IMPACT: International Journal of Research in Applied, Natural and Social Sciences (IMPACT: IJRANSS)
ISSN(E): 2321-8851; ISSN(P): 2347-4580
Vol. 2, Issue 2, Feb 2014, 117-124

QUORUM SENSING AND ITS DIFFERENT SIGNALS SYSTEMS IN BACTERIA

MUHAMMAD KAMRAN TAJ[1], WEI YUNLIN[2], ZOHRA SAMREEN[3], IMRAN TAJ[4], TAJ MUHAMMAD HASSANI[5] & JI XIU LING[6]

[1,2,6]Kunming University of Science and Technology, Yunnan, China

[3]Bolan Medical Hospital, Quetta, Balochistan, Pakistan

[4]Centre for Advanced Studies in Vaccinology and Biotechnology, UoB, Balochistan, Pakistan

[5]Food and Agriculture Organization, Balochistan, Pakistan

ABSTRACT

The chemical signal molecules called autoinducers are produced and released by the quorum sensing bacteria to levels dominating the increasing cell-population density. The attainment of minimal threshold stimulatory concentration of an autoinducer leads to an alteration in gene expression. Both Gram-positive and Gram-negative bacteria are capable of using quorum sensing communication circuits for regulating a diverse array of physiological activities. These activities include symbiosis, competence, virulence, conjugation, antibiotic production, sporulation, motility and biofilm formation. The Gram-negative bacteria use acylated homoserine lactones as autoinducers, while Gram-positive bacteria use processed oligo-peptides to communicate.

In the field of quorum sensing revealed, cell to cell communication via autoinducers both within and between bacterial species. The establishment of enormous data in this field suggests autoinducers acquiring specific responses from host organisms. Despite the difference in chemical signals, signal relay mechanisms and the target genes controlled by the bacterial quorum sensing systems, the ability to communicate with one another allows bacteria to coordinate the gene expression as well as the behaviour of the entire community. This process presumably confers upon the bacteria some of the qualities of higher organisms. The evolution of quorum sensing systems in bacteria thus could have been one of the early steps in the development of multicellularity.

KEYWORDS: Quorum, Sensing, Signals, Different, Systems

INTRODUCTION

Cellular signaling and communication are vital for the appropriate development and growth of every multicellular living organism. Because of the universal significance of cell communication it is quite evident that many of its fundamental aspects have been evolutionarily preserved between animals, plants and unicellular eukaryotes despite of the fact that more than 1 billion years ago these kingdoms diverged (Fletcher *et al*., 2007).

However, once it was thought that the ability to coordinate cellular behavior is restricted to eukaryotic organisms and there is only indirect bacterial perception of neighboring bacteria. But now due to the research done in the past 2 decades has revealed that bacteria also uses sophisticated communication systems in order to coordinate a variety of biological activities which include growth in biofilm communities and virulence factors production (Miller and Bassler, 2001).

Cell communication is population-density dependent in bacteria and in some other eukaryotic microorganisms and comprise production of and response to autoinducers which are small pheromone-like biochemical molecules. Microbes can express particular behaviors by intercellular signaling which results in differential gene regulation and this happens during growth in social communities only. This process has been termed quorum sensing to reflect the need for a sufficient population of microbes to activate the system (Fuqua *et al*., 1994).

In the past 30 years quorum sensing, which is the process of communication between bacterial cells, is one of the very significant discoveries in microbiology (Turovskiy *et al*., 2007). Signaling molecules involved in bacterial quorum sensing are divided into two groups. One group includes the fatty acid derivatives exploited by Gram-negative bacteria and the second group peptide derivatives are typically used by Gram-positive bacteria. Quorum sensing is ubiquitous in many known species of bacteria. Gram-negative bacteria which are pathogenic in human and plants including the genera *Pseudomonas, Brucella, Erwinia, Ralstonia, Vibrio, Agrobacterium, Enterobacter, Serratia, Yersinia, Bukholderia* and *Vibrio.* These genera utilize the mechanism of quorum-sensing in order to regulate synthesis of virulence factors (Williams, 2007). Bacteria included in genera *Enterococcus, Bacillus, Streptococcus, Streptomyces* and *Staphylococcus* make use of this mechanism for the production of antimicrobial peptides or exotoxin, formation of biofilms and development of genetic competence (Podbielski and Kreikemeyer, 2004).

Quorum sensing is used by the *Rhizobiumgenus* for nitrogen fixation. The symbiosome development and nodulation that is essential for nitrogen fixation is regulated by complex quorum-sensing systems in these symbiotic bacteria (Hoang *et al*., 2004). In extremophiles quorum sensing has also been described such as in the haloalkaliphilic *archeon Natronococcus occultus* and in *Halomonas* bacterial genus (Liamas *et al*., 2005), in *Acidithiobacillus ferrooxidans* (Rivas *et al*., 2007) and in the hyperthermophilic bacterium *Thermotoga maritima* (Johnson *et al*., 2005).

Bacterial community utilizes the system of interspecies quorum sensing in order to determine that how many of other and their own species are present in an area. Quorum sensing molecules are secreted by the bacteria which are increased in proportion to cell number. When these molecules hit a certain concentration, they activate the transcription of some specific genes for example virulence factors transcription. It has been revealed that bacteria not only interact with members of their own species via quorum sensing but also gather information about other species and there is a kind of some universal molecule which permits them to do so. This universal molecule is named as autoinducer 2 or AI-2 (Day and Maurelli, 2001).

Mechanisms of Quorum Sensing

The mechanism of quorum sensing is divided into 4 steps: (1) small biochemical signaling molecules production by the bacterial (2) signal molecules release in the surrounding environment, either passively or actively (3) specific receptors recognize these signaling molecules when concentration of these molecules exceeds a certain threshold, thus leading to (4) gene regulation changes (Sifri, 2008).

Quorum-Sensing Signals

Quorum sensing signaling systems are broadly classified into four main groups. Gram-negative bacteria utilizes two of these systems which involve autoinducer-1 (AI-1) and autoinducer-3 (AI-3), while on the other hand the third type of signaling system is used by Gram-positive cells involving the autoinducing polypeptide (AIP) system. These cell to cell

signaling are principally involved in intra species communication (Smith *et al*., 2004). However the fourth system is found in both Gram negative and Gram-positive bacteria using autoinducer-2 (AI-2). Quorum sensing which involves AI-2 is used mainly for interspecies communication (Schauder *et al*., 2001).

Autoinducer-1 or AI-1

Investigating the phenomenon of bioluminescence, the LuxIR system was discovered for the first time in *Vibrio fisheri* and presently the LuxI/LuxR system is considered to be the model system and is the base of other quorum-sensing systems (Nealson *et al*., 1970).

It is composed of LuxI which manufactures an N-AHL known as AI-1 and LuxR which is a transcription factor accountable for scheming gene expression in the company of the autoinducer. Autoinducers are synthesized by LuxI and its homologues by transfer of a fatty acid chain to SAM from an acylated acyl carrier protein (ACP) which results in the release of methyl thioadenosine and AHL (Schaefer *et al*., 1996).

LuxI homologues synthesize AHL composed of diverse fatty acid moieties which recognize specific ACPs by the enzyme synthases and result in intrageneric and intraspecies signaling. After synthesis, AI-1 is released into the adjacent environment by diffusion across the bacterial cell membrane. Environmental concentration of AI-1 rises with the increase in population. When there is high population density, AI-1 diffuses back into the cell because its local concentration is very high. Within the cell it binds to Lux resulting in the activation of the transcription of the luxCDABEGH operon located within the promoter region (Devine *et al*., 1989). Luciferase is the product of this operon that catalyzes a chemical reaction resulting in luminescence. Most of the Gram-negative bacteria have homologous LuxI/LuxR systems having the ability to produce specific AHLs. These signaling processes govern the expression of the virulence factors in many opportunistic pathogens like *Serratia marcescens* and *Pseudomonas aeruginosa*. *P. aeruginosa* have two systems which are homologous to LuxI/Lux. These systems not only control extracellular enzymes production and biofilm formation but also transcription of another quorum-sensing system i.e RhlI/RhlR thus adding further in the level of control by AHL signaling mechanism (De-Kievit and Iglewski, 2000).

Earlier it was thought that bacteria utilizes quorum sensing systems to regulate density of their population, however, studies on *Salmonella Typhimurium* and *E. coli* showed that this does not happen all the time. For example, *E. coli* and *S. Typhimurium* do not possess a LuxI homologue so they are not able to produce any AI- 1. However, they are capable of encoding a LuxR homologue called SdiA which when over expressed have a negative effect on those genes that are involved in cellular attachment in enterohemorrhagic *E. coli* (EHEC) (Kanamaru *et al*., 2000), while SdiA positively regulates numerous genes situated on the virulent plasmid of *S. Typhimurium* for example rck which is a protein concerned with the evasion of the immune response of host (Ahmer *et al*., 1998). Although the exact role of SdiA in pathogenesis is unclear, this protein allows EHEC and *S. Typhimurium* to alter gene expression in response to the presence of AI-1 produced by other bacteria (Michael *et al*., 2001).

Autoinducer-2 or AI-2

Most of the Gram-negative and Gram-positive bacteria use systems of quorum sensing that recognize an extracellular signal called as AI-2. It is synthesized from a SAM metabolism by- product. AI-2 synthesis from protein LuxS, a synthase encoded by luxS genes, in *V. harveyi* is good example. A series of steps are involved in synthesis of

AI-2 from SAM by LuxS including conversion of ribosehomocysteine into 4,5-dihydroxy-2,3- pentanedione (DPD) i.e a compound which cyclizes into numerous furanones in water presence and homocysteine (Schauder *et al*., 2001). Two separate AI-2-binding proteins cocrystalize to determine the structure of the AI-2 signals. These include a BAI-2 and R-THMF (Miller *et al*., 2004).

Bacterium releases AI-2 which accumulates in the cellular environment and can be detected by two different mechanisms. *V. harveyi* detects the form BAI-2. The presence of periplasmic AI-2 is detected by binding of the signaling molecule with LuxP which is a specific autoinducer binding protein. A phosphotransfer cascade is initiated when AI-2/LuxP complex interacts with LuxQ, a sensor kinase, thus resulting in luciferase production and luminescence. The cascade of LuxP/LuxQ has been recognized in *Vibrio* spp only. AI-2 is managed by a separate mechanism in *S. Typhimurium* and *E. coli.* In contrast to LuxP/LuxQ system, AI-2 is transported into the cytoplasm through Lsr (LuxS regulated) system of cell that initiates a cellular response. LsrB, a periplasmic protein, recognizes the signal and binds to the R-THMF which is a form of AI-2.

After binding, the Lsr ABC transporter that consists of LsrC and LsrA transports AI-2 into the cell where it is phosphorylated by LsrK. AI-2 in its phosphorylated form interacts with the LsrR that is a transcriptional repressor, in order to alleviate repression of the operon lsr which may up regulate other operons (Taga *et al*., 2001). In Gram-negative and Gram-positive bacteria a wide range of LuxS/AI-2 systems have been found which has proposed the idea that the AI-2 system is utilized for cross-species signaling process by organisms that live in mixed-species communities like biofilms (Xavier and Bassler, 2003).

Autoinducer-3 or AI-3

AI-3 was firstly described as a compound employed by the QseC system and is found in spent media which activates genes expression that are concerned in EHEC attachment to eukaryotic cells and subsequent rearrangement of actin (Sperandio *et al*., 2003).

But still the synthesis and structure of this signaling molecule is not well known. LuxS was supposed to be involved in the AI-3 production because of the fact that its synthesis was impaired in mutants of luxS. However, further studies revealed that impairment of AI-3 production in LuxS mutants was because of oxaloacetate used as a metionine precursor instead of SAM. The use of L-aspartate in the growth medium decreased the demand of oxaloacetate thus restored AI-3 production but had zero effect on production of AI-2 (Walters *et al*., 2006).

This study also revealed that a number of bacteria including nonpathogenic *Enterobacter cloacae* and *E.coli* and also pathogenic bacteria like *Klebsiella, Shigella* and *Salmonella* species can produce AI-3. Thus it is suggested that AI-3 might correspond to another cross-species signal. While the AI-3 has not been detected in Gram-positive bacteria till date. AI-3 is detected by using a 2-component system which comprises response regulator QseB and sensor kinase QseC. QseC autophosphorylate in the presence of periplasmic AI-3 and then phosphate is transferred to QseB that results in the upregulation of master flagellar regulator gene flhDC which are those genes responsible for biosynthesis of flagella and its motility (Clarke *et al*., 2006).

AI-3 presence is also related to the formation of EHEC's effacing and attaching lesions. This is accomplished by 5 different loci's up regulation in enterocyte effacement (LEE) operons that are present within the chromosome of EHEC

(Sperandio *et al*., 2003). However, the cascade accountable for regulation of these genes is still not clear completely, but most likely it involves QseA which is a regulator of LysR family and is influenced due to cell to cell signaling process. It is also involved in the frank upregulation of LEE genes (Sperandio *et al*., 2002).

Autoinducing Peptides

Autoinducing peptides responsible for cell to cell signaling are present absolutely in Gram positive bacteria. Prototypic Agr system is the base of these signaling processes which was described in *S. aureus* for the first time. The Gram-positive bacteria utilize a polypeptide signal in place of a smaller molecule. These polypeptides work as an autoinducer for organism that synthesizes and produces it while inhibits other organisms. This polypeptide signal is known as AIP and determined by the gene agrD. After its translation, N-terminal signal sequence targets the AgrD propeptide to the membrane.

After reaching the membrane, C-terminus of the propeptide is cleaved by AgrB that is a membrane-bound endopeptidase. Signal peptidase i.e. SpsB removes the propeptide's N-terminus which also includes the signal sequence. Lastly, a thiolactone ring having a free N-terminal tail is formed when the C-terminus of processed polypeptide is linked covalently to a centrally located cysteine. Both of these structures are essential for appropriate performance of the AIP in many cases. Signal receptor i.e AgrC recognizes AIP when it is released in the environment. AgrC consist of a transmembrane N-terminal domain in order to recognize specific AIPs and also a C-terminal histidine kinase domain that phosphorylates a response regulator called AgrA in the presence of the correct AIP.

Phosphorylated AgrA initiates transcription of selective genes after binding to direct repeats that are present in the promoter regions. One feature that is specific to the AIP/Agr system is the reality that an AIP which is produced by one strain of *Staphylococcus* will interfere with Agr system of another strain. This double role as an inhibitor and activator is associated to the AIP and AgrC interaction. The cyclic structure of AIP is necessary in order to interact with AgrC, while it is the N-terminal tail that is accountable for activation of AgrC. In fact removal of this tail results in the formation of a universal inhibitor which binds to AgrC but is not able to activate the Agr system (Lyon *et al*., 2000).

In many Gram-positive bacteria Agr system has associated to pathogenesis. AIP-directed regulation of genes by AgrA results in the making and release of many toxins by *S. aureus*, such as beta-hemolysins, alpha-hemolysins, delta-hemolysins, serine proteases and toxic shock syndrome toxin 1 (Parker and Sperandio, 2009). Another Gram-positive *Enterococcus faecalis* that take advantage from the use of AIP signal sensing. This organism uses a 2-component system homologous to the Agr system of *Staphylococcus* to sense the presence of AIP. When AIP is detected, the cell produces and releases 2 extracellular proteases, gelatinase and SprE (Qin *et al*., 2000).

CONCLUSIONS

The quorum sensing systems studies reveal that bacteria have used several languages for communicating within and between species. Interspecies and intraspecies cell to cell communication allows bacteria to manage different biological activities in order to act like multicellular organisms. Quorum sensing governs a variety of processes that reflect the definite needs of some communities living in distinctive niches. Susceptible hosts and rival bacteria have developed some natural strategies in order to hinder quorum sensing bacteria either by destruction of the chemical signal molecules or by production of autoinducer antagonists which impede bone fide signal molecule reorganization. In the same way,

bacteria and probably other eukaryotic hosts have developed strategy that supplement the quorum sensing abilities of the beneficial bacteria. These natural phenomena that improve disrupt quorum sensing are now used as model therapies in the designing of analogous synthetic strategies responsible for manipulating bacterial quorum sensing. Research in the field of biotechnology is now intended to develop molecules which are related to autoinducers structurally. These molecules have significant use as antimicrobial drugs intended at those bacteria which are used in quorum sensing for controlling virulence. Moreover, biotechnological approaches meant to utilize advantageous quorum sensing processes can be used in improving production of industrial natural products like antibiotics. Either with practical application or without it, ongoing study of quorum sensing systems in bacteria assures to give new insights in the mechanisms involved in the evolution of multicellular organisms, inter and intraspecies communication and inter and intracellular signal transmission.

ACKNOWLEDGEMENTS

I would also like to express my very great appreciation to professor Ban Bo and Wang Nan, for their valuable and constructive suggestions during the planning and development of this research work. My thanks and appreciations also go to my colleague in developing the article and people who have willingly helped me out with their abilities.

REFERENCES

1. Ahmer, B. M., Van-Reeuwijk, J., Timmers, C. D., Valentine, P. J., & Heffron, F. (1998). *Salmonella typhimurium* encodes an SdiA homolog, a putative quorum sensor of the LuxR family that regulates genes on the virulence plasmid. *J Bacteriol*, 180, 1185–93.
2. Clarke, M. B., Hughes, D. T., Zhu, C., Boedeker, E. C., & Sperandio, V. (2006). The QseC sensor kinase: A bacterial adrenergic receptor. *Proc Natl Acad Sci*, 103, 10420–5.
3. Day, W. A., & Jr-Maurelli, A. T. (2001). *Shigella flexneri* LuxS Quorum-Sensing System Modulates virB expression but is not Essential for Virulence. *Infect Immun*, 69(1), 15-23.
4. De-Kievit, T. R., & Iglewski, B. H. (2000). Bacterial quorum sensing in pathogenic relationships. *Infect Immun*, 68, 4839–49.
5. Devine, J. H., Shadel, G. S., & Baldwin, T. O. (1989). Identification of the operator of the *lux* regulon from the *Vibrio fischeri* strain ATCC7744. *Proc Natl Acad Sci*, 86, 5688–92.
6. Fletcher, M. P., Diggle, S. P., Camara, M., & Williams, P. (2007). Biosensor-based assays for PQS, HHQ and related 2-alkyl-4-quinolone quorum sensing signal molecules. *Nat Protoc*, 2, 1254–62.
7. Fuqua, W. C., & Winans, S. C. (1994). A LuxR-LuxI type regulatory system activates *Agrobacterium* Ti plasmid conjugal transfer in the presence of a plant tumor metabolite. *J Bacteriol*, 176, 2796–806.
8. Hoang, H. H., Becker, A., & Gonzalez, J. E. (2004). The LuxR homolog ExpR, in combination with the Sin quorum sensing system, plays a central role in *Sinorhizobium meliloti* gene expression. *J Bacteriol*, 186, 5460–72.
9. Johnson, M. R., Montero, C. I., Conners, S. B., Shockley, K. R., Bridger, S. L., & Kelly, R. M. (2005). Population density-dependent regulation of exopolysaccharide formation in the hyperthermophilic bacterium *Thermotoga maritime*. *Mol Microbiol*, 55, 664–74.

10. Kanamaru, K., Tatsuno, I., Tobe, T., & Sasakawa, C. (2000). SdiA, an *Escherichia coli* homologue of quorum-sensing regulators, controls the expression of virulence factors in enterohaemorrhagic *Escherichia coli* O157:H7. *Mol Microbiol*, 38, 805–16.
11. Liamas, I. E., Quesada, I. E., Martinez-Canovas, M. J., Gronquist, M., Eberhard, A., & Gonzalez, J. E. (2005). Quorum sensing in halophilic bacteria: detection of N-acyl-homoserine lactones in the exopolysaccharide-producing species of *Halomonas*. *Extremophiles*, 9, 333–41.
12. Lyon, G. J., Mayville, P., Muir, T. W., & Novick, R. P. (2000). 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, 13330–13335.
13. Michael, B., Smith, J. N., Swift, S., Heffron, F., & Ahmer, B. M. (2001). SdiA of *Salmonella enterica* is a LuxR homolog that detects mixed microbial communities. *J Bacteriol*, 183, 5733–42.
14. Miller, M. B., & Bassler, B. L. (2001). Quorum sensing in bacteria. *Ann Rev Microbio*, 55, 165–199.
15. Miller, S. T., Xavier, K. B., Campagna, S. R., Taga, M. E., Semmelhack, M. F., Bassler, B. L., & Hughson, F. M. (2004). *Salmonella typhimurium* recognizes a chemically distinct form of the bacterial quorum-sensing signal AI-2. *Mol Cell*, 15, 677–87.
16. Nealson, K., Platt, H., & Hastings, J. W. (1970). Cellular control of the synthesis and activity of the bacterial luminescent system. *J Bacteriol*, 104, 313–22.
17. Parker, T. C., & Sperandio, V. (2009). Cell-to-cell signaling during pathogenesis. *Cell Microbiol*, 11, 363–9.
18. Podbielski, A., & Kreikemeyer, B. (2004). Cell density-dependent regulation: basic principles and effects on the virulence of gram-positive *cocci*. *Intl J Infect Dis*, 8, 81–95.
19. Qin, X., Singh, K. V., Weinstock, G. M., & Murray, B. E. (2000). Effects of *Enterococcus faecalis fsr* genes on production of gelatinase and a serine protease and virulence. *Infect Immun*, 68, 2579–2586.
20. Rivas, M., Seeger, M., Jedlicki, E., & Holmes, D. S. (2007). Second acyl-homoserine lactone producing system in the extreme acidophile *Acidithiobacillus ferrooxidans*. *Appl Environ Microbiol*, 73, 3225–31.
21. Schaefer, A. L., Val, D. L., Hanzelka, B. L., Cronan-Jr, J. E., & Greenberg, E. P. (1996). Generation of cell-to-cell signals in quorum sensing: acyl homoserine lactone synthase activity of a purified *Vibrio fischeri* LuxI protein. *Proc Natl Acad Sci*, 93, 9505–9.
22. Schauder, S., Shokat, K., Surette, M. G., & Bassler, B. L. (2001). The LuxS family of bacterial autoinducers: biosynthesis of a novel quorum-sensing signal molecule. *Mol Microbiol*, 41, 463–76.
23. Sifri, C. D. (2008). Quorum Sensing: Bacteria Talk Sense. *Clin Infect Dis*, 47 (8), 1070-1076.
24. Smith, J. L., Fratamico, P. M., & Novak, J. S. (2004). Quorum sensing: a primer for food microbiologists. *J Food Protect*, 67, 1053–70.
25. Sperandio, V., Torres, A. G., Jarvis, B., Nataro, J. P., & Kaper, J. B. (2003). Bacteria-host communication: The language of hormones. *Proc Natl Acad Sci*, 100, 8951–6.

26. Taga, M. E., Semmelhack, J. L., & Bassler, B. L. (2001). 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, 777–93.

27. Turovskiy, Y., Kashtanov, D., Paskhover, B., & Chikindas, M. (2007). Quorum sensing: fact, fiction, and everything in between. *Adv Appl Microbiol*, 62, 191–234.

28. Walters, M., Sircili, M. P., & Sperandio, V. (2006). AI-3 synthesis is not dependent on *luxS* in *Escherichia coli*. *J Bacteriol*, 188, 5668–81.

29. Williams, P. (2007). Quorum sensing, communication and cross-kingdom signaling in the bacterial world. *Microbiology*, 153, 3923–38.

30. Xavier, K. B., & Bassler, B. L. (2003). LuxS quorum sensing: more than just a numbers game. *Curr Opin Microbiol*, 6,191–7.