IJBPAS, August, 2020, 9(8): 1812-1831

ISSN: 2277–4998

International Journal of Biology, Pharmacy and Allied Sciences (IJBPAS)

'A Bridge Between Laboratory and Reader'

www.ijbpas.com

QUORUM SENSING IN BACTERIAL VIRULENCE AND POSSIBILITIES FOR ITS CONTROL: A REVIEW

SARKAR S AND DAS RK*

Department of Chemistry, University of North Bengal, Darjeeling, West Bengal, India, 734013

***Corresponding Author: E Mail: rajeshnbu@gmail.com**

Received 6th Dec. 2019; Revised 4th Jan. 2020; Accepted 4th Feb. 2020; Available online 1st Aug. 2020

https://doi.org/10.31032/IJBPAS/2020/9.8.5128

ABSTRACT

Quorum sensing is a cell-cell communication process that allows bacteria to detect and respond to cell population density. Upon reaching a critical concentration, quorum sensing enables bacteria population to communicate and coordinate collective behaviour. This coordination mechanism allows bacteria to regulate gene expression accordingly through the use of signal molecules. Pathogenic bacteria use quorum sensing in the expression of virulence factors in disease and infection processes. Once a population reaches a threshold, relatively harmless bacteria overpower host defence mechanism activating genes regulating biofilm formation and virulence. Although it is very common among bacteria; molecular mechanisms, signal structures, gene regulons, and behavioural responses associated with quorum-sensing systems may greatly differ. Moreover, the way different types of bacteria apply quorum sensing varies widely. In this article we review the quorum-sensing circuits of *Staphylococcus aureus*, *Pseudomonas aeruginosa* to illustrate uniqueness of controlling virulence factors in each trait. Quorum sensing has been considered an attractive arena for the development of new antimicrobial therapeutics. Here we also discuss some of the recent techniques to encounter bacterial virulence based on quorum sensing inhibition mechanism.

Keywords: Quorum sensing, gene expression, virulence factors, *S.aureus*, *P. aeruginosa*, inhibition mechanism

INTRODUCTION

Bacteria are prokaryotes, single-celled organisms that thrive in diverse environments. For a long time they were believed to exist as individual cells that only consumed nutrients and multiply. The idea changed after the discovery of intercellular communication, like multicellular organisms, among bacteria. The bacteria when behave collectively as a group has certain advantages, for example, the ability to migrate to a more suitable environment for better nutrient supply and to adopt new model of growth, such as sporulation or biofilm formation which protect them from deleterious environments **[1]**. They communicate with each other through a code language which is based on self generated signal molecules called auto inducers (AIs). According to population density, bacteria can regulate their co-ordial behaviour using AI .This cell-cell communication system known as quorum sensing (QS) which can vary widely across different types of bacteria. In many organisms, QS regulates virulence function and is important for pathogenesis **[2]**. Numerous studies have been done to understand that how quorum sensing coordinates intra and inter species interactions. In this review, we discuss recent advancements in this field. The knowledge gathered from these studies has the potential to guide investigations of microbial sociality in natural settings and the design of new drugs and novel therapeutics.

Mechanism of quorum sensing

Bacterial cell-cell communication process known as quorum sensing that includes the production, detection and response to extra cellular small diffusible molecules. These molecules act as a signalling molecules and termed as autoinducers (AIs). Bacterium produces autoinducers at minimal levels continually and their concentration increases gradually with growth. AIs accumulate in environment as bacterial population density increases and reaches to critical concentration level which can be monitored and detected by bacteria. These signal molecules can diffuse through membranes and at critical concentration can bind to an active receptor inside bacterial cells. Bacteria then collectively alter gene expression to activate behaviours that are beneficial for their survival **[3-8]**.

Many classes of AIs have been discovered so far. QS signalling molecules can be divide into three major groups: N-acylhomoserine lactones (AHLs) are generated by Gram-negative bacteria, oligopeptides are used by Gram-negative bacteria and third interesting type autoinducer-2 (AI-2),a ribose derivative [4,5-dihydroxy-2,3-pentanedione (DPD)],

is used by both Gram-negative and Gram-positive bacteria **(Figure 1) [9, 10]**.

QS signalling plays a major role in bacterial virulence and thus QS systems might be a new target for controlling microbial virulence **[11-14]**.

QS in Gram-negative and Gram-positive bacteria

All known QS systems follow three basic principles though their molecular mechanism may differ. At first AIs are produced by community members. At low cell density (LCD), AIs diffuse away and are not detected as concentration falls below the threshold level. At high cell density (HCD) cumulative production leads the AIs concentration above threshold required for detection and response **[15]**. In second step, receptors that present in cytoplasm or in the membrane detect AIs. Final and third step activates gene expression and in addition to that results in more AI production. This positive feed-back autoinduction loop establishes synchrony in the population **[16, 17]**.

Gram-positive bacteria communicate using autoinducing peptides (AIPs) as signalling molecules. After production inside the cell AIPs are processed and secreted. At high cell density AIPs bind to a cognate membrane-bound two-component histidine kinase receptor. This activates receptor and through autophosphorylation passes phosphate to a cognate cytoplasmic response regulator. The phosphorylated response regulator in turn activates transcription of the genes in the QS regulon **(Figure 2A) [18-20]**. In some Gram-positive bacteria AIPs are carried back into the cell cytoplasm where they bind with transcription factors and modulate gene expression changes **(Figure 2B) [18, 21]**.

Gram-negative bacteria utilise acylhomoserine lactones (AHLs) as signalling molecules. There are other type of small molecules do exist. Production of them depends on S-adenosylmethionine as a substrate. As AIs are produced they diffuse through cell membranes. Concentration of AIs reaches to threshold level when cell density is high. AIs bind cytoplasmic receptors and activate transcription factors. AI-bound receptors modulate expression of the genes in the QS regulon **(Figure 2C) [7]**. In some Gram-negative bacteria AIs are detected by two-component histidine kinase receptors and the function is same as described earlier for Gram-positive bacteria **[18, 19]**.

Here, we review the quorum sensing mechanisms employed to regulate the collective production of virulence factors in case of two human pathogens- one for Gram-positive and one for Gram-negative. We also discuss the available strategies to restrain bacterial virulence using quorum sensing inhibitors.

Quorum sensing in *Staphylococcus aureus* virulence

Staphylococcus aureus, a Gram-positive bacteria, follows principles common to all QS circuits, production, detection and response to AIs. This organism uses oligopeptide as AIP and is detected by membrane bound two component signal transduction system **(Figure 3) [22]**.

Two component QS system is encoded by accessory gene regulator (*agr*) locus **[19]**. Oligopeptide (AIP) encoded by *agrD* is trimmed and secreted by a membrane-bound protein AgrB **[23-25]**. Active AIP is 7-9 aa , consists of 5-membered thiolactone ring **[26]**. Extracellular AIPs bind to a membrane-bound sensor kinase AgrC which gets autophosphorylated and leads to activation of AgrA **[23, 27, 28]**. The *agr* system regulates virulence and activates two promoters P2 and P3. These two promoters produce RNAII and RNAIII respectively **[16, 26]**. Phosphorylated AgrA promotes transcription at the promoters P2 and P3 with higher susceptibility towards P2 **[27, 30]**. Promoter P3 transcripts RNAIII, the most effective molecule of *agr* system **[26]**. RNAIII, 514 nt, functions as a mRNA for δ-toxin **[16, 31-33]**. 5' end of this regulatory RNA regulates α-haemolysin 3'end represses protein A synthesis **[29, 33]**. RNAIII decreases the expressions of cell surface virulence factors, and increases the transcription of several virulence factor including TSS toxin-1,hemolysins, proteases **[19,26]**.

The *agr* system regulates 23 genes encoded virulence factors which are of two types **[30]**. The first type responsible for attachments and immune invasion while second type involve in production of exoproteins and toxins **[34, 35]**. Activated *agr* system leads a bacterium from merely adhesive to an invasive pathogen **[26]**.

Four different types of *agr* polymorphism, named as I-IV, have been discovered **[Table1] [30, 36]**. Each type has a distinct AIP which able to activate corresponding receptor of same group **[36]**. However type I and IV capable of cross activate and type II and III capable of cross inhibit themselves **[30, 37, 38]**. This correlation between *agr* groups makes *S. aureus* more fatal but may leads to cross group inhibition therapies **[30, 39]**.

Several infections involving staphylococcal are not caused by free living individual but rather by community of interacting cells named biofilms. It may attach to a biotic or abiotic surface. It is a self producing matrix and has altered growth and gene expression profile compared to free living bacteria **[40]**. Biofilm production is a multi-step process. It begins with adhesion of bacteria to a surface after that attachment divide and form macrocolonies. The macrocolonies with various topographies develop into mature biofilms later. The final step of the

process is detachment of biofilm and significant for spreading of an infection **[41]**. There are two independent mechanism that *S.aureus* uses in biofilm formation, one involves extracellular polysaccharide, polysaccharide intercellular adhesion (PIA) and the another one is PIA-independent adhesive protein, *sarA* and *agr* global regulators **[19, 42]**. The *agr* plays vital role in biofilm formation. An *agr* mutant *S.aureus* has increased adherence and has detachment defect **[33, 43, 44]**. They produce robust static biofilm **[19]**. On contrary *agr* active counterpart has capability to regulate biofilm formation by reducing adherence and increasing production of δ-haemolysin and proteases both **[19]**. The *agr* plays different roles during infection. In some diseases specific *agr* groups are involved whereas other diseases may involve all four *agr* groups **[30]**.

RNAIII is the most effective molecule of *agr* system and has significant role in bacterial virulence. Therefore mechanism to inhibit RNAIII is challenging and several studies has been made on this topic. It is found that an RNAIII-inhibiting peptide (RIP) is able to inhibit *S.aureus* biofilm formation and toxin production **[45]**. RIP can prevent adherence and S. aureus virulence and has minimal adverse effect **[45, 46]**.

S. aureus is present in the normal human skin and found in 30% adult population. It can cause skin infections which may leads to pneumonia, bacteraemia, and sepsis **[47, 48]**. It is a very dangerous opportunistic bacteriam and has greater antibiotic resistance which imposes serious risk for Staphylococcus infection **[30, 46]**. Balaban and his associates had treated several cases with RIP and showed that it can prevent *S. aureus* growth in a urethral stent model. They also found that even after 2 days infection RIP was capable of suppressing bacterial growth. This antibody acts as a quorum sensing quencher **[46]**.

Quorum sensing in *Pseudomonas aeruginosa* virulence

P.aeruginosa is a Gram-negative bacterium and uses quorum sensing mechanism to express bacterial virulence. In this case three QS circuits are interlinked **(Figure 4) [49]**. Two of them are LuxI/LuxR type and third one is non-LuxI/LuxR-type known as *Pseudomonas* quinolone signal (PQS). *las* and *rhl,* homolog of LuxR, depend on production of AHLs as signalling molecules. In *las* system *lasI* gene encodes the enzyme that produces N-3-oxododecanoyl-homoserine lactone (3OC12-HSL) **(Table 2) [3]**. 3OC12-HSL when cell density reaches to threshold value binds and activate LasR which in turn binds to target promoters to regulate gene expression **[49]**. In similar fashion *rhlI*

gene of *rhl* system encodes enzyme to produce N-butyryl-homoserine lactone (C4-HSL). 3OC12-HSL binds with C4-HSL and activate transcriptional regulator, RhlR, to control the activity of target promoters **[49]**. At transcriptional or post transcriptional level *las* system dominates over *rhl* system **[50]**. In addition with LasR and RhlR an orphan receptor protein, QscR, which can detect 3OC12-HSL to control its self regulon **[8, 51, 52]**.

In *P. aeroginosa* quorum sensing regulates the expression of many virulence factors responsible for development of disease, involves in biofilm formation and controls the production of secreted factors like proteases as well as cell-associated factors like lipopolysaccaride & flagella **[8, 52-57]**. The two quorum sensing systems *las* and *rhl* regulate the production of various virulence factors such as elastase, alkaline proteases, exotoxin A, rhamnolipids, pyocianin, lectins, and superoxidase dismutase **[58, 59]**. These two QS systems also regulate the expression of antibiotic efflux pumps and make *P. aeruginosa* highly antibiotic resistant pathogen **[60]**.

In case of biofilm formation both lasI and rhlI play active role. Activity of lasI gene gets maximum at the day 4 and decreases between days 6 and 8 of biofilm development. Expression of rhlI fluctuates during biofilm formation **[61-64]**. lasI mutant traits do not produce normal biofilm whereas rhlI mutants are phenotypically different **[61]**. Moreover it is found that *P. aeruginosa rhlI* and *lasI* mutant strains result in less tissue destruction and decreases mortality rate in comparison with wild-type strains. This establish the importance of quorum sensing in *P.aeruginosa* pathogenesis **[59]**.

In addition to regulate expression of virulence factors some of the AIs affect immune responses to infection **[65]**. 3OC12-HSL inhibits lymphocyte proliferation, down-regulates TNF-α & IL-12 and activates T-cells to produce gamma-interferon **[49, 59, 65-67]**. This AI affect NFkB immune response regulator also **[68]**. The other QS regulators bind to LuxR are TraR. SdiR, and CviR **[69-72]**. According to the structures binding of these ligand to the receptor stabilizes folding of the hydrophobic core of the protein. Properly folded LasR and non-LuxR- type can dimerize, bind DNA and activate transcription **[73-75]**.

Pseudomonas quinolone signal (PQS), the third type QS circuit, secrets 2-heptyl-3hydroxyl-4-quinolone (HHQ) as AI to control virulence factor gene expression **[76-78]**. The AI belongs to 2-alkyl-4-quinolone (AQ) family produced by PqsA, PqsB, PqsC, PqsD and PqsH and is identified by the regulator PqsR. LasR activates expression of *pqsH* and *pqsR* whereas RhlR-C4HSL represses *pqsABCD*

and *pqsR* **[79, 80]**. PqsR controls pqs operon itself and results in a positive feedback loop **[77, 79, 81, 82]**. Hence PQS circuit involves to the lasI/LasR and RhlI/RhlR QS systems and influences virulence factor production **[79, 83, 84]**.

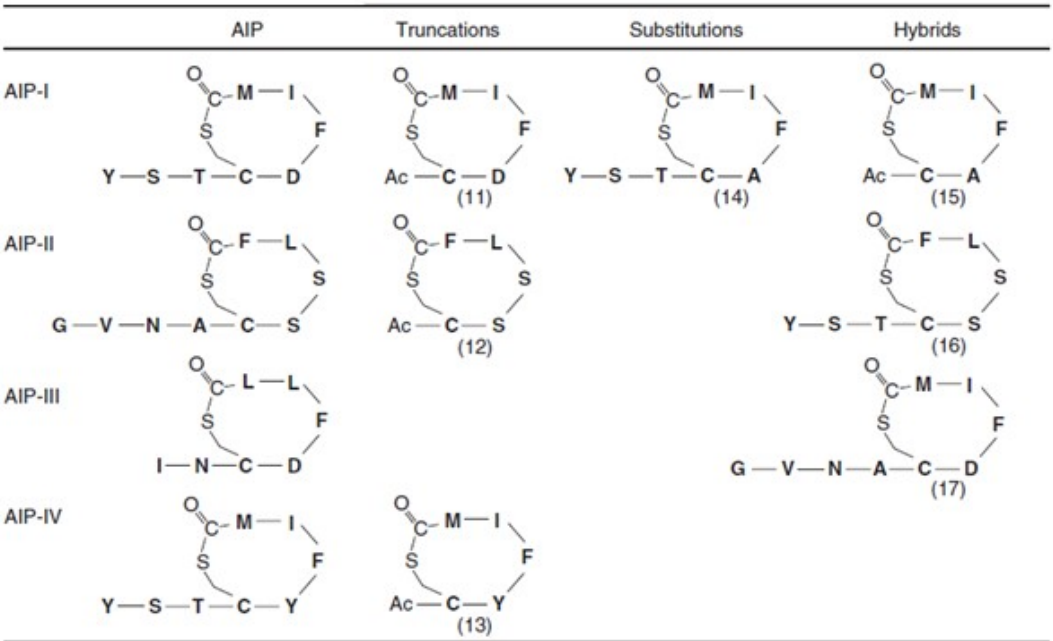
Transcriptome analysis found that 90 genes are regulated by *pqs* system **[82, 85]**. Moreover *pqs* controls biofilm formation and regulates several virulence factors including elastase, pyocyanin and LecAlectin. LecAlectin is essential for virulence in multiple hosts **[82, 86, 87]**. Apart from these PQS and HHQ down regulates the host immune response repressing immune response regulator NFkB **[88]**. In case of iron homeostasis PQS acts as an iron chelator and the process depends on activity of PqsR-PQS **[85, 89]**. These suggest that *pqs* system has significant role in *P. aeruginosa* pathogenesis.

QscR is an orphan LuxR homolog and have no partner LuxI homolog. It produces LasI,3OC12HSL when binds with AI **[90, 91]**. QscR forms dimmers with LasR and RhlR and make them inactive **[92]**. It may stop aberrant QS responses before quorum of cells has been reached.

The knowledge of *P. aeruginosa* QS circuits and inhibition technique may lead to discovery of novel antimicrobials therapeutics. A lot of studies have been performed to discover molecules that has the ability to inhibit quorum sensing in this organism. Some of the natural quorum sensing inhibitors are cyclic sulphur compounds **[93]**, halogenated furanones **[94]**, patulin and penicillin acid **[95]**. Garlic extracts and 4-nitro-pyridine-N-oxide also have the potential to inhibit quorum sensing in *P.aeruginosa* **[96]**. These molecules can decrease virulence expression and reduce the growth of biofilm formation. It is found that molecules derived from the plant species *Combretum albiflorum* can also be used as quorum sensing inhibitor **[97]**. Moreover based on these natural inhibitor some new inhibitory molecules have been synthesized and successfully used as potential drugs **[98-100]**.

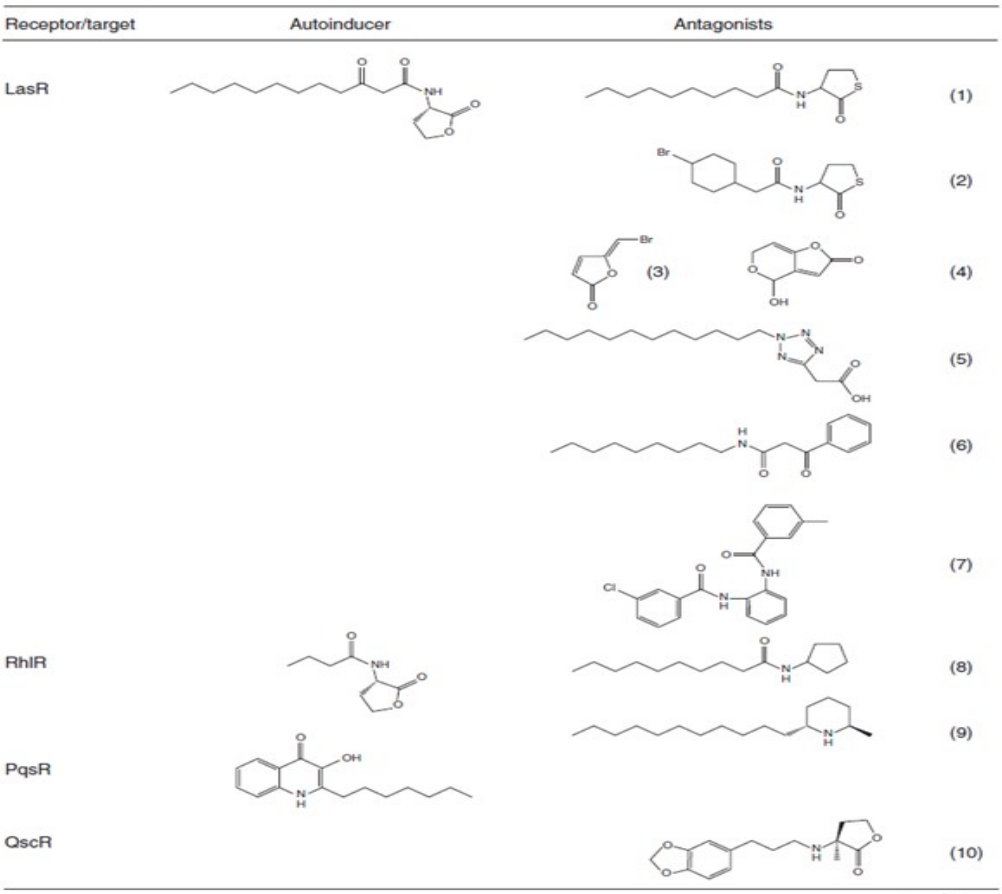
It is found that targeting regulators that affect both the LasI/LaR and RhlI/RhlR quorum sensing is most effective. Agonists and antagonists of QscR are the example of this .They can delay expression of virulence factors and lessen the overall quorum sensing response **[100-114]**. Some synthesized AHLs that target QscR also inhibit LasR predicts that there is a possibility of a compound that can target the entire *P.aeroginosa* quorum sensing systems **(Table 2, compound 10) [115]**.

Table: 1 *S. Aureus* AIPs and inhibitors



Inhibitors 11, 13, 15, 16, and 17 are discussed in [45]; inhibitor 12 is mentioned in [46]; and inhibitor 14 is described in [47]

Table 2: *P. aeruginosa* AIs and inhibitors



The three AIs (and their cognate receptors) are 3OC12HSL (LasR), C4HSL (RhIR), and PQS (PqsR). Inhibitors 1 and 2 [96]; inhibitor 3, a furanone [100-101]; inhibitor 4 is patulin [99]; inhibitors 5 and 6 [107]; inhibitor 7, a triphenyl derivative [108]; inhibitor 8 is C10-acyl-cyclopentylamine described in [109]; inhibitor 9 is solenopism [110]; and compound 10 is propanoyl homoserine lactone [112]

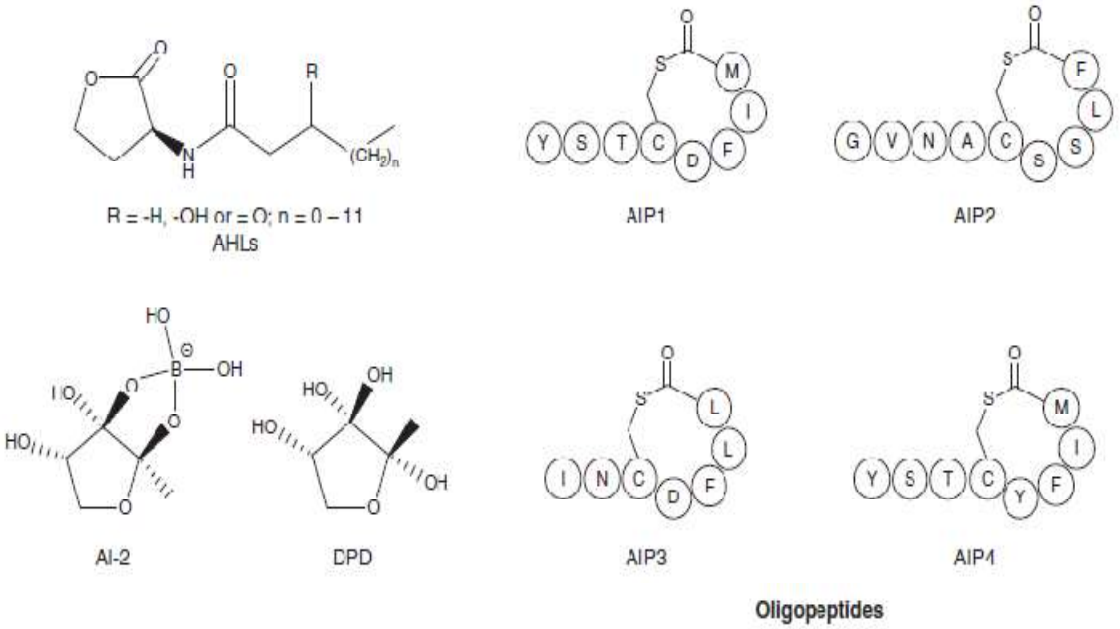


Figure 1: Examples of bacterial quorum sensing signalling molecules, representing the three major classes of autoinducers. AHLs: N-acyl homoserine lactones; Al-2: Autoinducer-2; AIP: Autoinducing peptide; DPD: 4, 5-dihydroxy-2, 3-pentanedione

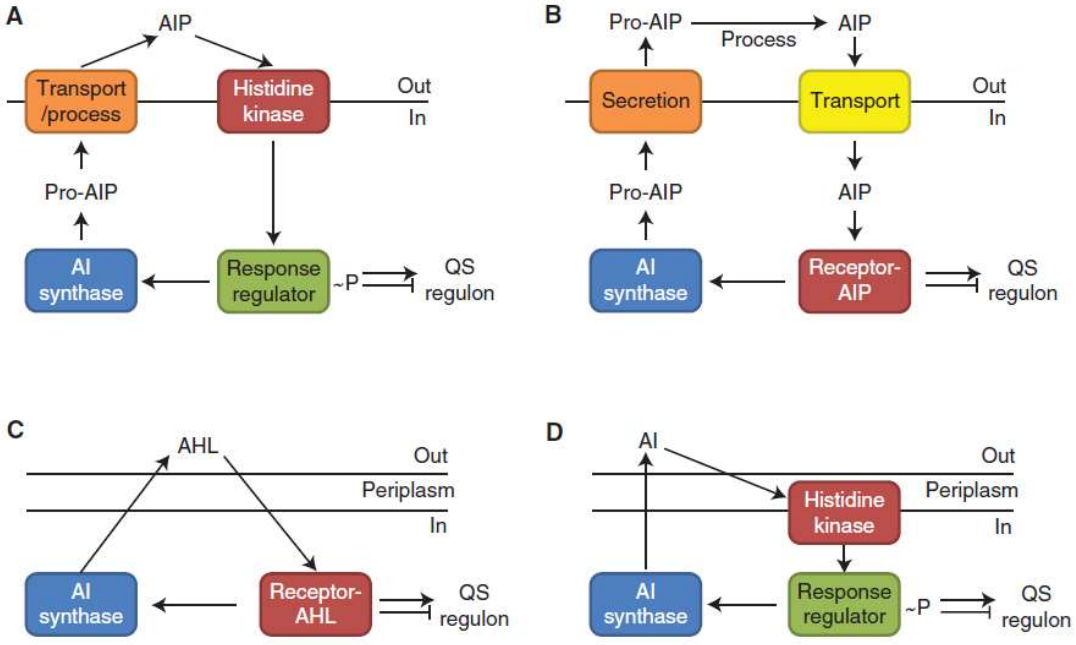


Figure 2: Canonical bacterial quorum-sensing (QS) circuits. Autoinducing peptide (AIP) QS in gram-positive bacteria by (A) two-component signalling, or (B) an AIP-binding transcription factor. Small molecule QS in Gram-negative bacteria by (C) a LuxI/LuxR-type system, or (D) two-component signalling

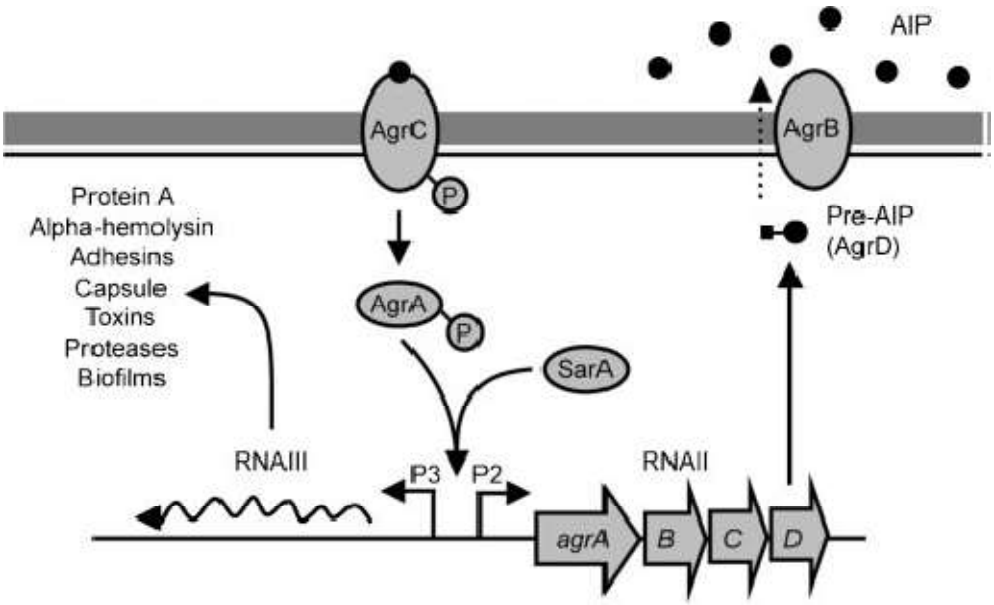


Figure 3: The accessory gene regulator (agr) quorum sensing system of *S. aureus*

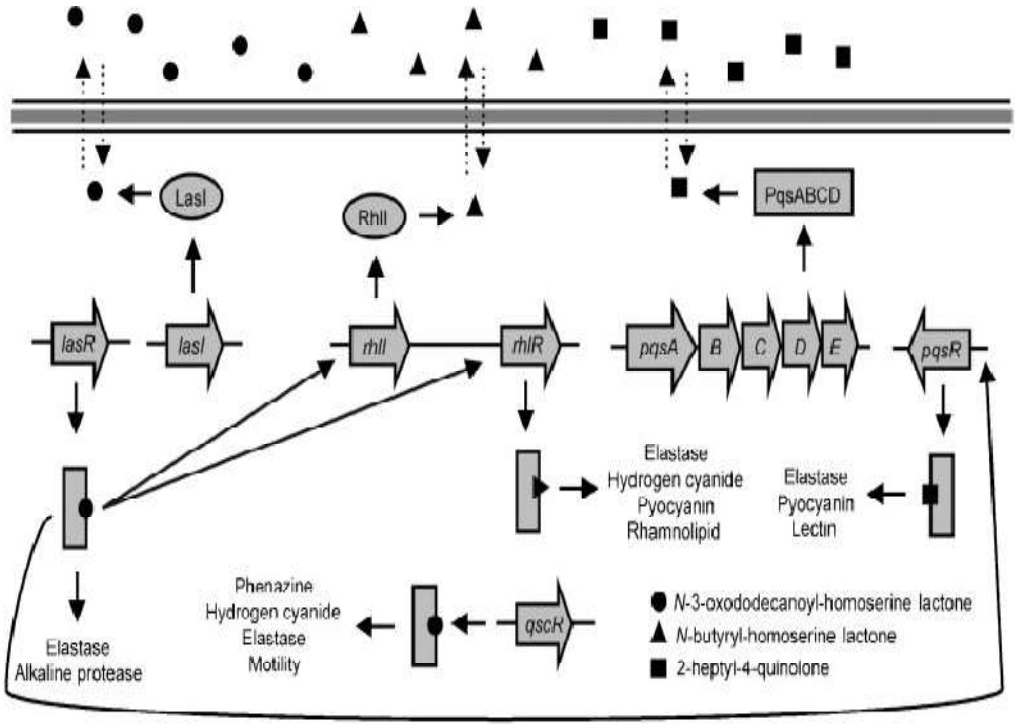


Figure 4: Quorum sensing control of gene expression in *P. aeruginosa*

CONCLUSION

A lot of experiments have been performed on bacterial quorum sensing so far and profound knowledge has been gathered about the mechanism used by bacteria to coordinate and regulate the virulence expressions. Nevertheless, discovery of new molecule and their effects on microbial virulence are still going on. Traditional antimicrobial therapy to combat bacterial virulence may lead to increase the resistance power of pathogenic bacteria. Thus quorum sensing inhibition represents the promising area to design new antivirulence drugs. The examples discussed here showed that inhibition of virulence through quorum sensing inhibition is possible and would be more practical. Nowadays challenge is lying in making novel therapeutics using this knowledge and overwhelm the uncontrolled and unwise use of harmful antibiotics.

Conflicts of interest

Authors declare that there is no potential conflict of interest.

ACKNOWLEDGEMENTS

The authors are grateful to the Departmental Special Assistance Scheme under the University Grants Commission, New Delhi and Head, all the members, Department of Chemistry, University of North Bengal, Darjeeling, India, for providing various facilities in connection with this review work.

REFERENCES

[1] Teresa R, De Kievit, Barbara H, Iglewski; Bacterial quorum sensing in pathogenic relationships. ASM 68, 2000, 4839-4849.

[2] Abisado RG, Benomar S, Klaus JR, Dandekar AA, Josephine RC; Bacterial quorum sensing and microbial community interactions. mBio 9, 2018, e02331-17.

[3] Rutherford ST, Bassler BL; Bacterial Quorum Sensing: Its Role in Virulence and Possibilities for Its Control. Cold Spring Harb Perspect Med 2, 2012, a012427. https://doi.org/10.1101/cshperspect.a012427

[4] Antunes LC, Ferreira RB, Buckner MM, Finlay BB; Quorum sensing in bacterial virulence. Microbiology 156, 2010, 2271-2282.

[5] Antunes LC, Ferreira RB; Intercellular communication in bacteria. Crit Rev Microbiol 35, 2009, 69–80.

[6] Bassler BL, Losick R; Bacterially speaking. Cell 125, 2006, 237–246.

[7] Fuqua WC, Winans SC, Greenberg EP; Quorum sensing in bacteria: the LuxR–LuxI family of cell density-responsive transcriptional regulators. J Bacteriol 176, 1994, 269–275.

[8] Fuqua C, Parsek MR., Greenberg EP Regulation of gene expression by cell-to-cell communication: acyl-homoserine lactone quorum sensing. Annu Rev Genet 35, 2001, 439–468.

[9] Waters CM, Bassler BL; Quorum sensing: cell-to-cell communication in bacteria. Ann Rev Cell Dev Biol 21, 2005, 319-346.

[10] Reading NC, Sperandio V; Quorum sensing: the many languages of bacteria. FEMS Microbiol Lett 254, 2006, 1 -11.

[11] Finch RG, Pritchard DI, Bycroft BW *et al*; Quorum sensing: a novel target for anti-infective therapy. J Antimicrob Chemother 42, 1998, 569 -571.

[12] Otto M; Quorum-sensing control in *Staphylococci* – a target for anti microbial drug therapy? FEMS Microbiol Lett 241, 2004, 135 -141.

[13] Smith RS, Iglewski BH; *Pseudomonas aeruginosa* quorum sensing as a potential antimicrobial target. J Clin Invest 112, 2003, 1460 -1465.

[14] Clatworthy AE, Pierson E, Hung DT; Targeting virulence: a new paradigm for antimicrobial therapy. Nat Chem Biol 3, 2007, 541 -548.

[15] Kaplan HB, Greenberg EP, Diffusion of autoinducer is involved in regulation of the Vibrio fischeri luminescence system. J Bacteriol 163, 1985, 1210–1214.

[16] Novick RP, Projan SJ, Kornblum J, Ross HF, Ji G, Kreiswirth B, Vandenesch F, Moghazeh S; The agr P2 operon: An autocatalytic sensory transduction system in *Staphylococcus aureus*. Mol Gen Genet 248, 1995, 446–458.

[17] Seed PC, Passador L, Iglewski BH; Activation of the *Pseudomonas aeruginosas* I gene by LasR and the Pseudomonas autoinducer PAI: An autoinduction regulatory hierarchy. J Bacteriol 177, 1995, 654–659.

[18] Ng WL, Bassler BL; Bacterial quorumsensing network architectures. Annu Rev Genet 43, 2009, 197–222.

[19] Novick RP, Geisinger E; Quorum sensing in staphylococci. Annu Rev Genet 42, 2008, 541–564.

[20] Taga ME, Semmelhack JL, Bassler BL; The LuxS dependent autoinducer AI-2 controls the expression of an ABC transporter that functions in AI-2 uptake in *Salmonella typhimurium*. Mol Microbiol 42, 2001, 777–793.

[21] Pottathil M, Lazazzera BA; The extracellular Phr peptide–Rap phosphatase signaling circuit of *Bacillus subtilis*. Front Biosci 8, 2003, d32–d45.

[22] Hoch JA, Silhavy TJ; Two-component signal transduction. ASM Press, Washington, DC, 1995.

[23] Ji G, Beavis RC, Novick RP; Cell density control of staphylococcal virulence mediated by an octapeptide pheromone. Proc Natl Acad Sci U S A 92, 1995, 12055–12059.

[24] Saenz HL, Augsburger V, Vuong C, Jack RW, Gotz F, Otto M; Inducible expression and cellular location of AgrB, a protein involved in the maturation of the staphylococcal

quorum-sensing pheromone. Arch Microbiol 174, 2000, 452–455.

[25] Zhang L, Lin J, Ji G; Membrane anchoring of the AgrD N-terminal amphipathic region is required for its processing to produce a quorum-sensing pheromone in Staphylococcus aureus. J Biol Chem 279, 2004, 19448–19456.

[26] Roux A, Payne SM, Gilmore MS; Microbial telesensing: probing the environment for friends, foes, and food. Cell Host Microbe 6, 2009, 115–124.

[27] Koenig RL, Ray JL, Maleki SJ, Smeltzer MS, Hurlburt BK; *Staphylococcus aureus* AgrA binding to the RNAIII-agr regulatory region. J Bacteriol 186, 2004, 7549–7555.

[28] Lina G, Jarraud S, Ji G, Greenland T, Pedraza A, Etienne J, Novick RP, Vandenesch F; Transmembrane topology and histidine protein kinase activity of AgrC, the agr signal receptor in *Staphylococcus aureus*. Mol Microbiol 28, 1998, 655–662.

[29] Morfeldt E., Taylor D, von Gabain A, Arvidson S; Activation of alpha-toxin translation in *Staphylococcus aureus* by the trans-encoded antisense RNA, RNAIII. EMBO J 14, 1995, 4569–4577.

[30] George EA, Muir TW; Molecular mechanisms of agr quorum sensing in virulent staphylococci. Chem BioChem 8, 2007, 847–855.

[31] Balaban N, Novick RP; Translation of RNAIII, the *Staphylococcus aureus* agr regulatory RNA molecule, can be activated by a 39-end deletion. FEMS Microbiol Lett 133, 1995, 155–161.

[32] Benito Y, Kolb FA, Romby P, Lina G, Etienne J, Vandenesch F; Probing the structure of RNAIII, the *Staphylococcus aureus* agr regulatory RNA, and identification of the RNA domain involved in repression of protein A expression. RNA 6, 2000, 668–679.

[33] Kong KF, Vuong C, Otto M; Staphylococcus quorum sensing in biofilm formation and infection. Int J Med Microbiol 296, 2006, 133–139..

[34] Bowden MG, Chen W, Singvall J, Xu Y, Peacock SJ, Valtulina V, Speziale P, Hook M; Identification and preliminary characterization of cell-wall-anchored proteins of *Staphylococcus epidermidis*. Microbiology 151, 2005, 1453–1464.

[35] Yao Y, Vuong C, Kocianova S, Villaruz AE, Lai Y, Sturdevant DE, Otto M ; Characterization of the *Staphylococcus epidermidis* accessory-gene regulator response: quorum-sensing regulation of resistance to human innate host defense. J Infect Dis 193, 2006, 841–848.

[36] Ji G, Beavis R, Novick RP; Bacterial interference caused by autoinducing peptide variants. Science 276, 1997, 2027–2030.

[37] Mayville P, Ji G, Beavis R., Yang H., Goger M., Novick RP, Muir TW;

Structure–activity analysis of synthetic autoinducing thiolactone peptides from *Staphylococcus aureus* responsible for virulence. Proc Natl Acad Sci U S A 96, 1999, 1218–1223.

[38] Otto M, Sussmuth R., Jung G, Gotz F; Structure of the pheromone peptide of the *Staphylococcus epidermidis* agr system. FEBS Lett 424, 1998, 89–94.

[39] Fleming V, Feil E, Sewell AK, Day N, Buckling A Massey RC; Agric interference between clinical *Staphylococcus aureus* strains in an insect model of virulence. J Bacteriol 188, 2006, 7686–7688.

[40] Donlan RM, and Costerton JW; Biofilms: survival mechanisms of clinically relevant microorganisms. Clin Microbiol Rev 15, 2002, 167–193.

[41] Parsek MR, Tolker-Nielsen T; Pattern formation in *Pseudomonas aeruginosa* biofilms. Curr Opin Microbiol 11, 2008, 560–566.

[42] Lauderdale KJ, Boles BR., Cheung AL, Horswill AR; Interconnections between Sigma B, agr, and proteolytic activity in *Staphylococcus aureus* biofilm maturation. Infect Immun 77, 2009, 1623–1635.

[43] Vuong C, Saenz HL, Gotz F, Otto M; Impact of the agr quorum-sensing system on adherence to polystyrene in *Staphylococcus aureus*. J Infect Dis 182, 2000, 1688–1693.

[44] Yarwood JM, Bartels DJ, Volper EM, Greenberg EP; Quorum sensing in *Staphylococcus aureus* biofilms. J Bacteriol 186, 2004, 1838–1850.

[45] Lyon GJ, Wright JS, Muir TW, Novick RP.; Key determinants of receptor activation in the agr autoinducing peptides of *Staphylococcus aureus*. Biochemistry. 41, 2002, 10095–10104.

[46] Lyon GJ, Mayville P, Muir TW, Novick RP.; Rational design of a global inhibitor of the virulence response in *Staphylococcus aureus*, based in part on localization of the site of inhibition to the receptor-histidine kinase AgrC. Proc Natl Acad Sci. 97, 2000, 13330–13335.

[47] McDowell P, Affas Z, Reynolds C, Holden MT, Wood SJ, Saint S, Cockayne A, Hill PJ, Dodd CE, Bycroft BW, et al.; Structure, activity and evolution of the group I thiolactone peptide quorum-sensing system of Staphylococcus aureus. Mol Microbiol. 41, 2001, 503–512.

[48] Cirioni O, Ghiselli R., Minardi D, Orlando F, Mocchegiani F, Silvestri C, Muzzonigro G, Saba V, Scalise G et al; RNAIII-inhibiting peptide affects biofilm formation in a rat model of staphylococcal ureteral stent infection. Antimicrob Agents Chemother 51, 2007, 4518–4520.

[49] Balaban N, Cirioni O, Giacometti A, Ghiselli R., Braunstein JB, Silvestri C, Mocchegiani F, Saba V, Scalise G; Treatment of *Staphylococcus aureus* biofilm infection by the quorum-sensing inhibitor RIP. Antimicrob

Agents Chemother 51, 2007, 2226–2229.

[50] Lowy FD; *Staphylococcus aureus* infections. N Engl J Med 339, 1998, 520–532.

[51] Massey RC, Horsburgh MJ, Lina G, Hook M, Recker M (2006) The evolution and maintenance of virulence in *Staphylococcus aureus*: A role for host-to-host transmission? Nat Rev Microbiol 4:953–958.

[52] De Kievit TR, Iglewski BH; Bacterial quorum sensing in pathogenic relationships. Infect Immun 68, 2000, 4839–4849.

[53] Latifi A, Foglino M, Tanaka K, Williams P, Lazdunski A; A hierarchical quorum-sensing cascade *Pseudomonas aeruginosa* links the transcriptional activators LasR and RhIR (VsmR) to expression of the stationary-phase sigma factor RpoS. Mol Microbiol 21, 1996, 1137–1146.

[54] Chugani SA., Whiteley M., Lee KM, D'Argenio D, Manoil C, Greenberg EP; QscR, a modulator of quorum-sensing signal synthesis and virulence in *Pseudomonas aeruginosa*. Proc Natl Acad Sci U S A 98, 2001, 2752–2757.

[55] Schuster M, Greenberg EP; A network of networks: quorum-sensing gene regulation in *Pseudomonas aeruginosa*. Int J Med Microbiol 296, 2006, 73–81.

[56] Dekimpe V, De´ ziel E; Revisiting the quorum-sensing hierarchy in *Pseudomonas aeruginosa*: the transcriptional regulator RhlR regulates LasR-specific factors. Microbiology 155, 2009, 712–723.

[57] Diggle SP, Cornelis P, Williams P, Camara M; 4- Quinolone signalling in *Pseudomonas aeruginosa*: old molecules, new perspectives. Int J Med Microbiol 296, 2006, 83–91.

[58] Swift S, Downie JA, Whitehead NA, Barnard AM, Salmond GP, Williams P; Quorum sensing as a population-density dependent determinant of bacterial physiology. Adv Microb Physiol 45, 2001, 199–270.

[59] Wagner VE, Bushnell D, Passador L, Brooks AI., Iglewski BH; Microarray analysis of *Pseudomonas aeruginosa* quorum sensing regulons: effects of growth phase and environment. J Bacteriol 185:, 2003, 2080–2095.

[60] Whitehead NA, Barnard AM, Slater H, Simpson NJ, Salmond GP; Quorum-sensing in Gram-negative bacteria. FEMS Microbiol Rev 25, 2001, 365–404.

[61] Schuster M, Lostroh CP, Ogi T, Greenberg EP; Identification, timing, and signal specificity of *Pseudomonas aeruginosa* quorum-controlled genes: a transcriptome analysis. J Bacteriol 185, 2003, 2066–2079.

[62] Smith RS, Iglewski BH; *P.aeruginosa* quorum-sensing systems and virulence. Curr Opin Microbiol 6, 2003, 56–60.

[63] Driscoll JA, Brody SL, Kollef MH; The epidemiology, pathogenesis and treatment of *Pseudomonas aeruginosa* infections. Drugs 67, 2007, 351–368.

[64] Davies DG, Parsek MR., Pearson JP, Iglewski BH, Costerton JW, Greenberg EP; The involvement of cell-to-cell signals in the development of a bacterial biofilm. Science 280, 1998, 295–298.

[65] Patriquin GM, Banin E., Gilmour C, Tuchman R, Greenberg EP, Poole K; Influence of quorum sensing and iron on twitching motility and biofilm formation in *Pseudomonas aeruginosa*. J Bacteriol 190, 2008, 662–671.

[66] Yoon SS, Hennigan RF, Hilliard GM, Ochsner UA., Parvatiyar K, Kamani MC, Allen HL, DeKievit TR, Gardner PR et al; *Pseudomonas aeruginosa* anaerobic respiration in biofilms: relationships to cystic fibrosis pathogenesis. Dev Cell 3, 2002, 593–603.

[67] De Kievit TR, Gillis R., Marx S, Brown C, Iglewski BH; Quorum-sensing genes in *Pseudomonas aeruginosa* biofilms: their role and expression patterns. Appl Environ Microbiol 67, 2001, 1865–1873.

[68] Smith RS, Harris SG, Phipps R, Iglewski B; The *Pseudomonas aeruginosa* quorum-sensing molecule N-(3-oxododecanoyl) homoserine lactone contributes to virulence and induces inflammation in vivo. J Bacteriol 184, 2002, 1132–1139.

[69] Tateda K, Ishii Y, Horikawa M, Matsumoto T, Miyairi S, Pechere JC, Standiford TJ, Ishiguro M, Yamaguchi K; The *Pseudomonas aeruginosa* autoinducer N-3-oxododecanoyl homoserine lactone accelerates apoptosis in macrophages and neutrophils. Infect Immun 71, 2003, 5785–5793.

[70] Telford G, Wheeler D, Williams P, Tomkins PT, Appleby P, Sewell H, Stewart GS, Bycroft BW, Pritchard DI; The *Pseudomonas aeruginosa* quorum-sensing signal molecule N-(3-oxododecanoyl)-L-homoserine lactone has immune modulatory activity. Infect Immun 66, 1998, 36–42.

[71] Kravchenko VV, Kaufmann GF, Mathison JC, Scott DA., Katz AZ, Grauer DC, Lehmann M, Meijler MM, Janda KD, Ulevitch RJ; Modulation of gene expression via disruption of NF-kB signaling by a bacterial small molecule. Science 321, 2008, 259–263.

[72] Vannini A, Volpari C, Gargioli C, Muraglia E, Cortese R, De Francesco R, Neddermann P, Marco SD (2002) The crystal structure of the quorum sensing protein TraR bound to its autoinducer and target DNA. EMBO J 21, 2002, 4393–4401.

[73] Zhang RG, Pappas KM, Brace JL, Miller PC, Oulmassov T, Molyneaux JM, Anderson JC, Bashkin JK, Winans SC, Joachimiak A; Structure of a bacterial quorum sensing transcription factor complexed with pheromone and DNA. Nature 417:971–974.

[74] Yao Y, Martinez-Yamout MA, Dickerson TJ, Brogan AP, Wright PE, Dyson HJ; Structure of the Escherichia

coli quorum sensing protein SdiA: Activation of the folding switch by acyl homoserine lactones. J Mol Biol 355, 2006, 262–273.

[75] Chen G, Swem LR, Swem DL, Stauff DL, O'Loughlin CT, Jeffrey PD, Bassler BL, Hughson FM; A strategy for antagonizing quorum sensing. Mol Cell 42, 2011, 199–209.

[76] Zhu J, Winans SC; The quorum-sensing transcriptional regulator TraR requires its cognate signaling ligandfor protein folding, protease resistance, and dimerization.Proc Natl Acad Sci 98, 2001, 1507–1512.

[77] Kiratisin P, Tucker KD, Passador L; LasR, a transcriptional activator of *Pseudomonas aeruginosa* virulence genes, functions as a multimer. J Bacteriol 184, 2002, 4912–4919.

[78] Urbanowski ML, Lostroh CP, Greenberg EP; Reversibleacyl-homoserine lactone binding to purified Vibrio fischeri LuxR protein. J Bacteriol 186, 2004, 631–637.

[79] Gallagher LA, McKnight SL, Kuznetsova MS, Pesci EC, Manoil C; Functions required for extracellular quinolone signaling by *Pseudomonas aeruginosa*. J Bacteriol184, 2002, 6472–6480.

[80] De'ziel E, Lepine F, Milot S, He J, Mindrinos MN, TompkinsRG, Rahme LG; Analysis of *Pseudomonas aeruginosa*4-hydroxy-2-alkylquinolines (HAQs) reveals arole for 4-hydroxy-2-heptylquinoline in cell-to-cell communication. Proc Natl Acad Sci U S A 101, 2004, 1339–1344.

[81] Xiao G, He J, Rahme LG; Mutation analysis of the *Pseudomonas aeruginosa* mvfR and pqs ABCDE gene promoters demonstrates complex quorum-sensing circuitry. Microbiology 152, 2006b, 1679–1686.

[82] Xiao G, Deziel E, He J, Lepine F, Lesic B, Castonguay MH, Milot S, Tampakaki AP, Stachel SE, Rahme LG; MvfR, a key *Pseudomonas aeruginosa* pathogenicity LTTR-class regulatory protein, has dual ligands. Mol Microbiol 62, 2006a, 1689–1699.

[83] Diggle SP, Matthijs S, Wright VJ, Fletcher MP, Chhabra SR, Lamont IL, Kong X, Hider RC, Cornelis P, Camara M, et al; The *Pseudomonas aeruginosa* 4-quinolone signal molecules HHQ and PQS play multifunctional roles in quorum sensing and iron entrapment. Chem Biol 14:, 2007, 87–96.

[84] Cao H, Krishnan G, Goumnerov B, Tsongalis J, Tompkins R, Rahme LG; A quorum sensing-associated virulence gene of *Pseudomonas aeruginosa* encodes a LysR-like transcription regulator with a unique self-regulatory mechanism. Proc Natl Acad Sci U S A 98, 2001, 14613–14618.

[85] De´ziel E, Gopalan S, Tampakaki AP, Lepine F, Padfield KE, Saucier M., Xiao G, Rahme LG; The contribution of MvfR to *Pseudomonas aeruginosa* pathogenesis and quorum sensing circuitry regulation: multiple quorum

sensing-regulated genes are modulated without affecting lasRI, rhlRI or the production of N-acyl-L-homoserine lactones. Mol Microbiol 55, 2005, 998–1014.

[86] Hazan R., He J, Xiao G., Dekimpe V, Apidianakis Y, Lesic B, Astrakas C, De´ziel E., Le´ pine F , Rahme LG; Homeostatic interplay between bacterial cell–cell signaling and iron in virulence. PLoS Pathog 6, 2010, e1000810.

[87] McGrath S, Wade DS, Pesci, EC; Dueling quorum sensing systems in *Pseudomonas aeruginosa* control the production of the Pseudomonas quinolone signal (PQS). FEMS Microbiol Lett 230, 2004, 27–34.

[88] Bredenbruch F, Geffers R., Nimtz M., Buer J, Ha¨ ussler S; The *Pseudomonas aeruginosa* quinolone signal (PQS) has an iron-chelating activity. Environ Microbiol 8, 2006, 1318–1329.

[89] Rahme LG, Tan MW, Le L, Wong SM, Tompkins RG, Calderwood SB, Ausubel FM; Use of model plant hosts to identify *Pseudomonas aeruginosa* virulence factors. Proc Natl Acad Sci U S A 94, 1997, 13245–13250.

[90] Rahme LG, Ausubel FM, Cao H, Drenkard E, Goumnerov BC, Lau GW, Mahajan-Miklos S, Plotnikova J, Tan MW; Plants and animals share functionally common bacterial virulence factors. Proc Natl Acad Sci U S A 97, 2000, 8815–8821.

[91] Kim K, Kim YU, Koh BH, Hwang SS, Kim SH, Lepine F, ChoYH, Lee GR; HHQ and PQS, two *Pseudomonas aeruginosa* quorum-sensing molecules, down-regulate the innate immune responses through the nuclear factor-kB pathway. Immunology 129, 2009b, 578–588.

[92] Oglesby AG, Farrow JMIII, Lee JH, Tomaras AP, Greenberg EP, Pesci EC, Vasil ML; The influenceof iron on *Pseudomonas aeruginosa* physiology: a regulatory link between iron and quorum sensing. J BiolChem 283, 2008, 15558–15567.

[93] Lequette Y, Lee JH, Ledgham F, Lazdunski A, Greenberg EP ; A distinct QscR regulon in the *Pseudomonas aeruginosa* quorum-sensing circuit. J Bacteriol 188, 2006, 3365–337.

[94] Lintz MJ, Oinuma K, Wysoczynski CL, Greenberg EP, Churchill ME; Crystal structure of QscR, a *Pseudomonas aeruginosa* quorum sensing signal receptor. Proc Natl Acad Sci 108:, 2011, 15763–15768.

[95] Ledgham F, Ventre I, Soscia C, Foglino M, Sturgis JN, Lazdunski A; Interactions of the quorum sensing regulator QscR: interaction with itself and the other regulators of *Pseudomonas aeruginosa* LasR and RhlR. Mol Microbiol 48, 2003, 199–210.

[96] McInnis CE, Blackwell HE; Thiolactone modulators of quorum sensing revealed through library design

andscreening. Bioorg Med Chem. 19, 2011, 4820–4828.

[97] Persson T, Hansen TH., Rasmussen TB, Skinderso ME, Givskov M, Nielsen J (2005) Rational design and synthesis of new quorum-sensing inhibitors derived from acylated homoserine lactones and natural products from garlic. Org Biomol Chem 3, 2005, 253–262.

[98] Givskov M., de Nys R., Manefield M., Gram L, Maximilien R, Eberl L, Molin S, Steinberg PD, Kjelleberg S; Eukaryotic interference with homoserine lactone-mediated prokaryotic signalling. J Bacteriol 178, 1996, 6618–6622.

[99] Rasmussen TB, Skindersoe ME, Bjarnsholt T, Phipps RK, Christensen KB, Jensen PO, Andersen JB, Koch B, Larsen TO; Identity and effects of quorum-sensing inhibitors produced by Penicillium species. Microbiology. 151, 2005b, 1325–1340.

[100] Hentzer M, Riedel K, Rasmussen TB, Heydorn A, Andersen JB, Parsek MR, Rice SA, Eberl L,Molin S, Hoiby N, et al.; Inhibition of quorum sensing in *Pseudomonas aeruginosa* biofilm bacteria by a halogenated furanone compound. Microbiology. 148, 2002, 87–102.

[101] Wu H, Song Z, Hentzer M, Andersen JB, Molin S, Givskov M, Hoiby N.; Synthetic furanones inhibit quorum-sensing and enhance bacterial clearance in *Pseudomonas aeruginosa* lung infection in mice. J Antimicrob Chemother 53, 2004, 1054–1061.

[102] Rasmussen TB, Skindersoe ME, Bjarnsholt T, Phipps RK, Christensen KB, Jensen PO, Andersen JB, Koch B, Larsen TO; Identity and effects of quorum-sensing inhibitors produced by Penicillium species. Microbiology 151, 2005b, 1325–1340.

[103] Vandeputte OM, Kiendrebeogo M, Rajaonson S, Diallo B, Mol A, El Jaziri, M, Baucher M; Identification of catechin as one of the flavonoids from Combretum albiflorum bark extract that reduces the production of quorum-sensing-controlled virulence factors in *Pseudomonas aeruginosa* PAO1. Appl Environ Microbiol 76, 2010, 243–253.

[104] Kim C, Kim J, Park HY, Park HJ, Lee JH, Kim CK, Yoon J; Furanone derivatives as quorum sensing antagonists of *Pseudomonas aeruginosa*. Appl Microbiol Biotechnol 80, 2008, 37–47.

[105] Kim C, Kim J, Park HY, Lee JH, Park HJ, Kim CK, Yoon J; Structural understanding of quorum-sensing inhibitors by molecular modeling study in *Pseudomonas aeruginosa*. Appl Microbiol Biotechnol 83, 2009a, 1095–1103.

[106] Liu HB, Lee JH, Kim JS, Park S; Inhibitors of the *Pseudomonas aeruginosa* quorum-sensing regulator,

QscR. Biotechnol Bioeng 106, 2010, 119–126.

[107] Muh U, Schuster M, Heim R, Singh A, Olson ER, Greenberg EP.; Novel *Pseudomonas aeruginosa* quorumsensing inhibitors identified in an ultra-high-throughput screen. Antimicrob Agents Chemother. 50, 2006b, 3674–3679.

[108] Muh U, Hare BJ, Duerkop BA, Schuster M, Hanzelka BL, Heim R, Olson ER, Greenberg EP.; A structurally unrelated mimic of a *Pseudomonas aeruginosa* acyl-homoserine lactone quorum-sensing signal. Proc Natl Acad Sci. 103, 2006a, 16948–16952.

[109] Ishida T, Ikeda T, Takiguchi N, Kuroda A, Ohtake H, Kato J.; . Inhibition of quorum sensing in *Pseudomonas aeruginosa* by N-acyl cyclopentylamides. Appl Environ Microbiol. 73, 2007, 3183–3188.

[110] Park J, Kaufmann GF, Bowen JP, Arbiser JL, Janda KD.; Solenopsin A, a venom alkaloid from the fire ant Solenopsis invicta, inhibits quorum-sensing signaling in *Pseudomonas aeruginosa*. J Infect Dis. 198, 2008, 1198–1201.

[111] Lee JH, Lequette Y, Greenberg EP; Activity of purified QscR, a *Pseudomonas aeruginosa* orphan quorum-sensing transcription factor. Mol Microbiol 59, 2006, 602–609.

[112] Mattmann ME, Geske GD, Worzalla GA, Chandler JR, Sappington KJ, Greenberg EP, Blackwell HE; Synthetic ligands that activate and inhibit a quorum-sensing regulator in *Pseudomonas aeruginosa*. Bioorg Med Chem Lett 18, 2008, 3072–3075.

[113] Mattmann ME, Shipway PM, Heth NJ, Blackwell HE; Potent and selective synthetic modulators of a quorum sensing repressor in *Pseudomonas aeruginosa* identified from second-generation libraries of N-acylated L-homoserine lactones. ChemBioChem 12, 2011, 942–949.

[114] Amara N, Mashiach R, Amar D, Krief P, Spieser SA, Bottomley MJ, Aharoni A, Meijler MM; Covalent inhibition of bacterial quorum sensing. J Am Chem Soc 131, 2009, 10610–10619.

[115] Mattmann ME, Blackwell HE; Small molecules that modulate quorum sensing and control virulence in *Pseudomonas aeruginosa*. J Org Chem 75, 2010, 6737–6746.