ISBN: 978-81-19149-94-0

7. Quorum Sensing, The Signalling Pathway in Bacteria

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Abstract:

Quorum sensing is term given to the cell-to-cell communication that occurs between the bacterial population. It occurs through various diffusible chemical signals that effect the gene regulation when the bacterial cell density is high. Quorum sensing changes the metabolic and behavioral activities of a community. It is used by both gram negative and gram-negative bacteria and involves the production of extracellular signalling molecule called autoinducers. Quorum sensing is utilized by the bacteria for various activities such as sporulation, virulence, biofilm production, anti-biotic production etc. Among pathogenic bacteria Pseudomonas aeruginosa utilizes quorum sensing for regulating virulence factors. Quorum sensing is also being considered as a means to exploit for antimicrobial therapy to control bacterial infections. Gram negative uses N-acyl homoserine lactone as autoinducer, quorum sensing with the help of HSLs occurs in a cell density and growth dependent manner. Whereas, gram positive bacteria secrete processed peptied to be used as autoinducers. In this chater, we will discuss the basic mechanism of quorum sensing in gram negative and gram-positive bacteria with appropriate examples. The biofilm production by pathogenic bacteria is often connected with quorum sensing. It enables the bacteria to up regulate or down regulate the secretion of extracellular polymeric substances to increase their competitive ability against different strains present withing the biofilm or against other species.

Keywords:

Quorum sensing, bacteria, cell to cell commuication, biofilm production, autoinducers.

7.1 Introduction:

Bacteria exist as individual cells. They have the unique ability to undergo intercellular communications with other bacterial cells, which proves that they can coordinate with each other. Because of these capabilities bacteria can behave collectively as a group and perform important functions such as migration to a favourable environment, sporulation, antibiotic production and biofilm production etc. (Kievit and Iglewski, 2000).

This phenomenon of coordinated behaviour is known as Quorum sensing. It is known as the regulation of gene expression in response to changes in cell-population density. It requires the use of chemicals signalling pathway via molecules called autoindiucers.

The concentration of autoinducers increase with increase in cell density (Miller and Bassler, 2001). When a single bacterium releases autoinducers (AIs) into the environment, their concentration is too low to be detected. The detection of a minimal threshold concentration of the chemical leads to an alteration in gene expression of another bacteria in the vicinity. When sufficient bacteria are present, autoinducer concentrations reach a threshold level that allows the bacteria to sense a critical cell mass and, in response, to activate or repress target genes. Most of the bacteria identified that utilize quorum-sensing systems are associated in some way with plants or animals. Quorum sensing, is used by both Gram-negative and Gram positive bacteria to regulate a variety of physiological functions. Studies show that quorum sensing maintains both intra- and inter-species cell–cell communication, and it plays a major role in enabling bacteria to perform complex community functions. Bacteria are also known to regulate their phenotype with the help of various components of quorum sensing.

Gram-positive and Gram-negative bacteria use quorum sensing communication for an array of physiological activities such as symbiosis, virulence, competence, conjugation, antibiotic production, motility, sporulation, and biofilm formation.

The autoinducer used by gram-negative bacteria is acylated homoserine lactone and gram-positive use processed oligo peptides. this type of communications occurs at both inter and intra species level. The autoinducers produced by the bacteria function to elicit a specific response from the host. The nature of the chemicals signals and the mechanism with which it works differs in every organism but the essence remains the same i.e. to regulate coordiante gene expression of a larger group of bacteria and ultimately have an effect on the behaviour of the entire community.

7.2 Quorum Sensing in Gram Negative Bacteria:

Quorum sensing in gram negative bacteria occurs with the use of N-acyl homoserine lactones (NHL) as autoinducers or signalling molecules. These molecules have a tendency to bind to a transcriptional activator at high concentrat, which will thereby lead to the expression of the target genes. AHLs were discovered by with the help of biosensors. These biosensors, consisting of quorum sensing controlled promoter connected to a reporter such as lacZ or the lux operon, were used to screen spent culture supernatants. Biosensors also contain a functional R protien but are devoid of AHL synthase enzyme and hence, promote activity depending on the presence of exogenous AHL. Even though the R protiens are used extensively and are highly sensitive to AHL,but some problems do exist. R protiens are able to be responsive for a large number of AHL molecules, but only at high concentration of AHL molecules. Identified AHL molecules contains 4 to 14 carbon acyl sides chains and an oxo, a hydroxy or no substituion at the third carbon. Some gram-negative bacteria also contain alternate signalling molecules other than the AHLs. For example, R. solanacearum produces 3-hydroxypalmitic acid methyl ester in combination with AHLs as signalling molecules to regulate virulence among the population. (Flavier et al., 1997).

In Pseudomonas aeruginosa most common type of autoinducer produced is a 2-heptyl-3-hydroxy-4-quinolone. Along with this, a new form of autoinducer has been identified that is produced by the bacterium called as PQS (*Pseudomonas* quinolone signal) (Pesci et al., 1999).

Another molecule, butyrolactones have also been isolated from Pseudomonas aureofaciens vulture supernatants. A new family of diketopiperazines (DKPs) has been discovered in *P. aeruginosa*, *Pseudomonas fluorescens*, *Pseudomonas alcaligenes*, *Enterobacter agglomerans, and Citrobacter freundii* (Holden et al., 1999). Many of these molecules were capable of activating the the LuxR based biosensors, but the DKPs were found to affect negatively regulate the N-3 homoserine lactone mediated bioluminescence, which suggests that they might be competing with the LuxR binding (Prasad, 1995).

7.2.1 The LuxI/LuxR Quorum Sensing Systems:

The system of LuxI/LuxR quorum sensing has been used to control cell density dependent functions for over 30 species of gram-negative bacteria (Swift et al., 1999). This system utilizes HSL as autoinducer. It is synthesized on a luxi homologue and a luxR homologue as well, encoding for a transcriptional activator protien which is responsible for detection of the autoinducer (HSL) and lead to the expression of the output. R. solanacearum uses quorum sensing to control virulence of the plant cell and for the production of cell wall degrading enzymes. Its system is known as SolI/SolR system which is regulated by a LysR like regulator called the PhcA, which responds to an autoinducer (3-hydroxy-palmitic acid methyl ester). It is controlled by RpoS, which is stationary factor (Flavier et al., 1997). Quroum sensing can sometimes be responsiable for both bacterial as well as host signals. This is observed in Agrobacterium tumefaciens, a crown call causing bacterium. Here, the opine hormones secreted by the plant interacts with the bacterial protien called OccR and regulates the expression of Lux R homologue TraR (Winnas et al., 1999).

Bioluminescent qourum sensing system if the marine bacetrium *V. fischeri* is one of the most studied systems. Here the bacterium is known to be in a symbiotic relationship with a eukaryotic host. The host (squid) has a specialised light organ in which the pure culture of a strain of *V. fischeri* resides. In this particular type of association, the host provides the bacterium with a nutrient rich environment to live and the bacteria provides the host with light. The emission is directly related with the cell population density of the bacteria in the host organ, which is in turn controlled by quorum sensing. The bacterial culture inside the host organ reaches a population sensity of upto 1011 cells per ml (10^7) and produces and releases an autoinducer into the environment. This hormone gets trapped inside the light organ with the bacteria. This autoinducer molecule inside the light organ acts as signalling molecule. This accumulation of the hormone gives signal to the bacteria of being present inside the host and not outside in the water, as the light organ of the host is the only place where the hormone can be accumulated. When *V. fischeri* detects the presence of the autoinducer it leads to the emission of light (Engebrecht et al., 1983). There are various enzymes required for the production of light of these, the luciferase enzyme is encoded by luxCDABE which is an inherent part of a larger operon called the luxICDABE (Lee et al., 1993). LuxI and LuxR protiens constitute the quorum sensing system. The autoinducer synthase enzyme (LuxI) helps in the production of HSL, N-(3-oxohexanoyl)-homoserine lactone (Eberhard et al., 1983). Whereas, LuxR has two functions, first to bind with the autoinducer and second to activate trasncription of the luxICDABE operon. In situations where the population of the bacteria is not sufficient, a operon is transcribed at a low base level producing a low level of light. Kaplan ad Greenber in 1985 states that, the HSL autoinducer diffuses across the cell membrane therefore, equal concentration of HSL is present in the extracellualr and intercellular environment.

With the growth of the culture of *V. fischeri*, the concentration of the autoinducer also grows up to a threshold level of 1-10 µg/ml, which is required for detection and binding by the LuxR protien. Interaction of luxR with HSL reveals the LuxR DNA binding domain, which allows it to bind to the luxICDABE promoter and activate transcription, beacuase of this there is a substantial increase in the concentration of both autoinducer production and light emission. The LuxRHSL complex may also sometimes act negatively to regulate the expression of luxR. The negative feedback decreases luxICDABE expression.

7.2.3 Quorum Sensing in Gram Positive Bacteria:

Quorum sensing is an important phenomenon observed in many gram positive bacteria. The signalling molecules of gram positive bactria differ from those found in the gram negative bateria. Quourum sensing is used to perform various function in gram positive bacteria such as DNA uptake in B. subtilis, virulence in *S. aureus*, conjugation in *Enterococcus faecalis* and microcin production in bacteria such as *Lactobacillus sake* and *Carnobacterium piscicola*. Unlike gram negative bacteria which employs the usse of LuxI/LuxR signalling pathway and HSLs as signalling molecule gram positive bacteria use processed peptide signalling molecule through a ABC (ATP-binding cassette) exporter protien. The signal generated by the peptides is recognised by two component sensor kinase protien which will further interact with cytoplasmic protiens. This mechanism is called the phosohorelay cascade (Kleerebezem et al., 1997; Novik and Muir, 1999). Lazazerra and Grossman (1998) explained the QS mechanism as observed in *B. subtilis.* They proposed that the two processed peptide signals enables the bacteria to choose between becoming competent for foreign DNA uptake or to undergo sporulation. Research has not been able to understand the science behind the srection machinery for these two peptides. One of the two peptide ComX is known to activate the ComP/ComA system to allow the the bateria to transition towards a more transformable condition. Peptide CSF (commonly called competence and sporulation factor) is imported by an ABC transporter. Turgay et al (1998) reported that different concentration of the CSF will lead to different result such that, at high CSF concentration competence will be inhibited and sporulation favoured whereas, at low concentration of CSF competence development is favoured. To summarize we must understand that in gram positive bacteria, secreted peptides function as autoinducers which in high concentration are detected by two component sensor kinases. The interaction between the two initiates a series of phosphoryl events which results in phosphorylation of the regulator protein. This phosphorylation activates the regulator protien which allows it to bind to the DNA and alter the transcription of the target gene.

S. aureus is another example of the gram positive bacteria undergoing quorum sensing. Dunny and Leonard (1997) reported that the virulence of the bacteria depends on cell associated protiens like protien A, collagen and fibronectin-binding protien, proteases, alpha toxin etc. During infection, the attachment of the bacterium to the host is of utmost importance and is favoured by surface protiens such as collagen and fibronectin-binding protien along with protein A required for defence. When the concentration of the bacterium at the host surface reaches a certain level, the expression of these surface protiens is known to decrease giving way for the production of secreted protiens (Ji et al., 1997). The genetic basis for this depends on two pleiotropic regulatory loci called agr (accessory gene regulator (Morfeldt et al., 1988) and sar (staphylococcal accessory gene regulator). The agr locus consists of two divergently transcribed operons, RNAII and RNAIII).

The RNAII operon constitutes the agrBDCA genes which encode for signal transducer (AgrC) and resonse regulator (AgrA) and AgrB and AgrD, which together generate the signal molecule. The AgrC signal transducer is autophosphorylated in response to the signal molecule, this leads to phoosphorylation of the AgrA response regulator. AgrA helps in the transcription of RNAIII, which upregulatos the expression of various *S. aureus* protiens and positively regulates the agrBDCA locus. This locus leads to a rapid increase in the production and transport of octopeptide signal molecule. The second locus sar, produces the sar gene product (sarA) whose main function is to regulate DNA-binding protien to induce expression of both RNAII and RNAIII operons of the agr locus.

7.2.4 Evolution of Quorum Sensing in Bacterial Biofilms:

An important example of coordinated social behaviour in bacteria is the production of biofilms. This phenomenon involves the secretion of polymers used to envelope the communities of cells attached to the surface of the host. This polymer is secreted only when the bacterial population reaches a certain level. Nadell et al (2008) used individual based simulations to study the competitions between different strains of bacteria throughout evolution, which differ in their secretions of these biofilm producing polymers and quorum sensing phenotypes. It is known that polymer secretion is activated at high cell density which starts the biofilm formation. This is necessary as it allows the bacteria to provide a nutrient rich medium for the growth of its newer generation. It was unclear as to why quorum sensing is used again to stop the polymer secretions at high cell density. The researchers were able to establish that the reason for this termination lies in the fact that, once biofilm production has been stopped the resources can then me redirected towards growth of the bacteria, but this was only possible for a limited time frame. Therefore, it was theorized that the polymer secretion termination will evolve when it coincides with dispersal events. They suggested that the variation in quorum sensing can be attributed to the requirement of bacteria in chronic or acute biofilm infections. For example, *V. chlorae*, uses biofilm production to overcome production and then subsequently terminates it at high cell density and leads to disease which ultimately helps in the dispersal of the bacteria from the host.

7.3 Role of AHL in Biofilm Formation:

Biofilms are known to be aggregation of microorganisms which attach themselves to a solid surgace in a matrix of extracellular biopolymers. McLean et al (1997) reported that AHL or N-acyl-L-homoserine lactones play an important role in the biofilm production of many bacteria. They have been detected from many aquatic biofilms. They play asignificant role in the virulence of the bacteria. AHL negatively impacts the biosynthesis of extracellular polysaccharide (Koutsoudis et al., 2006). EPS is functionaly involved in the virulence of the bacteria. The function of the EPS inculde protecting the bacteria from the host defences, and aid in the formation of lesions by water-soaking and lead to wilting by blocking the free flow of water in the vascular system of the plant.

Psuedomonas syringae causes the brown spot in beans and is another pathogen which utilises the AHL dependent EPS production (Quinones et al., 2005). Other than the plant pathogenic bacteria, certain human bacterias also produce AHL-dependent biofims, which

is commonaly observed in patients of cystic fibrosis, a genetic defect. Cystic fibrosis is a condition which regulates the transport of chloride ion in the chloride ion channel. A defect in cystic fibrosis gene leads to secretions of mucoid which leads to chronic bacterial infections in the lungs. *P. aeruginosa* forms a biofilm in the lungs of the patient in an AHL dependent process (Dickschat, 2010).

7.4 Conclusion:

Bacteria's ability to coordinate behavious at cell density has many advantages. Pathogenic microorganisms need to regulate virulence factors throughout the infection process, which helps in their pathogenicity. One of the most important aspect of pathogenic bacteria is to evade the defence of its host. As of now quorum sensing plays the most important role in helping the bacteria to overcome the defence responses of the host by timely expression of immuntiy related protiens. Quorum sensings allows the bacteria to multiply in number to an appropriate level and then develop the virulence factors, and put forward a coordinated and planned attack to overwhelm the host defences. There are a number of bacteria which make use of the complex system of quorum sensing, and for this precise humans have found ways to manioulate and exploit it for their own benefit. Strategies can be planned out to manipulate the quorum sensing and hample the virulence of the disease causing pathogen. Quorum sensing if used by human pathogenic bacteria can be utilised against it, by exploiting the signalling molecules such as AHL to control human infections. The discovery that *P. aeruginosa* uses quorum sensing to regulate biofilm production suggests that agents capable of blocking quorum sensing may also be useful for preventing biofilm formation. The recent production of AHLs in plants represents an exciting new approach to controlling crop diseases as well as to manipulating plant-microbe interactions for improved crop production in the future.

Bacteria in general, have optimized quorum sensing to regulate a number of activities and in every case, quorum sensing gives the bacteria capability to be communicate with each other and alter their genetic response to a stimuli. Bacteria have evolves in such a way to use quorum sensing in an interspecific as well as species specific manner. This ability of the bacteria provides it with extra benefit to be able to adapt to various environmental conditions, grow competetively and survive, using autoinducers. New antimicrobial strategies can be designed in the future, to manipulate quorum sensing mechanism of the bacteria for human benefit. The challenge faced by the clinicians in the future will be to understand the complex nature of autoinducer-based signalling and develop effective therapeutic startegies.

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