QSI) by natural and synthetic products (Adonizio et al. 2006; Steindler and Venturi 2007). An example of a biomonitor commonly used is *C. violaceum* CV026, a mutant strain derived from wild type *C. violaceum* ATCC 31532, which is unable to produce AHL but retains the ability to respond to exogenous AHLs (McClean et al. 1997).

Other phenotypes studied in *C. violaceum* that are under the regulation of QS include biofilm formation, cell aggregation, chitinase production, and exoprotease production (Oca-Mejía et al. 2014; Chernin et al. 1998).

2.2.4 QS in *Aeromonas hydrophila*

Aeromonas hydrophila is a Gram-negative opportunistic pathogen that is capable of infecting a wide variety of hosts, which include terrestrial and aquatic animals in addition to humans. Its pathogenicity typically includes minor skin infections or gastroenteritis in humans. Furthermore, *A. hydrophila* is present in raw milk and is an important spoilage bacterium due to its ability to grow and to present proteolytic activity in chilled foods. *A. hydrophila* has the homologous *luxRI* genes termed *ahyRI*, and the QS mechanism is mediated by C4-HSL and C6-HSL. Phenotypes regulated by QS in *A. hydrophila* included biofilm formation (Ponce-Rossi et al. 2016), proteolytic activity related with serine protease and metalloprotease (Khajanchi et al. 2009; Martins et al. 2018; Ponce-Rossi et al. 2016; Swift et al. 1999), and virulence (Khajanchi et al. 2009).

The effect of QS on the virulence of *A. hydrophila* was demonstrated using mutants in *ahyI* and, or *ahyR* genes and therefore incapable of synthesizing and/or detecting AHLs. A double mutant *ΔahyRI* of *A. hydrophila* SSU presented reduced protease production, less biofilm formation, and attenuation of virulence in mice (Khajanchi et al. 2009). Mutants of *A. hydrophila* AH-1N in *ahyI* or *ahyR* genes were used to challenge burbot (*Lotalota*) and resulted in higher survival of larvae when compared to challenge with the wild type (Natrah et al. 2012). The addition of the signal molecule C4-HSL restored the virulence of the QS mutant. These results with mutants *A. hydrophila* in QS are examples of models for studying this mechanism applied to other bacterial genera and also to elucidate when and how cellular communication is involved in the regulation of important phenotypes in pathogenesis. This knowledge can be useful in the development of specific and promising strategies to block this communication for the control of pathogens.

2.2.5 QS in *Serratia*

Species of *Serratia* are widely dispersed in the environment, including soil, water, plant surfaces, and the gastrointestinal tract of several animals, including humans. Many species are part of the spoilage microbiota of diverse foods, and some have been related to outbreaks and opportunistic infections (Doulgeraki et al. 2012; Mahlen 2011). A range of AHLs and genes for production and regulation have been described in *Serratia*, and four systems have already been studied: SmaI/SmaR in *Serratia* sp. ATCC 39006, SwrI/SwrR in *S. marcescens* MG1, SpII/SpIR in *S. plymuthica*, and SprI/SprR in *S. proteamaculans* (Van Houdt, Givskov, and Michiels 2007). Not all *Serratia* species have a homologous LuxIR system and produce AHL. In addition, there is considerable strain-dependent variation in both the ability to synthesize AHLs and in the nature of the AHL produced (Wei and Lai 2006).

Several phenotypes have been described as being regulated by QS in *Serratia*, such as virulence, biofilm formation and sloughing, butanediol fermentation, biosynthesis of antibiotic, and production of lipase, protease, chitinase, and the prodigiosin pigment (Christensen et al. 2003; Rice et al. 2005; Thomsonet al. 2000; Van Houdt et al. 2006). In *S. marcescens* strain SS-1, the QS system SpnIR is located in a mobile transposon, and that means lateral gene transfer may play an important role in the transfer of QS units between different bacterial genera and species (Wei et al. 2006). This may have significant implications for the diversity of the mechanism, and the acquisition of such a mobile QS system may allow the bacterium to bypass a possible specific disruption of their native QS system as long as the new signal-receptor complex is capable of activating target gene expression (Defoirdt, Boon, and Bossier, 2010).

2.2.6 QS in *Burkholderia*

The genus *Burkholderia* is very diverse and contains more than 30 species that occupy different niches, having agricultural, biotechnological, and clinical importance (Coenye and Vandamme 2003). All species of *Burkholderia* investigated encode at least one QS system that relies on AHL signal molecules for coordinated gene expression and is usually referred to as a CepI/CepR system (Eberl 2006). This system is present in the collectively called *Burkholderia cepacia* (Bcc) complex, which includes at least nine species recognized as problematic opportunistic pathogens in patients with cystic fibrosis and in immunocompromised individuals (Mahenthiralingam, Urban, and Goldberg 2005).

The CepI/CepR system positively regulates different functions, such as the production of exoproteases, siderophores, swarming motility, and biofilm production, besides contributing to the virulence of *Bcc* complex (Venturi et al. 2004). The detection of AHL in sputum and mucopurulent respiratory secretions in patients with cystic fibrosis provides clinical evidence of the occurrence of the mechanism during infection (Chambers et al. 2005; Middleton et al. 2002). In addition, the analysis of sequential strains of cystic fibrosis patients, obtained several years apart, indicates that the QS genes are maintained and expressed during chronic infections (McKeon et al. 2011).

A more complex QS system than in other *Burkholderia* species with more than one LuxI/LuxR homologue and numerous AHL-signaling molecules was described in the *Bptm* group, which includes the non-pathogenic soil saprophyte *Burkholderia thailandensis* and the pathogens *Burkholderia pseudomallei* and *Burkholderia mallei*, the causative agents of melioidosis and glanders, respectively (Breck et al. 2009; Ulrich 2004; Ulrich et al. 2004). In *B. thailandensis* and *B. pseudomallei*, AHL QS systems are described as QS-1, QS-2, and QS-3 made up of AHL synthase/AHL receptor pairs BpsI1/BpsR1, BpsI2/BpsR2, and BpsI3/BpsR3 respectively, besides two additional solo AHL receptors (R4 and R5).

As in the *Bcc* complex, the QS mechanism is also related to virulence, biofilm formation, and production of biomolecules that provide fitness advantages in the *Bptm* group (Mott, Panchal, and Rajamani 2017). Furthermore, recent findings about the mechanisms of QS have drawn attention because of their influence on the regulation of physiology and microbial metabolism, in order to provide strategies for competitiveness and at the same time perpetuating the species through cooperative behaviors (Abisado et al. 2018; Majerczyk et al. 2014a, 2014b). In *B. pseudomallei* and *B. thailandensis*, QS induces the production and excretion of oxalate in the stationary phase, which becomes a shared resource with the whole population and protects cells from self-intoxication and killing as a result of ammonia production (Goo et al. 2012).

2.2.7 QS in Gram-Negative Phytopathogens

Phytopathogenic bacteria benefit from QS mechanisms to control gene expression related to virulence and colonization of hosts (Von Bodman, Bauer, and Coplin 2003). Important phytopathogens such as *Agrobacterium tumefaciens*, *Pantoea stewartia*, *Pectobacterium carotovora*, *P. syringae*, *P. aeruginosa*, *Ralstonia solanacearum*, *Xanthomonas campestres*, among others use QS to fine tune plant-microbe interactions. These pathogens have developed abilities to colonize the ryzosphere or aerial surfaces of plants in order to circumvent plant defenses and cause diseases by using a plethora of virulence factors.

Agrobacterium tumefaciens is an alpha-proteobacterium that belongs to the Rhizobiaceae family, which includes plant pathogens and nitrogen-fixing microbes (Slater et al. 2009; Wood et al. 2001). The bacterium is found in soil and can cause crown gall disease in dicotyledonous plants at wounded sites (Winans 1992). The illness is usually non-fatal and is characterized by the growth of tumors which can reduce crop productivity (Escobar and Dandekar 2003).

The tumor inducing principle is as a piece of DNA that is transferred from the bacteria to the plant cells and is linked to the presence of the so-called tumor inducing plasmid (Ti plasmid) (Chilton et al. 1977). Research with Ti plasmids has impacted many different fields including plant biology, agriculture, biotechnology, and molecular biology (Binns 2002; Escobar and Dandekar 2003).

The Ti-plasmid is a large circular replicon that carries the transferred DNA (also known as transforming or T-DNA) and most genes required for tumorigenesis (Pinto, Pappas, and Winans 2012; White and Winans 2007). The T-DNA carries a set of genes responsible for plant cell proliferation and another

set of genes required for the synthesis of opines which support bacterial growth (Zhu et al. 2000). The Ti-plasmid also codes for the transport and catabolism of opines produced in the tumors. In fact, Ti-plasmids are usually classified according to the type of opines that are encoded in the T-DNA.

Infection starts when bacterial cells containing the Ti plasmid encounter a plant wounded site, which releases compounds such as amino acids, organic acids, and sugars that activate the transfer of the T-DNA from bacterial to plant cells. Once the T-DNA is transported to the nucleus, it can integrate into the plant genome and initiate expression of the tumor inducing and opine synthase genes (Escobar and Dandekar 2003; Pappas 2008; Zhu et al. 2000).

Interestingly, *luxI* and *luxR* homologues known as *traI* and *traR* are found within the Ti plasmid and regulate plasmid copy number, Ti plasmid conjugation, and entry exclusion as well as increased tumorigenesis in plants infected with bacteria containing these plasmids (Cho, Pinto, and Winans 2009; Fuqua and Winans 1994; Pinto, Pappas, and Winans 2012). The crystal structure of TraR complexed with 3-oxo-octanoyl-L-homoserine lactone bound to the *tra* box DNA has been solved by two groups (Zhang et al. 2002; Vannini et al. 2002). The protein binds DNA as a dimer and both the N-terminal and C-terminal domains contribute to protein dimerization (Pinto and Winans 2009). Several studies have further confirmed and extended the structural predictions, broadening the understanding of TraR transcription activation and making it a pivotal model for the LuxR family of transcriptional regulators.

Pectobacterium (previously classified as *Erwinia*) is a genus of Gram-negative phytopathogenic bacteria that belongs to the Enterobacteriaceae family (Davidsson et al. 2013). These bacteria can cause soft rot and degradation of plant cell wall polysaccharides in commercially important plants such as those destined for food (especially crop potatoes) and for ornamental purposes (Joshi et al. 2016; Park et al. 2012). In fact, pectobacteria encode for a large number of plant cell wall-degrading enzymes (PCWDEs) which are under control of QS (Barnard et al. 2007). The PCWDEs are usually cellulases, hemicellulases, pectinases, and proteinases, mainly secreted by type II secretion systems (Davidsson et al. 2013).

The three main species that can cause soft rot are *P. carotovorum*, *P. atrosepticum*, and *P. parmentieri*. The species *P. carotovorum* is further divided into *P. carotovorum* subsp. *brasiliense* (*Pcb*), *P. carotovorum* subsp. *carotovorum* (*Pcc*), and *P. carotovorum* subsp. *odoriferum* (*Pco*) (Li et al. 2018). One *luxI* homologue and two or more *luxR* homologues are found in these organisms. Signaling in *Pectobacterium* is usually mediated by 3-oxo-hexanoyl homoserine lactone (3OC6HSL), 3-oxo-octanoyil homoserine lactone (3OC8HSL), and autoinducer-2(AI-2), which regulate the expression of PCWDEs, contributing to the soft rot phenotype (Põllumaa, Alamäe, and Mäe 2012).

Signaling molecules vary according to the subspecies and the strain type, as well as the *luxI/luxR* pair. For instance, different homologues have been found, and for the case of *Pcc* strain SCC3193 they are named ExpI/ExpR1/ExpR2. On the other hand, in strain *Pcc* EC153 they have been named AhlI/ExpR; while in *Pcc* ATCC390048, the homologues are CarI/CarR and ExpR1/VirR. These QS systems are responsible for controlling the production of PCWDEs and virulence factors, in addition to the production of carbapenem antibiotics in *Pcc* ATCC390048 (Põllumaa, Alamäe, and Mäe 2012). A pioneer work by Dong et al. (2001) demonstrated that inactivation of QS through enzymatic hydrolysis of AHLs rendered transgenic tobacco plants resistant to *Pcc* infections, bringing interesting insights into the role of QS inactivation in controlling bacterial infections.

2.3 Bacteria with Incomplete Auto-Inducer-1 Quorum Sensing Mechanisms

Some Proteobacteria belonging to the Enterobacteriaceae family, such as *Escherichia coli* and *Salmonella*, presents incomplete AI-1 QS mechanism. These bacteria do not have the *luxI*-homologous gene encoding AI-1 synthase; consequently, there is no synthesis of AHLs. However, a LuxR homolog, known as SdiA (cell division inhibition suppressor), which shows an amino acid sequence similar to that of the LuxR-type transcriptional activators, is present and allows the detection of signal molecules produced by other bacterial species leading to the regulation of gene expression (Michael et al. 2001; Dyszel et al. 2010; Smith and Ahmer 2003; Smith et al. 2008). In *Salmonella* and *E. coli* some phenotypes regulated by QS have been described.

2.3.1 QS in *E. coli*

Escherichia coli is a very diverse bacterial species belonging to the family Enterobacteriaceae, which comprises Gram-negative bacilli, and inhabits the lower gastrointestinal tract of humans and other animals. This bacterium is a paradigm of bacterial versatility and comprises harmless commensal as well as different pathogenic variants with the ability to cause either intestinal or extraintestinal diseases (Leimbach, Hacker, and Dobrindt 2013). *E. coli* strains regulate their virulence gene expression in response to a variety of environmental factors and can use QS to modulate gene expression. Different intercellular signaling systems have been identified: the LuxR homolog SdiA, the LuxS/AI-2 system, an AI-3 system, and a signaling system mediated by indole.

The SdiA protein played a role in the regulation of *ftsQAZ* cell division genes in *E. coli* (Ahmer et al. 1998), and increases of up to four-fold in the *ftsQAZ* expression were reported (Dyszel et al. 2010). In contrast, SdiA repressed the expression of virulence factors in enterohemorrhagic *E. coli* O157:H7 (EHEC) (Kanamaru et al. 2000), conferred multidrug resistance and increased levels of AcrAB (Dyszel et al. 2010; Rahmati et al. 2002), and increased the acid tolerance of *E. coli* upon exposure to AHLs (Van Houdt et al. 2006). SdiA also decreases early *E. coli* biofilm formation 51-fold, enhances acid resistance, and is required to reduce *E. coli* biofilm formation in the presence of AHLs (Lee et al. 2007). The quinolone resistance, expression of *acrAB* and *ftsQAZ* were not increased by chromosomal *sdiA* and/or AHL in *E. coli* K-12 or EHEC (Dyszel et al. 2010). However, using plasmid-encoded *sdiA* a two-fold change in response to some antibiotics was observed, an increase of up to two-fold in *acrA* expression and four-fold in *ftsQAZ* expression (Dyszel et al. 2010).

The *sdiA* mutants of atypical enteropathogenic *E. coli* (aEPEC) were capable of forming thicker biofilm structures and showed increased motility when compared to the wild type and complemented strains (Culler et al. 2018). These authors also demonstrated increased *csgA*, *csgD*, and *fliC* transcription on mutant strains. Biofilm formation, as well as *csgD*, *csgA*, and *fimA* transcription decreased on wild type strains by the addition of AHL. These results indicate that SdiA participates on the regulation of these phenotypes in aEPEC and that AHL addition enhances the repressor effect of this receptor on the transcription of biofilm and motility related genes (Culler et al. 2018). In *sdiA* mutant of EHEC, the expression of the glutamate decarboxylase acid-resistance system genes (*gad* genes) was dramatically decreased, even in the absence of AHLs and, consequently, this mutant was less resistant to acidic environments than wild-type of EHEC (Dyszel et al. 2010; Hughes et al. 2010).

The rumen of cattle harbors AHLs, and these chemical signals can be sensed in part through SdiA to modulate gene expression in EHEC, leading to successful colonization of these animals (Hughes et al. 2010). The presence of SdiA seems to be essential in EHEC colonization of the bovine intestine since *sdiA* transposon insertion mutants were not recovered or were recovered at low levels in the feces of old Friesian bull calves (Dziva et al. 2004). The *sdiA* mutant was detected in feces of only one of the four calves at low levels (10^2 CFU/g feces) from days 19 to 27 post-inoculation, whereas the fecal shedding of the wild-type strain persisted at approximately four-logs in all four calves. AHLs activated expression of the *gad* genes and repressed expression of the locus of enterocyte effacement (LEE) of EHEC (Hughes et al. 2010). Of note, the arginine acid-resistance system (*adi*) was not regulated by SdiA or the addition of AHLs (Hughes et al. 2010). Sharma and Bearson (2013) confirmed that SdiA represses *ler*, which encodes a positive transcriptional regulator of LEE in response to AHLs and reduces adherence of EHEC to HEp-2 cells.

Phenotypes regulated by SdiA protein in the absence of AHLs in *Salmonella* and *E. coli* have been reported (Dyszel et al. 2010; Hughes et al. 2010; Nguyen et al. 2015; Smith and Ahmer 2003). For instance, SdiA of EHEC is constitutively activated by the binding of molecule 1-octanoyl-*rac*-glycerol (OCL) in the absence of AHLs (Nguyen et al. 2015). The OCL is a monoglycerol present in prokaryotes and eukaryotes and is used as an energy source and substrate for the synthesis of membrane and a signaling molecule (Alvarez and Steinbüchel 2002; Liu et al. 2012). However, the activation of SdiA from EHEC by AHLs conferred greater stability and affinity to DNA, albeit not affecting *sdiA* gene transcription (Nguyen et al. 2015). Additionally, these authors observed conformational changes of EHEC SdiA protein complexed with different ligands such as: OCL in the absence of AHLs; *N*-(3-oxo-hexanoyl)-L-homoserine lactone (3-oxo-C6-HSL); and *N*-(3-oxo-octanoyl)-L-homoserine lactone (3-oxo-C8-HSL).

2.3.2 QS in *Salmonella*

Salmonella is a genus of rod-shaped, Gram-negative, facultative anaerobic bacteria belonging to the family Enterobacteriaceae, and it is considered the main foodborne bacterial pathogen. It is an important cause of gastrointestinal diseases worldwide, and complications can lead to death. In this pathogen, the communication by QS can be mediated by three types of AIs, called AI-1, AI-2, and AI-3 (Ahmer 1998; Hughes and Sperandio, 2008). In *Salmonella*, SdiA was described for the first time by Ahmer et al. (1998) and they showed that, in *Salmonella enterica* serovar Typhimurium, this protein is able to partially activate the promoter two of the *ftsQAZ* operon and suppress *ftsZ* responsible for cell filamentation, unlike what occurs in *E. coli*. The AHLs regulate the expression of the *rck* operon (resistant to complement killing), which codes for *pefI*, *srgD*, *srgA*, *srgB*, *rck*, and *srgC* genes, and it is found in plasmids influencing virulence of *Salmonella* Typhimurium (Ahmer et al. 1998; Michael et al. 2001; Smith and Ahmer 2003; Soares and Ahmer 2011).

Genes related to virulence such as *hilA*, *invA*, and *invF* present on the pathogenicity island PAI-1, and genes involved in the formation of biofilm by *Salmonella* Enteritidis were more expressed in the presence of exogenous AHLs (Campos-Galvão et al. 2016). A global analysis carried out on the influence of AHL on proteins of *Salmonella* Enteritidis showed that the abundance of proteins involved in translation (PheT), transport (PtsI), metabolic processes (TalB, PmgI, Eno and PykF), and response to stress (HtpG and Adi) increased while the abundance of other proteins related to translation (RplB, RplE, RpsB, and Tsf), transport (OmpA, OmpC, and OmpD), and metabolic processes (GapA) decreased in the presence of AI-1 (Almeida et al. 2017a). It was hypothesized that these changes observed in cells in the middle of logarithmic phase in presence of AHL are correlated with those into the early stationary phase of growth, without AHL. In other organisms, the effect of AHLs in anticipating the stationary phase responses was confirmed by global analysis, such as the transcriptome of *P. aeruginosa* (Schuster et al. 2003) and *B. thailandensis* (Majerczyk et al. 2014a), as well as the metabolomes of *Burkholderia glumae*, *B. pseudomallei*, and *B. thailandensis* (Goo et al. 2012).

The suggestion that QS signal anticipated a stationary phase response in *Salmonella* was reinforced when cells were cultivated in anaerobic condition in the presence of *N*-dodecanoyl-homoserine lactone (C12-HSL), and the fatty acid profiles were altered and similar to those of cells at late stationary phase (Almeida et al. 2017a). The presence of C12-HSL increased the abundance of thiol related proteins such as Tpx, Q7CR42, Q8ZP25, YfgD, AhpC, NfsB, YdhD, and TrxA, as well as the levels of free cellular thiol in late log phase, suggesting that these cells have greater potential to resist oxidative stress (Almeida et al. 2018). Additionally, the LuxS protein which synthesizes the AI-2 signaling molecule was differentially abundant in the presence of C12-HSL. The increased abundance of NfsB protein in the presence of C12-HSL suggested that the cells may be susceptible to the action of nitrofurans or that AHLs present some toxicity. Overall, the presence of C12-HSL altered important pathways related to oxidative stress and stationary phase response in *Salmonella* (Almeida et al. 2018).

The role of AHLs in *Salmonella* pathogenicity was suggested when *N*-hexanoyl homoserine lactone (C6-HSL) and *N*-octanoylhomoserine lactone (C8-HSL) increased the invasion of HEp-2 cells by *Salmonella* Typhimurium at 37°C (Nesse et al. 2011) whereas C8-HSL increased adhesion of *S. enterica* serovar Typhi containing plasmid pRST98, which harbors the virulence gene *rck*, to HeLa cells after 1 h at 37°C in the presence

of 5% CO_2 gas (Liu et al. 2014). Biofilm formation by *S. enterica* serovar Enteritidis PT4 578 in polyestyrene surface was positively regulated by C12-HSL in anaerobiose, even though no growth changes were observed in planktonic cells (Almeida et al. 2017b; Campos-Galvão et al. 2016). *N*-butyrilhomoserine lactone (C4-HSL) and C6-HSL also increased biofilm formation by *Salmonella* Typhimurium on polyestyrene (Aswathanarayan and Vittal 2016).

On the other hand, a cell free supernatant (CFS) rich in AHLs, AI-2, and other unknown compounds of *Y. enterocolitica* and *Serratia proteamaculans* altered growth of different phage types of *Salmonella* Enteritidis and *Salmonella* Typhimurium under aerobiose (Dourou et al. 2011). Similarly, the CFS of *P. aeruginosa* containing AHLs and different metabolites decreased growth of nine serovars of *S. enterica* (Wang et al. 2013). Conversely, the CFS of *Hafnia alvei* containing AHLs, as well as the addition of synthetic *N*-3-oxo-hexanoyl homoserine lactone (3-oxo-C6-HSL) to the growth medium in aerobioses did not influence biofilm formation by *Salmonella* Typhimurium (Blana et al. 2017). It is noteworthy that in these studies, the CFS of different bacteria contained metabolites other than AHLs which might have interfered in the detection of the AI's subtle effects in the cells.

2.4 Quorum Quenching of Autoinducer-1

A large variety of microorganisms have adopted the QS communication system leading to the expression of specific genes in order to coordinate certain basic cellular functions in response to changes in the environment (Fuqua, Parsek, and Greenberg 2001; Whitehead et al. 2001; Federle and Bassler 2003). Some phenotypes regulated by AI-1 of QS mechanisms are shown in Table 2.1.

Such coordinate functions in the bacterial population can provide competitive advantages to microorganisms to remain in ecological niches. Likewise, a microorganism's ability to neutralize QS signaling from its competitors can also significantly increase its competitive strength in the ecosystem (Zhang and Dong 2004). Besides the ability of many bacteria to produce and utilize AHL-based communication systems, inhibitors and quorum quenching (QQ) enzymes have been identified from different sources, including both prokaryotic and eukaryotic organisms (Hentzer et al. 2003; Zhang 2003; Zhang and Dong 2004; Uroz, Dessaux, and Oger 2009). Therefore, the interruption of this communication system or the activity of the QS mechanism in bacteria can lead to the attenuation of the microbial virulence (Whiteley, Lee, and Greenberg 1999; De Kievit and Iglewski 2000; Smith and Iglewski 2003).

Natural and synthetic compounds with QQ action have gained interest as potential attractive strategies for controlling bacterial pathogenesis. One of the first mechanisms most studied is related to chemical compounds quorum sensing inhibitors (QSI) acting as antagonists and interfering with the transcriptional regulator structure. Plants are potential sources of antimicrobials with QSI activity due to the production of a broad spectrum of secondary metabolites, such as phenolic compounds, alkaloids, terpernoids, and polyacetylenes, among other classes (Givskov et al. 1996; Zhang et al. 2002; Vattem et al. 2007; Sybiya Vasantha Packiavathy et al. 2012). Another mechanism that strongly interferes or even eliminates the functions regulated by QS is related to the production of enzymes capable of degrading the AHL signal molecules (Uroz, Dessaux, and Oger 2009). The potential for biological decomposition of these signals is interesting because other bacteria sharing the same local environment as quorum-sensitive bacteria could gain a competitive advantage by degrading acyl-HSL signals. Four types of enzymes have been shown to possess an ability to degrade QS signals-AHLs: lactonases and decarboxylases hydrolyze lactone ring, whereas acylase and deaminase cleave the acyl side chain (Kalia 2013).

2.5 Concluding Remarks

There is a growing interest in QS mechanism in the bacterial world and its implications for biotechnology, medicine, ecology, and agriculture. The great challenge of QS studies is expected to be an insightful observation and practical application of chemical signaling that occurs in the natural world. Scientists need to develop intelligent and sophisticated strategies to study such an intricate network of interactions at the crossroads of chemistry, physics, and biology. Most of the research that led to the current understanding of QS used well-controlled pure culture and mixed culture in laboratories, but the borders need to be broadened to understand the impact and exploitation of QS in complex microbial communities.

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