

Quorum Sensing in Bacteria and a Glance on *Pseudomonas aeruginosa*

Mehrdad Moosazadeh Moghaddam^{1,2*}, Samaneh Khodi¹ and Ali Mirhosseini¹

¹Applied Biotechnology Research Center, Baqiyatallah University of Medical Sciences, Tehran, Iran

²National Institute of Genetic engineering and Biotechnology, Tehran, Iran

Abstract

Quorum sensing is a key behaviour-coordination mechanism employed by many bacteria to regulate gene expression in accordance with population density through the use of signal molecules, known as autoinducers. The quorum sensing is used by Bacteria populations to communicate and coordinate their group interactions, which is typically applied by pathogens in infection processes. Generally, the quorum sensing pathways in bacteria are composed of several main parts, including bacteria populations, signal molecules, protein activators and target genes. In this system, the bacteria secrete the signal molecules into the environment and the concentration increases gradually as the bacteria population grows. In a certain concentration threshold, the molecules become detectable to the bacteria populations, and then activate target genes that regulate various behaviours, such as virulence factors. In *Pseudomonas aeruginosa*, the expression of many virulence factors appears to be controlled by quorum sensing. So, according to the role of this mechanism in the regulation and production of many virulence factors, the function of quorum sensing is required for *Pseudomonas aeruginosa* to cause disease and infection. In this article, we discussed the quorum sensing mechanism in Gram negative and positive bacteria with a closer look at the *Pseudomonas aeruginosa*.

Keywords: Quorum sensing; *Pseudomonas aeruginosa*; Autoinducer; Cell-to-Cell signaling

Introduction

Pseudomonas aeruginosa is an opportunistic pathogen that primarily infects patients with immune deficiency such as neutropenia. Generally, the pathogenesis of *Pseudomonas* is complex and multifactorial, since the bacterium is both invasive and toxigenic, can cause a range of infections in humans (Table 1) [1-3]. *Pseudomonas* clearance is often challenging due to immunodeficiency in patients with infection, multidrug resistancy and sometimes β -lactamase production and the inactivation of third-generation cephalosporin [4,5]. *P. aeruginosa* produces a variety of cellular structures and products involved in the ability to cause disease through enforcing the adhesion, protecting the phagocytosis, modifying the immune response or destroying the host tissue. Generally, the effective factors involved in the development of infection by this bacterium can be divided into two categories: Extracellular virulence factors and structural virulence factors. Extracellular factors include pigments that are active in Fe absorption, prevent the growth of other bacterial species and degrade factors including protease, hemolysins and toxins (Figure 1) [6,7].

Major structural factors in this bacterium are pili, flagellum and LPS. In addition to these factors, *P. aeruginosa* has also other capabilities that enhance bacteria pathogenesis, including a broad-spectrum Beta-Lactamase (ESBLs) of class A-B and D that is one of

the major causes of high bacterial resistance to most commonly used antibiotics; an exopolysaccharid known as biofilm matrix and secreted

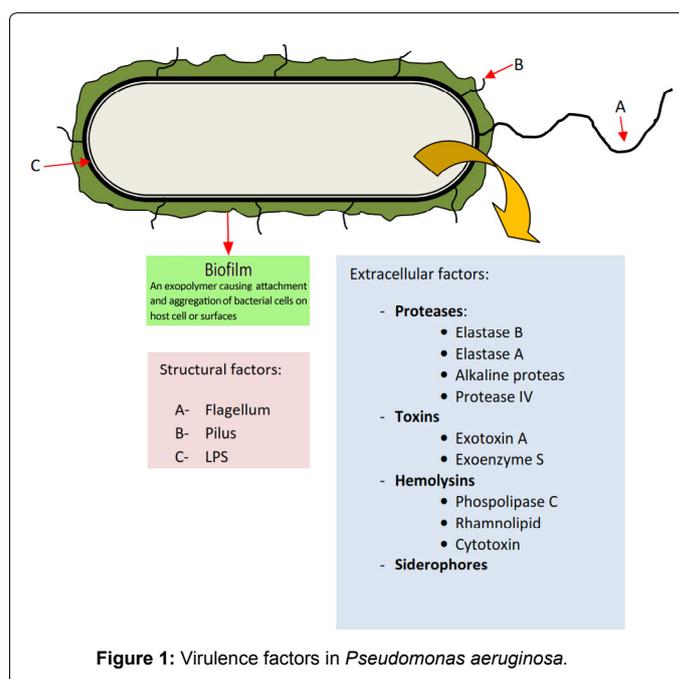


Figure 1: Virulence factors in *Pseudomonas aeruginosa*.

Infection	Related Risk Factors
Pneumonia	Old age, Chronic obstructive pulmonary disease, Cystic fibrosis, Mechanical ventilation
Bacteremia	Immunocompromised
Urinary tract	Use of urinary catheter
Soft tissue	Burns, Open wounds, Postsurgery
Diabetic foot	Diabetes, Impaired microvascular circulation
Keratitis	Extended contact lens wear, Contaminated contact lens solution
Otitis externa	Tissue injury, Water blockage in ear canal
Otitis media folliculitis	Improperly cleaned hot tubs

Table 1: Common infections causing by *Pseudomonas aeruginosa* [3].

*Corresponding author: Mehرداد Moosazadeh Moghaddam, Applied Biotechnology Research Center, Baqiyatallah University of Medical Sciences, Tehran, Iran, P.O. Box 19395-5487, Tel: +982182482549; E-mail: mm.genetics@gmail.com

Received April 25, 2014; Accepted June 13, 2014; Published June 20, 2014

Citation: Moghaddam MM, Khodi S, Mirhosseini A (2014) Quorum Sensing in Bacteria and a Glance on *Pseudomonas aeruginosa*. Clin Microbial 3: 156. doi:10.4172/2327-5073.1000156

Copyright: © 2014 Moghaddam MM, et al. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

by mucoid strains of *P. aeruginosa* into its surroundings which helps to the attachment and aggregation of bacteria cells to mucin on the host cells, especially in respiratory infections such as cystic fibrosis (Figure 1); and having a mechanism to escape the immune system based on the Cell-to-Cell signaling [4,8-13].

Cell-to-cell signaling and immune escape mechanisms

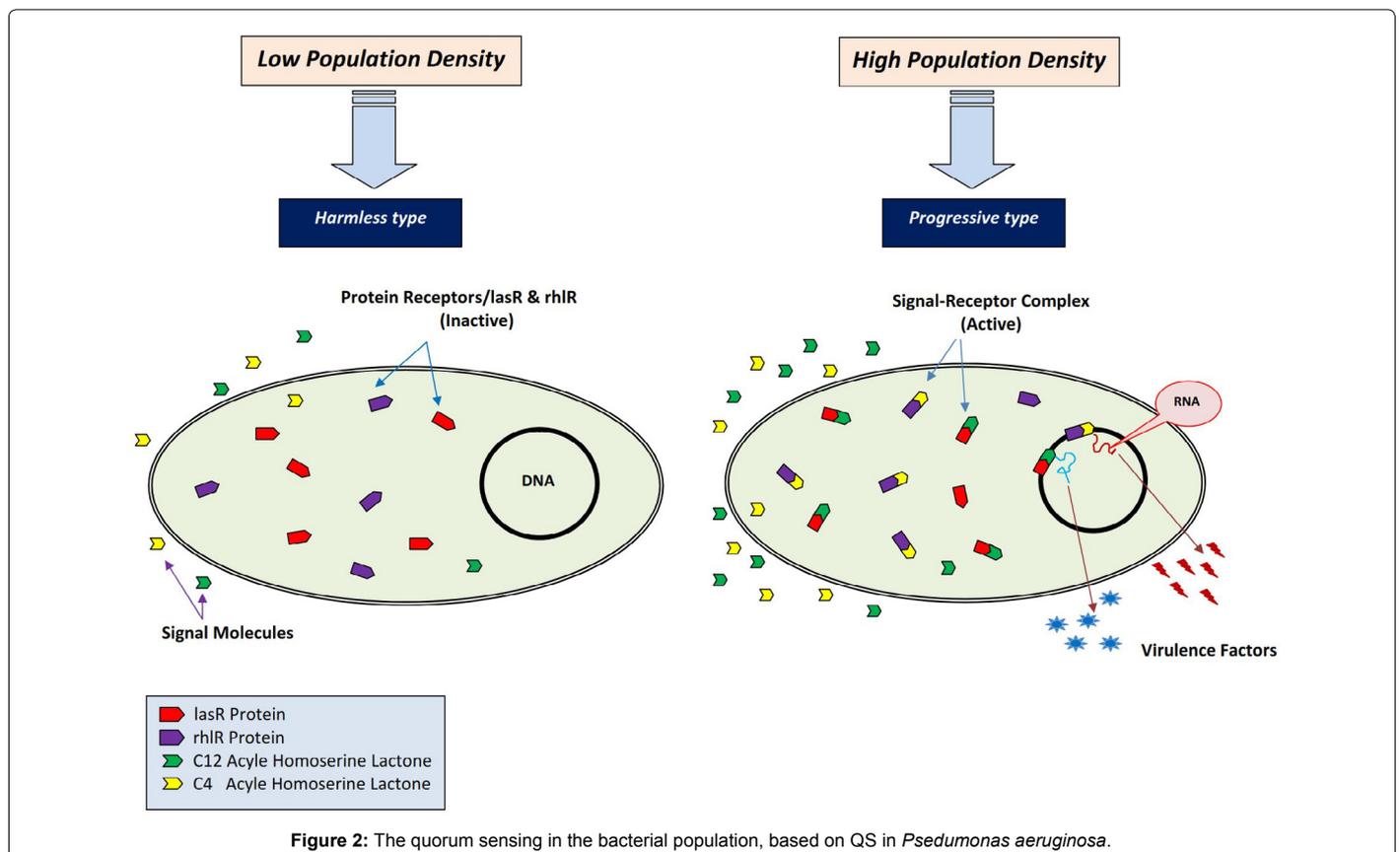
In general, the bacteria control their environmental systems and cell populations through intracellular communications to have the best performance and response according to the demographic and the environmental conditions [14,15]. Pathogenic bacteria take the best advantages of communication capability, as an example, they can overcome the host immune system barrier using the community, in this case, they estimate the cell density using these intercellular signals and given the concentration of signal transmitter factors, and when the density reaches its minimum extent in which the immune system cannot simply cope, bacteria will release the virulence factors, thus, the host immune system will be prevented to deliver rapid responses. The process is controlled by a system in bacteria called "Quorum Sensing" [16-20].

Quorum sensing system in bacteria

Bacteria are known as one of the simplest and the most primitive life forms, which have the single-cell life and their reproduction, feeding and communication mechanisms are identified as basic and simple patterns, however, the complex and interesting mechanisms are provided by studies in recent decades that control bacterial behavior similar to what occurs in multicellular organisms. This mechanism is controlled by quorum sensing system that enables intraspecies and interspecies communication, which involves in terms of biofilm

formation, food shortages and environmental stress conditions, such as disinfectants, antibiotics, bacterial colonization, the identification of annoying species, the establishment of normal intestinal flora and the prevention of harmful intestinal flora, etc [21-24]. An important reason to study this system is to evaluate the mechanisms and to identify the factors affecting on it, in order to inhibit the communication by producing the antagonist of factors and to prevent the biofilm formation, antibiotic resistance spread and chronic and treatment-resistant infections [25-27].

As was pointed out, the bacteria often live in the communities, not individual. They can communicate by producing and responding to a series of messages. Quorum sensing is one of the main forms of communication, a two-component term in which quorum means "threshold" and sensing means "feel" that is briefly called QS system. The system is a cell to cell signaling mechanism by which bacteria control the expression of some genes depends on the cell density, a process by which bacteria determine the cell density population and the information is used to regulate the gene expression, so QS system is an example of multicellular behavior in the unicellular world of bacteria [28,29]. The bacteria produce some chemical molecules in the QS phenomenon that are often spreadable and have a low molecular weight that aggregate in the surrounding, these messenger molecules are called "Autoinducer" or "Self-inducer". It is assumed that the balance of chemical messages between organisms and cells is a unique property in eukaryotic cells but recent studies indicate that many bacteria use a group of messenger molecules to coordinate the behaviors. Once the concentration of molecules outside the bacteria exceeds the threshold, the signaling pathways are activated and the bacteria response the messages by altering the gene expression and modulating physiological processes in a collective mode (Figure 2) [30-32].



Quorum sensing in Gram-negative bacteria

Over the past decade, quorum sensing complex have been identified in more than 25 Gram-negative bacteria species. A wide range of major processes in these bacterial species is regulated by quorum sensing, such as bioluminescence, Swarming, Swimming, Twitching, Antibiotics biosynthesis, the growth and spread of biofilm, Conjugation, Sporulation, Virulence markers production, etc [33-36]. Among these species QS system in *Vibrio fischeri*, *Pseudomonas aeruginosa* and *Agrobacterium tumefaciens* are more considered. However, the studies show the evolution of system and the compliance with the specific requirements of each of the bacteria.

The most studies on quorum sensing are performed on bioluminescence property in *Vibrio fischeri*. The bacteria lives in symbiosis with eukaryotes, the eukaryotic host provides a nutrient-rich environment for symbiotic bacteria, and in turn, the bacteria produces light that is used to avoid predation and to prey capture. The quorum sensing system in *Vibrio fischeri* is composed of two regulatory proteins, LuxI and LuxR, which LuxI protein is an autoinducer synthase enzyme and LuxR is a promoter binding protein. Generally, LuxI protein is responsible to biosynthesize a specific messenger molecule called acyl homoserine lactones (AHL/acyl-HSL) or autoinducer. The concentration of autoinducer increases with increasing the cell population. When the concentration reaches a threshold, the autoinducer enters the bacterial cell and binds a protein called R protein. The autoinducer and R protein complex activates the transcription of target gene, and therefore, the bacteria efficiently coordinate the gene expression using quorum sensing system with cell population fluctuations (Figure 2) [32,37,38]. In recent studies, more special systems than LuxI/R are identified, for example, two pairs of genes in *P. aeruginosa* (LasI and LasR) have been

identified that control the expression of virulence factors. Studies on LuxI and LuxR homologous genes in different bacteria show a similar system and performance (Table 2) [20,39].

Autoinducers

As previously mentioned, autoinducers are small diffusible signaling molecules which are produced by bacteria in the quorum sensing system. These autoinducers (AI) are divided into two categories, autoinducers type-1 or AI-1, which are species-specific and employed for intraspecies communication. Autoinducers type-2 or AI-2 are not only applied for interspecies communication, but also enable the bacteria to communicate microorganisms such as fungi and protozoa to coordinate their own behavior in a population of microorganisms. AI-1 is composed of a acyl homoserine lactone ring (AHL) with an carbon chain which is different in Gram-negative bacteria, therefore, makes the autoinducer thoroughly specific for the species by a series of changes on C3 (locating OXO or Hydroxyl group on it). But, the AI-2 is a furanosyl borate diester. It is noteworthy to mention that the AHL production is only limited to the Proteobacteria of which only 21 species, comprising only 4% of the species, produce AI-2 (Figures 3 and 4) [30,40-44].

Quorum sensing in Gram-positive bacteria

After changes, the peptides produced in bacterial cells are employed by an ABC channel and secreted into the extracellular space. The induction pathway in the recipient bacterium, called Two-component signal-transduction, is comprised of two components: Two-component sensor kinase, located in the bacterial cell membrane, acts as a receptor

Organism	Major signal molecule	Regulatory proteins	Phenotype
<i>Vibrio fischeri</i>	3-Oxo-C ₆ -HSL	<i>LuxI/LuxR</i>	Bioluminescence
<i>Vibrio harveyi</i>	3-Hydroxy-C ₄ -HSL	<i>LuxLM/LuxN</i>	Bioluminescence
<i>Vibrio anguillarum</i>	?	<i>VanI/VanR</i>	3-Oxo-C ₁₀ -HSL
<i>Pseudomonas aeruginosa</i>	3-Oxo-C ₁₂ -HSL	<i>LasI/LasR</i>	Multiple extracellular enzymes, RhlR, Xcp, biofilm formation
	C ₄ -HSL	<i>RhlI/RhlR</i>	Multiple extracellular enzymes, rhamnolipid, RpoS, secondary metabolites
<i>Pseudomonas aureofaciens</i>	C ₆ -HSL	<i>PhzI/PhzR</i>	Phenazine antibiotics
<i>Agrobacterium tumefaciens</i>	3-Oxo-C ₈ -HSL	<i>TraI/TraR</i>	Ti plasmid conjugation
<i>Erwinia carotovora</i>	3-Oxo-C ₆ -HSL	<i>ExpI/ExpR</i>	Exoenzymes
subsp. <i>carotovora</i>	?	<i>CarI/CarR</i>	Carbapenem antibiotics
<i>Erwinia chrysanthemi</i>	3-Oxo-C ₆ -HSL	<i>ExpI/ExpR</i>	Pectate lyases
	C ₆ -HSL		
<i>Erwinia stewartii</i>	3-Oxo-C ₆ -HSL	<i>EsaI/EsaR</i>	Exopolysaccharide, virulence factors
<i>Rhizobium leguminosarum</i>	C ₆ -HSL	<i>RhlI/RhlR</i>	RhiABC rhizosphere-expressed genes, nodulation
	C ₈ -HSL	?	
	3-Hydroxy-7-cis-C ₁₄ -HSL	<i>CinI/CinR</i>	Quorum sensing regulatory cascade
<i>Rhizobium etli</i>	Multiple, unconfirmed	<i>RaiI/RaiR</i>	Restriction of number of nitrogen-fixing nodules
<i>Chromobacterium violaceum</i>	C ₆ -HSL	<i>CviI/CviR</i>	Exoenzymes, antibiotics, cyanide, violacein
<i>Burkholderia cepacia</i>	C ₆ -HSL	<i>CepI/R</i>	Protease, siderophores
<i>Aeromonas hydrophila</i>	C ₄ -HSL	<i>AhyI/AhyR</i>	Exoprotease production
<i>Aeromonas salmonicida</i>	C ₄ -HSL	<i>AsaI/AsaR</i>	Extracellular protease
<i>Ralstonia solanacearum</i>	C ₈ -HSL	<i>SolI/SolR</i>	?
<i>Serratia liquifaciens</i>	C ₄ -HSL	<i>SwrI/SwrR</i>	Extracellular protease, swarming
<i>Rhodobacter sphaeroides</i>	7-cis-C ₁₄ -HSL	<i>CerI/CerR</i>	Dispersal from bacterial aggregates
<i>Enterobacter agglomerans</i>	3-Oxo-C ₆ -HSL	<i>EagI/EagR</i>	?
<i>Escherichia coli</i>	?	<i>?/SdiA</i>	Cell division, attachment and effacing lesion formation
<i>Yersinia enterocolitica</i>	C ₆ -HSL	<i>YenI/YenR</i>	?
<i>Yersinia pseudotuberculosis</i>	C ₆ -HSL	<i>YesI/YesR</i>	?

Table 2: The homologous genes of Lux system in various bacteria [60].

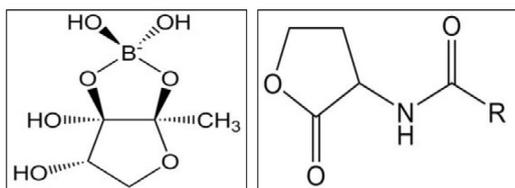


Figure 3: Right) Autoinducer type-2 structure, Left) Autoinducer type-1 structure (R: carbon chain).

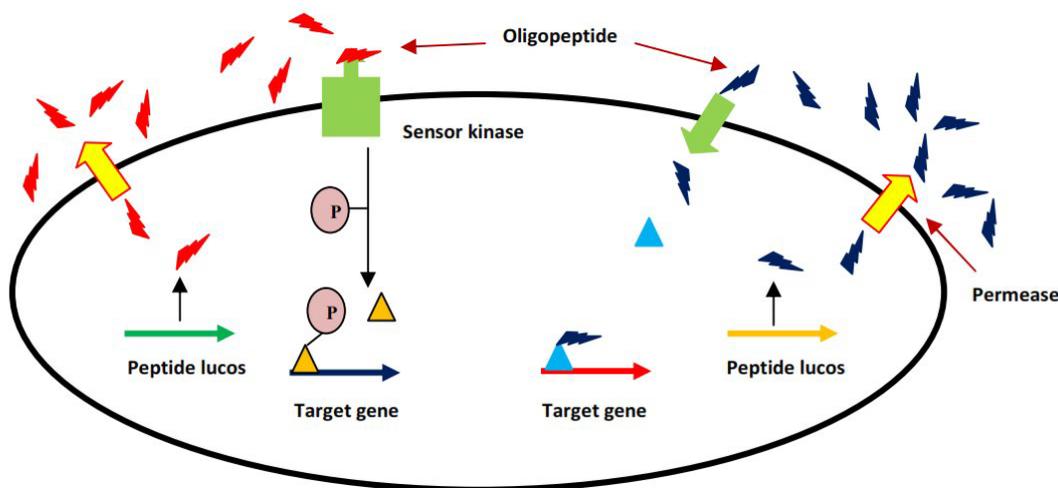


Figure 4: The performance of messenger peptides in QS system in Gram-positive bacteria.

for peptide messenger and triggers the phosphorylation of component B located in the cytoplasmic space. Two-component response regulator, component B, that its phosphorylation causes the activation and regulation of gene expression [45,46].

In the alternative method, the peptide is transferred as a messenger molecule by active transport inside the bacteria using a specific Permease located in the cell membrane, then; the peptide binds a cognate regulatory protein and triggers the regulation of gene expression.

As an example of communication in Gram-positive bacteria, comE/comD system in *Streptococcus pneumoniae* can be mentioned in which the recipient bacterium is potent to receive a foreign DNA. This potential is temporary and occurs only during the logarithmic phase in which the bacteria uptake the DNA regardless its sequence and the species of origin [47-49]. The TraR/TraI virulence system in phytopathogen *Agrobacterium tumefaciens* and hybrid messenger system in *Vibrio harveyi* are another examples of the role of quorum sensing in pathogenesis. In *Agrobacterium tumefaciens* Ti plasmid transport is controlled by quorum sensing system [50].

Quorum sensing in *Pseudomonas aeruginosa*

The quorum sensing system of *P. aeruginosa* is composed of two pairs of genes as main constitutive genes. One pair consists of LasI and LasR genes, encoding C12-HSL autoinducer synthase and R protein, respectively. Another pair called Rhl is composed of RhlI and RhlR genes, encoding synthase and R protein, respectively (Figure 5). The synthase enzyme of this pair of QS system produces C4-HSL autoinducer, but produces a small amount of N-hexanoyl-L-homoserine-lactone (HHL) [51]. There is also a regulatory gene named QScR in this system that produces a regulatory protein which functions as an inhibitor of LasR

protein. The functions of the genes of QS system in *P. aeruginosa* are inter-related and do not operate independently, in other words, it can be said that the expression of Rhl genes is under regulation of Las genes which is performed through the R protein of LasR gene [52].

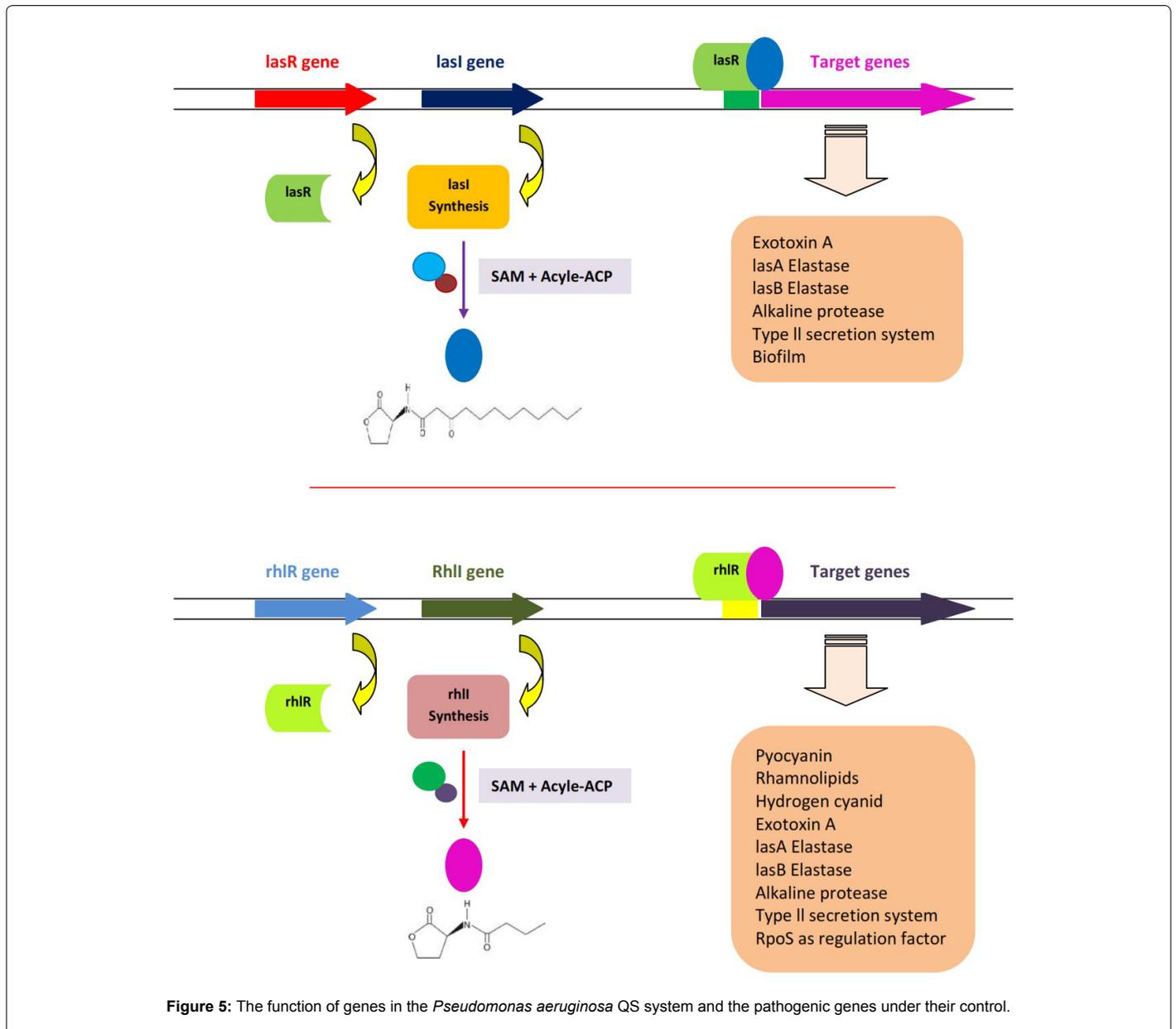
The main genes that control by QS in *P. aeruginosa*

Many genes in *P. aeruginosa* are regulated and expressed by quorum sensing system including pathogenesis genes such as Alkaline protease, Pyocyanin, pyoverdine, cyanide, lipase, twitching movement, alginate, azurin, chitinase, catalase, superoxide dismutase, lasA, lasB, XCP transport machine (a mechanism to deliver bacterial virulence factor), etc [53]. Studies have shown that the Las gene controls the production of virulence factors such as lasB Elastase, lasA Elastase, Alkaline protease and Toxin A. The Rhl gene controls the production of factors such as Rhamnolipids, Elastase, Pyocyanin, Cyanide, moreover, the control can be carried out combined with the Las gene (Figure 5). The function of Las gene was firstly evaluated by the enzyme activity of lasB elastase, it is why that it was named Las gene in the QS system of *P. aeruginosa*, and the reason for the naming of Rhl gene is its major role in the Rhamnolipid production. Rhl controls an operon called RhlAB encoding Rhamnosyltransferase enzyme which is essential in the Rhamnolipid production [54].

Regarding the QS system of *P. aeruginosa*, the followings are noteworthy [32,55,56]:

This system is not compatible, meaning that there is no interaction between the products of these two systems;

Some bacterial genes are under the control of both pairs of genes, and some others are controlled exclusively by one gene;



These genes are Auto-regulated so that LasR protein/C12-HSL is a positive regulator of the LasI gene and regulates the expression of RhIR gene, and RhlR protein/C4-HSL regulates the RhII gene transcription;

The R protein of Las system forms a multimer that can bind the Las-Box upstream of the target gene when is bound to the C12-HSL autoinducer. The R protein is a two-domain polypeptide consisting of an N-terminal that binds its autoinducer and a C- terminal that allows the R protein to bind the target gene. In the absence of any autoinducer, N-terminal domain inhibits C-terminal to bind the target gene;

The C-terminal domain contains a helix-turn-helix motif by which binds to the Las-Box in 40 bp upstream of the ATG start codon. Las-Box consists of 20 base pair palindrome. The amino acids in the C-terminal domain involve in polymerization;

The R protein of Rhl system forms a dimer and binds to the DNA in the presence or absence of C4-HSL, but when it is bound to the autoinducer, it can affect the expression of target genes;

It is necessary to have an appropriate concentration of autoinducer in the environment to regulate the genes under the control of QS system, but not sufficient. Simply, the QS-controlled gene expression is not only dependent on the threshold concentration of autoinducer in the environment, but the bacterial growth phase is also important and when the bacteria is not in the appropriate growth condition, the presence of a threshold concentration do not induces the expression of target gene.

As was pointed out, the QS systems play a major role in the regulation of expression of genes by the molecules messengers or autoinducers. Studies have shown that C12-HSL autoinducer causes the IL-8 production in human lung structural cells such as fibroblasts and bronchial epithelial. The autoinducer also stimulates the production of Cyclooxygenase-2 and Prostaglandin E2 in lung fibroblast cells thereby plays a role in inflammation. Moreover, the molecule can induce apoptosis in neutrophils and macrophages. Therefore, the above

phenomena lead to the suggestion that the autoinducers besides the regulation of the expression gene producing virulence factors are also a modulator factor for the host immune system function [57,58].

Also, another signal molecule has been discovered in *P. aeruginosa* called 2-heptyl-3-hydroxyl-4-quinolone. Its structure is not among the homoserine lactone family and is designated Pseudomonas quinolone signal (PQS), which controls the expression of lasB gene beside the Las and Rhl system. The expression of PQS depends on the LasR and also induces the expression of RhlR gene, therefore, the PQS acts as a link between Las and Rhl systems [59].

Autoinducer synthesis in *P. aeruginosa*

In this bacterium, the autoinducer is produced by the products of the LasI and RhlI genes which are synthase enzymes. These enzymes utilize S-adenosylmethionine (SAM) and Acyl-acyl carrier protein (Acyl-ACP) substrates to synthesize the autoinducers. The Acyl-ACP is also a mediating factor in fatty acid synthesis process. In the autoinducer synthesis process, SAM binds to the ACP by forming an amide bond between the amino group of homocysteine and the Acyl group of ACP. Subsequently, the autoinducer is produced by the release of Methylthioadenosine and the production of lactone (Figure 6) [60,61].

The regulation of QS in *P. aeruginosa*

Considering that the QS system plays an important role in controlling the bacterial genes, therefore, it is important to control and regulate its function. The regulation can be done by the bacteria or by the external factors (Figure 7).

This regulation can be done as follows:

The regulation of the transcription of LasI and LasR genes that induces changes in the amount of the production of synthase and R protein.

The regulation by the molecular analogs of autoinducers produced by other bacteria or eukaryotic cells.

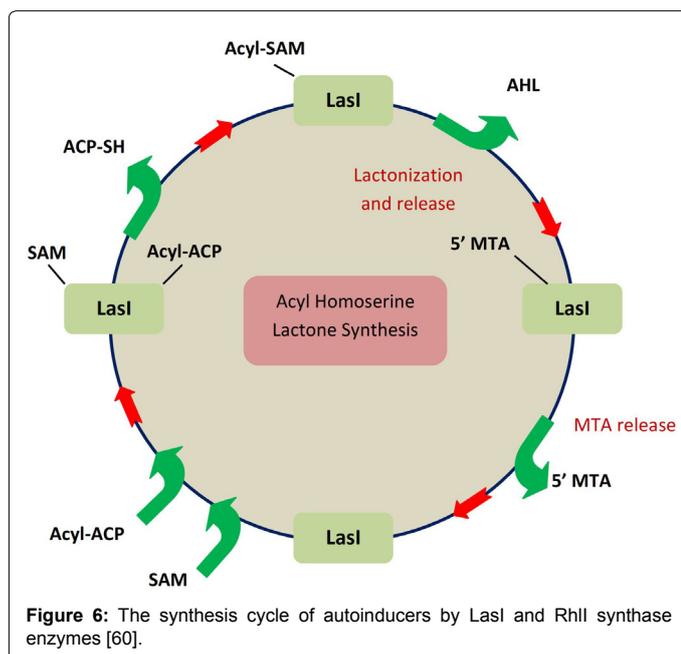


Figure 6: The synthesis cycle of autoinducers by LasI and RhlI synthase enzymes [60].

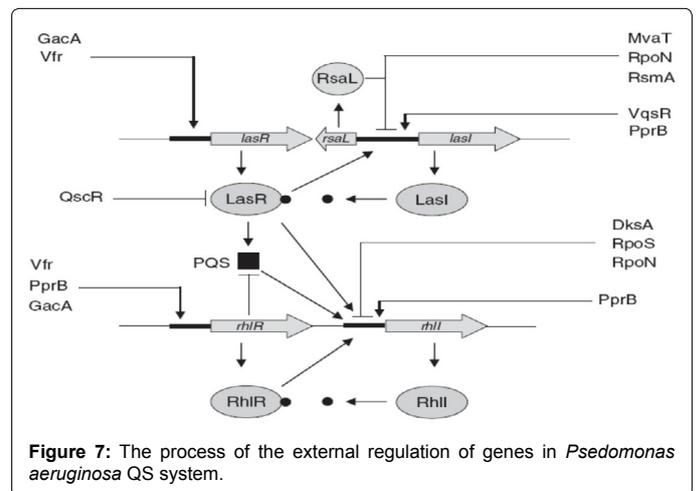


Figure 7: The process of the external regulation of genes in *Pseudomonas aeruginosa* QS system.

The regulation by the molecules that can compete with R protein as Agonist or Antagonist.

Enzymatic destruction like AHL degradation enzymes.

In *Pseudomonas aeruginosa*, salR, vatM, posR, smaR, ponR genes are the suppressor of the expression of LasR and LasI genes in the low density cells.

The inactivation of anti-activator protein QScR encoded by QScR gene is triggered by interacting with LasR protein. In high bacterial cell density, the GacA and PprB genes are also a positive regulators of LasR and RhlR genes (Table 3) [55,62].

Developing chemical modifications of autoinducers may also be involved in regulating the function of QS system, for instance, the acetylation of homoserine lactone stabilizes the molecular in pH 5 to 6. As was mentioned, the enzyme activities are also involved in regulating QS system, for example, AHL lactonase which was first identified in *Variovorax paradoxus* helps the organism to utilize AHL as the only source of carbon and energy, consequently causes the AHL degradation, or AHL-acylase in *P. aeruginosa* by which the bacteria is able to degrade and utilize AHL with long-chains as the only source of carbon and energy, therefore, the enzyme may have a regulatory role. Homoserine lactones are also hydrolysed and inactivated through alkalines.

Studies in humans have shown that the epithelial cells of the lung tissue in healthy individuals normally secrete a substance which degrades the autoinducers effective in the quorum sensing process of *P. aeruginosa* by an enzymatic function and people who are genetically deficient in the production of this inhibitors enzyme shows a higher percentage of cystic fibrosis disease.

The other interesting point is the direct effect of some antibiotics in the regulation of QS system activity, for example, the studies have shown that, in the case of *P. aeruginosa*, azithromycin antibiotic accelerates the improvement of disease by interfering with the elastase production and the expression of lasR and lasI genes [63].

Quorum sensing as drug target

Due to the spread of antibiotic resistance that today has become one of the main challenges in the field of infectious diseases, therapies avoiding the indiscriminate use of antibiotics are paid special attention. On the other hand, given to the importance of the quorum sensing phenomenon in controlling pathogenicity of bacteria such as *P.*

Regulators	Quorum sensing targeting	Description
<i>RpoS</i>	Negative transcriptional regulator of <i>RhlI</i>	Considerable overlap between RpoS and QS regulators
<i>RpoN</i>	Negative transcriptional regulator of <i>LasR</i> and <i>RhlR</i>	In minimal medium RpoN positively regulates <i>RhlI</i> expression
<i>RsaL</i>	Negative transcriptional regulator of <i>LasI</i>	In competition with LasR C ₁₂ AHL for <i>LasI</i> transcription; Avoids early activation of QS
<i>MvaT</i>	Negative transcriptional regulator/target currently unknown	Avoids early activation of QS
<i>QscR</i>	Negative regulator (antiactivator) of <i>LasR</i> protein	Avoids early activation of QS; LuxR-family member
<i>RsmA</i>	Negative transcriptional regulator of <i>LasI</i>	Avoids early activation of QS
<i>DksA</i>	Negative transcriptional regulator of <i>RhlI</i>	Avoids early activation of QS
<i>Vfr</i>	Positive transcriptional regulator of <i>LasR</i> and <i>RhlR</i>	Binds to <i>LasR</i> promoter, not known if also binds to <i>RhlR</i> promoter
<i>VqsR</i>	Positive transcriptional regulator of <i>LasI</i>	Considerable overlap between VqsR and QS regulators; LuxR-family member
<i>GacA/GacS</i>	Positive transcriptional regulator of <i>LasR</i> and <i>RhlR</i>	Two-component signal transduction system; Environmental stimulus unknown
<i>PprB</i>	Positive transcriptional regulator of <i>LasI</i> , <i>RhlI</i> and <i>RhlR</i>	Response regulator of a two-component signal transduction system; Overlap of PprB and QS regulators; Environmental stimulus unknown
<i>PQS</i>	Positive transcriptional regulator of <i>RhlI</i>	PQS is a signal molecule; its production is regulated by the Las system
<i>AlgR2</i>	Negative transcriptional regulator of <i>LasR</i> and <i>RhlR</i>	Also major regulator of alginate production; Homologous to <i>E. coli</i> Rsd protein which is an anti-sigma 70 factor.

Table 3: How the effective genes influence the process of the external regulation of genes in *P. aeruginosa* QS system [60].

aeruginosa, a new generation of antibiotics can be imagined which are designed based on the inhibition of QS system. It is not far from the notion that these inhibitors can be used as a synergism with other medications to reduce their dose. Biofilm that causes resistance against many commonly used antibiotics is regarded as one of the main challenges in treatment. As mentioned, quorum sensing system plays a fundamental role in the regulation, control and formation of biofilm and many virulence factors. Therefore, it is possible that the inhibition of QS regulatory process for removing and reducing the drug resistance in infectious bacteria to be effective.

In 2003, three independent research groups studied the genes under the control of *P. aeruginosa* QS system, and investigated 388, 315 and 163 genes in the bacterial genome. Studies were performed using the bacteria in which their QS system had been inactivated by mutation and the gene induction was controlled by foreign autoinducers. After studying the gene activity in the presence or absence of autoinducer, 97 genes were reported that jointly are controlled by the QS system (The differences in the results are probably due to the differences in the composition and condition of test medium and ambient oxygen). The studies showed that about 10% of the bacterial genomes are under the control of QS system. Therefore, it was suggested that the bacterial QS system can be considered as a suitable target in order to research about the control of bacterial infection [64-66]. This goal can be achieved using several methods including: I) The blockage of R proteins activation by HLA antagonists or HLA antibodies and HLA degradation by chemical or enzymic destruction; II) The metabolism inhibition of AHL by compounds that can compete with substrates which are used in the synthesis of autoinducers; III) Inhibition of regulatory factors that have a positive effect on QS genes; IV) Using antisense oligos against main genes of the QS system [67-69].

Quorum sensing signals as biosensor markers

Based on studies, QS signals can be used as markers for the presence of pathogenic bacteria in clinical and environmental samples by bacterial whole-cell QS biosensors. Generally these biosensors are composed of two main parts: a plasmid construct containing an AHL-responsive transcriptional regulator, regulatory promoter, reporter gene; and bacterial host cell (Figure 8A). However, because QS-deficient mutants can be developed by pathogenic bacteria after colonization in the host, so QS signals should not be employed as the only inputs for microbial biosensors, but can be used for detection of pathogenic bacteria in contaminated environments and

products such as groundwater, dairy, and meat products. For example pSB406 and pSB1075 plasmids, and *E. coli* JM109 as host cell, are QS biosensor system which are used for detection of C4-HSL and C12-HSL autoinducers of *P. aeruginosa*, respectively. In this system each plasmid is comprised of a R protein producing gene (*LasR* for pSB1075, *RhlR* for pSB406) and *LuxCDABE* operon gene of *Vibrio fischeri* as a luminescence reporter gene that is activated by specific *LasR/RhlR* protein-AHL complex (Figure 8B). It is noteworthy to mention that GRAS hosts like lactic acid bacteria (LABs) (*Lactobacillus*, *Leuconostoc*) can be used as carrier host of reporter plasmid for safe applications [70-72].

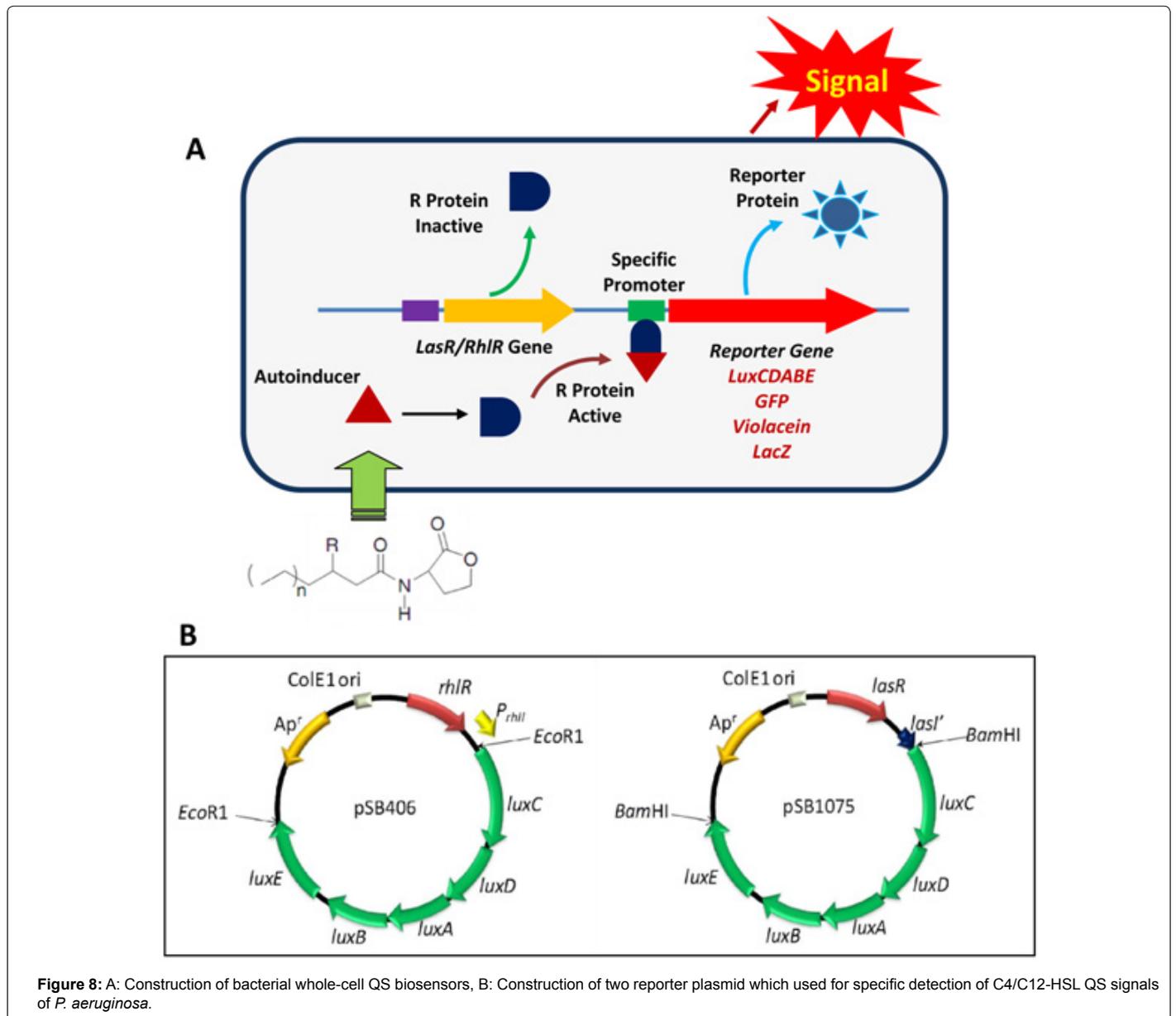
Quorum sensing signals and anticancer therapy

The 3-oxo-C12-HSL QS signal of *P. aeruginosa* inhibits proliferation and induces apoptosis in human breast cancer cell, therefore, it can be considered as an anticancer drug. However this feature may be prevented due to some side effects, for example studies showed that 3-oxo-C12-HSL can lead to macrophage apoptosis. Nevertheless, this QS signal is a good starting point for developing synthetic AHL homologs with anticancer toxicity and reducing side effects [68,70].

On the other hand, studies in mouse models have shown that some bacteria such as *E. coli*, *Bifidobacterium longum*, and attenuated strains of *Vibrio cholerae*, *Salmonella typhimurium*, and *Listeria monocytogenes* have a tendency to localization and proliferation in solid tumors and metastases, perhaps through immune system surveillance, which provides barrier against the host immune system [71,72]. Therefore, bacterial aggregation can be used to produce biosensors that target cancer cells which can be achieved by designing synthetic genetic circuits to recognize cancer microenvironment conditions or cancer cell surface antigens [73]. Based on this concept, in a study by Anderson et al. a genetic network was developed in *E. coli* in which the *Yersinia pestis* *invasin* gene was regulated by the *V. fischeri* *LuxI/R* system. Followed by high HSL concentrations, *invasin* will express and allows *E. coli* to bind and invade mammalian cancer-derived cells displaying β 1-integrin cell surface receptors. Therefore, whole-cell QS biosensors and gene delivery vehicles can be designed to recognize cancer cell aggregations *in vivo* [74].

Quorum quenching and biological control

The application of Quorum Quenching (QQ) strategy may be an alternative approach to control bacterial pathogens which employ the



AHL based QS mechanism to regulate pathogenicity. The strategy includes several methods to achieve an artificially increased level of AHLs such as the introduction of a gene coding AHL synthase directly to the plant cells, the employment of AHL-degrading bacteria to protect plants and heterologous expression of genes encoding AHL-degrading enzymes in pathogen cells or in plant tissue. Therefore, bacteria misinterpret the population size and the misinterpretation leads to the production of virulence determinants long before the pathogen population is large enough to sustain infection [75].

Several studies showed that the application of bacterial cells producing AHL degrading enzymes can be as biological control agents of plant bacterial diseases. The first example of such purposeful usage for the attenuation of infection symptoms development in plants was transgenic potato and tobacco plants expressing the gene encoding AiiA lactonase manifested strong resistance against infection by *P. Carotovorum* subsp. *carotovorum*. Heterologous expression of the *aiiA* gene encoding the AiiA lactonase from *Bacillus* sp. in plant pathogens

cells *P. carotovorum*, *Burkholderia thailandensis* and *Erwinia amylovora* diseased symptoms development. In order to silence QS system of *P. aeruginosa*, the same approach was used to reduce 3-oxo-C12-HSL followed by preventing the accumulation C4-HSL. This result was also observed by addition of purified AhlM protein to the growth medium of *P. aeruginosa*. Consequently, the AHL degrading enzymes together with QS inhibitors may be successfully be applied to disrupt bacterial cell to cell communication and to control bacterial infections [76].

Conclusion

Many bacteria use quorum sensing as a multicellular system to coordinate gene expression according to the density of their local population. By this mechanism bacteria can regulate metabolic, host interactions and environmental processes. So this system can be used as a useful target in medicine and other applications such as the production of biochemicals, microbial biosensors and mixed-species fermentations.

References

1. Driscoll JA, Brody SL, Kollef MH (2007) The epidemiology, pathogenesis and treatment of *Pseudomonas aeruginosa* infections. *Drugs* 67: 351-368.
2. Stover CK, Pham XQ, Erwin AL, Mizoguchi SD, Warrener P, et al. (2000) Complete genome sequence of *Pseudomonas aeruginosa* PAO1, an opportunistic pathogen. *Nature* 406: 959-964.
3. Gellatly SL, Hancock RE (2013) *Pseudomonas aeruginosa*: new insights into pathogenesis and host defenses. *Pathog Dis* 67: 159-173.
4. Tenover FC (2006) Mechanisms of antimicrobial resistance in bacteria. *Am J Med* 34: 34-73.
5. Moghaddam MM, Abolhassani F, Babavalian H, Mirnejad R, Azizi-Barjini K, et al. (2012) Comparison of in vitro antibacterial activities of two cationic peptides CM15 and CM11 against *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Vibrio cholerae*, *Acinetobacter baumannii*, and *Escherichia coli*. *Probiotics Antimicrob Proteins* 4: 133-139.
6. Veessenmeyer JL, Hauser AR, Lisboa T, Rello J (2009) *Pseudomonas aeruginosa* virulence and therapy: evolving translational strategies. *Crit Care Med* 37: 1777-1786.
7. Bjarnsholt T, Jensen PØ, Jakobsen TH, Phipps R, Nielsen AK, et al. (2010) Quorum sensing and virulence of *Pseudomonas aeruginosa* during lung infection of cystic fibrosis patients. *PLoS One* 5: e10115.
8. Antunes LC, Ferreira RB, Buckner MM, Finlay BB (2010) Quorum sensing in bacterial virulence. *Microbiology* 156: 2271-2282.
9. Zavascki AP, Carvalhaes CG, Picão RC, Gales AC (2010) Multidrug-resistant *Pseudomonas aeruginosa* and *Acinetobacter baumannii*: resistance mechanisms and implications for therapy. *Expert Rev Anti Infect Ther* 8: 71-93.
10. Høiby N, Bjarnsholt T, Givskov M, Molin S, Ciofu O (2010) Antibiotic resistance of bacterial biofilms. *Int J Antimicrob Agents* 35: 322-332.
11. Livermore DM (2002) Multiple mechanisms of antimicrobial resistance in *Pseudomonas aeruginosa*: our worst nightmare? *Clin Infect Dis* 34: 634-640.
12. Breidenstein EB, de la Fuente-Núñez C, Hancock RE (2011) *Pseudomonas aeruginosa*: all roads lead to resistance. *Trends Microbiol* 19: 419-426.
13. Sadikot RT, Blackwell TS, Christman JW, Prince AS (2005) Pathogen-host interactions in *Pseudomonas aeruginosa* pneumonia. *Am J Respir Crit Care Med* 171: 1209-1223.
14. Decho AW, Norman RS, Visscher PT (2010) Quorum sensing in natural environments: emerging views from microbial mats. *Trends Microbiol* 18: 73-80.
15. Horswill AR, Stoodley P, Stewart PS, Parsek MR (2007) The effect of the chemical, biological, and physical environment on quorum sensing in structured microbial communities. *Anal Bioanal Chem* 387: 371-380.
16. Li YH1, Tian X (2012) Quorum sensing and bacterial social interactions in biofilms. *Sensors (Basel)* 12: 2519-2538.
17. Bjarnsholt T, Tolker-Nielsen T, Høiby N, Givskov M (2010) Interference of *Pseudomonas aeruginosa* signalling and biofilm formation for infection control. *Expert Rev Mol Med* 12: e11.
18. de Kievit TR, Iglewski BH (2000) Bacterial quorum sensing in pathogenic relationships. *Infect Immun* 68: 4839-4849.
19. Deep A, Chaudhary U, Gupta V (2011) Quorum sensing and Bacterial Pathogenicity: From Molecules to Disease. *J Lab Physicians* 3: 4-11.
20. Waters CM, Bassler BL (2005) Quorum sensing: cell-to-cell communication in bacteria. *Annu Rev Cell Dev Biol* 21: 319-346.
21. Rutherford ST, Bassler BL (2012) Bacterial quorum sensing: its role in virulence and possibilities for its control. *Cold Spring Harb Perspect Med* 2.
22. Dandekar AA, Chugani S, Greenberg EP (2012) Bacterial quorum sensing and metabolic incentives to cooperate. *Science* 338: 264-266.
23. Zhang LH, Dong YH (2004) Quorum sensing and signal interference: diverse implications. *Mol Microbiol* 53: 1563-1571.
24. Miller MB, Bassler BL (2001) Quorum sensing in bacteria. *Annu Rev Microbiol* 55: 165-199.
25. Köhler T, Perron GG, Buckling A, van Delden C (2010) Quorum sensing inhibition selects for virulence and cooperation in *Pseudomonas aeruginosa*. *PLoS Pathog* 6: e1000883.
26. Mattmann ME, Blackwell HE (2010) Small molecules that modulate quorum sensing and control virulence in *Pseudomonas aeruginosa*. *J Org Chem* 75: 6737-6746.
27. Raina S, De Vizio D, Odell M, Clements M, Vanhulle S, et al. (2009) Microbial quorum sensing: a tool or a target for antimicrobial therapy? *Biotechnol Appl Biochem* 54: 65-84.
28. Häussler S (2010) Multicellular signalling and growth of *Pseudomonas aeruginosa*. *Int J Med Microbiol* 300: 544-548.
29. Foster KR (2010) Social behaviour in microorganisms. *Social Behaviour: Genes, Ecology and Evolution* Cambridge: Cambridge University Press p:331-356.
30. Fuqua C, Parsek MR, Greenberg EP (2001) Regulation of gene expression by cell-to-cell communication: acyl-homoserine lactone quorum sensing. *Annu Rev Genet* 35: 439-468.
31. Ng WL, Bassler BL (2009) Bacterial quorum-sensing network architectures. *Annu Rev Genet* 43: 197-222.
32. Eglund KA, Greenberg EP (1999) Quorum sensing in *Vibrio fischeri*: elements of the luxI promoter. *Mol Microbiol* 31: 1197-1204.
33. Atkinson S, Chang CY, Sockett RE, Cámara M, Williams P (2006) Quorum sensing in *Yersinia enterocolitica* controls swimming and swarming motility. *J Bacteriol* 188: 1451-1461.
34. Daniels R, Vanderleyden J, Michiels J (2004) Quorum sensing and swarming migration in bacteria. *FEMS Microbiol Rev* 28: 261-289.
35. Annos BA, Fratamico PM, Smith JL (2009) Quorum sensing in biofilms: why bacteria behave the way they do. *J Food Sci* 74(1):R1-R14.
36. Von Bodman SB, Bauer WD, Coplin DL (2003) Quorum sensing in plant-pathogenic bacteria. *Annu Rev Phytopathol* 41: 455-482.
37. Stevens AM, Greenberg EP (1997) Quorum sensing in *Vibrio fischeri*: essential elements for activation of the luminescence genes. *J Bacteriol* 179: 557-562.
38. Visick KL, Foster J, Doiño J, McFall-Ngai M, Ruby EG (2000) *Vibrio fischeri* lux genes play an important role in colonization and development of the host light organ. *J Bacteriol* 182: 4578-4586.
39. Juhas M, Eberl L, Tümmler B (2005) Quorum sensing: the power of cooperation in the world of *Pseudomonas*. *Environ Microbiol* 7: 459-471.
40. Parsek MR, Greenberg EP (2000) Acyl-homoserine lactone quorum sensing in gram-negative bacteria: a signaling mechanism involved in associations with higher organisms. *Proc Natl Acad Sci U S A* 97: 8789-8793.
41. Schuster M, Sexton DJ, Diggie SP, Greenberg EP (2013) Acyl-homoserine lactone quorum sensing: from evolution to application. *Annu Rev Microbiol* 67: 43-63.
42. Xavier KB, Bassler BL (2005) Interference with AI-2-mediated bacterial cell-cell communication. *Nature* 437: 750-753.
43. Vendeville A, Winzer K, Heurlier K, Tang CM, Hardie KR (2005) Making 'sense' of metabolism: autoinducer-2, LuxS and pathogenic bacteria. *Nat Rev Microbiol* 3: 383-396.
44. Xavier KB, Bassler BL (2003) LuxS quorum sensing: more than just a numbers game. *Curr Opin Microbiol* 6: 191-197.
45. Kleerebezem M, Quadri LE, Kuipers OP, de Vos WM (1997) Quorum sensing by peptide pheromones and two-component signal-transduction systems in Gram-positive bacteria. *Mol Microbiol* 24: 895-904.
46. Sturme MH, Kleerebezem M, Nakayama J, Akkermans AD, Vaughn EE, et al. (2002) Cell to cell communication by autoinducing peptides in gram-positive bacteria. *Antonie Van Leeuwenhoek* 81: 233-243.
47. Lyon GJ, Novick RP (2004) Peptide signaling in *Staphylococcus aureus* and other Gram-positive bacteria. *Peptides* 25: 1389-1403.
48. Claverys JP, Martin B (2003) Bacterial "competence" genes: signatures of active transformation, or only remnants? *Trends Microbiol* 11: 161-165.
49. Martin B, Soulet AL, Mirouze N, Prudhomme M, Mortier-Barrière I, et al. (2013) ComE/ComE-P interplay dictates activation or extinction status of pneumococcal X-state (competence). *Mol Microbiol* 87: 394-411.
50. White CE, Winans SC (2007) Cell-cell communication in the plant pathogen *Agrobacterium tumefaciens*. *Philos Trans R Soc Lond B Biol Sci* 362: 1135-1148.

51. Girard G, Bloemberg GV (2008) Central role of quorum sensing in regulating the production of pathogenicity factors in *Pseudomonas aeruginosa*. Future Microbiol 3: 97-106.
52. Ledgham F, Ventre I, Soscia C, Foglino M, Sturgis JN, et al. (2003) Interactions of the quorum sensing regulator QscR: interaction with itself and the other regulators of *Pseudomonas aeruginosa* LasR and RhlR. Mol Microbiol 48: 199-210.
53. Schuster M, Lostroh CP, Ogi T, Greenberg EP (2003) Identification, timing, and signal specificity of *Pseudomonas aeruginosa* quorum-controlled genes: a transcriptome analysis. J Bacteriol 185: 2066-2079.
54. Pearson JP, Pesci EC, Iglewski BH (1997) Roles of *Pseudomonas aeruginosa* las and rhl quorum-sensing systems in control of elastase and rhamnolipid biosynthesis genes. J Bacteriol 179: 5756-5767.
55. Pesci EC, Pearson JP, Seed PC, Iglewski BH (1997) Regulation of las and rhl quorum sensing in *Pseudomonas aeruginosa*. J Bacteriol 179: 3127-3132.
56. Seed PC, Passador L, Iglewski BH (1995) Activation of the *Pseudomonas aeruginosa* lasI gene by LasR and the *Pseudomonas* autoinducer PAI: an autoinduction regulatory hierarchy. J Bacteriol 177: 654-659.
57. Smith RS, Fedyk ER, Springer T, Mukaida N, Iglewski BH, et al. (2001) IL-8 production in human lung fibroblasts and epithelial cells activated by the *Pseudomonas* autoinducer N-3-oxododecanoyl homoserine lactone is transcriptionally regulated by NF- κ B and activator protein-2. J Immunol 167: 366-374.
58. Smith RS, Kelly R, Iglewski BH, Phipps RP (2002) The *Pseudomonas* autoinducer N-(3-oxododecanoyl) homoserine lactone induces cyclooxygenase-2 and prostaglandin E2 production in human lung fibroblasts: implications for inflammation. J Immunol 169: 2636-2642.
59. Pesci EC, Milbank JB, Pearson JP, McKnight S, Kende AS, et al. (1999) Quinolone signaling in the cell-to-cell communication system of *Pseudomonas aeruginosa*. Proc Natl Acad Sci U S A 96: 11229-11234.
60. Parsek MR, Val DL, Hanzelka BL, Cronan JE Jr, Greenberg EP (1999) Acyl homoserine-lactone quorum-sensing signal generation. Proc Natl Acad Sci U S A 96: 4360-4365.
61. Li Z, Nair SK (2012) Quorum sensing: how bacteria can coordinate activity and synchronize their response to external signals? Protein Sci 21: 1403-1417.
62. Venturi V (2006) Regulation of quorum sensing in *Pseudomonas*. FEMS Microbiol Rev 30: 274-291.
63. Kalia VC (2013) Quorum sensing inhibitors: an overview. Biotechnol Adv 31: 224-245.
64. Amara N, Krom BP, Kaufmann GF, Meijler MM (2011) Macromolecular inhibition of quorum sensing: enzymes, antibodies, and beyond. Chem Rev 111: 195-208.
65. Kalia VC, Purohit HJ (2011) Quenching the quorum sensing system: potential antibacterial drug targets. Crit Rev Microbiol 37: 121-140.
66. Smith RS, Iglewski BH (2003) *Pseudomonas aeruginosa* quorum sensing as a potential antimicrobial target. J Clin Invest 112: 1460-1465.
67. Rasmussen TB, Givskov M (2006) Quorum sensing inhibitors: a bargain of effects. Microbiology 152: 895-904.
68. Miyairi S, Tateda K, Fuse ET, Ueda C, Saito H, et al. (2006) Immunization with 3-oxododecanoyl-L-homoserine lactone-protein conjugate protects mice from lethal *Pseudomonas aeruginosa* lung infection. J Med Microbiol 55: 1381-1387.
69. Hirakawa H, Harwood CS, Pechter KB, Schaefer AL, Greenberg EP (2012) Antisense RNA that affects *Rhodospseudomonas palustris* quorum-sensing signal receptor expression. Proc Natl Acad Sci U S A 109: 12141-12146.
70. Umesha S, Shivakumar J (2013) Bacterial quorum sensing and its applications in biotechnology. Int J Pharm Bio Sci 4: 850-861.
71. Winson MK, Swift S, Fish L, Throup JP, Jørgensen F, et al. (1998) Construction and analysis of luxCDABE-based plasmid sensors for investigating N-acyl homoserine lactone-mediated quorum sensing. FEMS Microbiol Lett 163: 185-192.
72. Steindler L, Venturi V (2007) Detection of quorum-sensing N-acyl homoserine lactone signal molecules by bacterial biosensors. FEMS Microbiol Lett 266: 1-9.
73. Yu YA, Shabahang S, Timiryasova TM, Zhang Q, Beltz R, et al. (2004) Visualization of tumors and metastases in live animals with bacteria and vaccinia virus encoding light-emitting proteins. Nat Biotechnol 22: 313-320.
74. Anderson JC, Clarke EJ, Arkin AP, Voigt CA (2006) Environmentally controlled invasion of cancer cells by engineered bacteria. J Mol Biol 355: 619-627.
75. Dong Y, Wang LH, Zhang LH (2007) Quorum-quenching microbial infections: mechanisms and implications. Philos Trans R Soc Lond B Biol Sci 362: 1201-1211.