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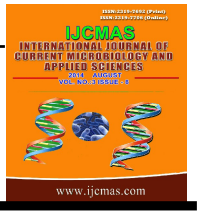

Original Research Article

Exploiting Quorum Sensing to inhibit the Bacterial Pathogens

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Keywords

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A B S T R A C T

A wide range of Gram-positive and Gram-negative bacteria infest humans by exhibiting various virulent traits. Prolonged use of antibiotics has eventually led to antibiotic-resistant mutants and has prompted research on new and efficient disease control strategies. One such convincing strategy is to interfere with the quorum sensing mechanism or cell-cell communication via different quorum-quenching approach. Gram-negative bacteria that employ N-acyl-homoserine lactones as quorum molecules, being one of the most pathogenic groups are extensively studied for deducing a number of quorum sensing signals, receptors and inhibitors. Gram-positive bacteria employ oligo-peptides to communicate within the species. Exploitation of quorum sensing mechanism is one of the most promising strategies to inhibit and control bacterial pathogens. It involves use of various quorum inhibitors like halogenated furanones and quorum inhibiting enzymes to interfere the virulence promoting factors. This review article focuses on the different signal molecules used by different bacteria to communicate within the species, quorum mechanism and inhibition that provide the intriguing possibility of therapeutic drug discovery and future applications.

Introduction

Quorum Sensing (QS) was first observed in luminous marine bacterium *Photobacterium fischeri* (*Vibrio fischeri*). Kenneth H. Nealson and John W. Hastings (Juan E *et.al*; 2006) observed that the marine bacteria do not luminescence until and unless they reach a high population density.

QS is a type of bacterial communication process which depends upon the bacterial population density where small easily diffusible signaling molecules activates the expression of thousands of genes that control diverse functions like bioluminescence, virulence, bio-film formation, production of antibiotic compounds, competence, conjugation and sporulation etc. Bacterial cell-cell communication of this type is termed as Quorum Sensing where Quorum implies "gathering of the minimal number of members of an organization to conduct a process" (Bhardwaj AK *et.al*; 2013). Signal mediated QS gene expression in both Gram-

positive and Gram negative bacteria are similar (Williams P; 2000), but the molecular mechanisms of expression and the signal molecules differ variedly. The components of QS system are typically encoded by chromosomal genes, which are believed to be acquired via horizontal gene transfer (Gray KM *et.al* ; 2001).

Despite of the differences in regulatory components and different ways to conduct the mechanism, the mechanism of QS systems depends on three basic principles:-

1) Members of the bacterial community produce autoinducers (AIs) or the signaling molecules. At low cell density, AIs diffuse away due to concentrations below the threshold required for detection. At high cell density, the increase in the production of AIs leads to high concentration enabling easy detection and response.

2) AIs are detected by the receptors suspended in the cytoplasm or embedded in the bacterial membrane.

3) Along with the activation of gene expression for co-operative behaviours of the bacteria, the detection of AIs results in further the activation of AI production among the bacterial population (Novick RP *et.al* ; 1995).

Key Players in Bacterial Communication

Autoinducers- Autoinducers are usually small molecules that either diffuse freely across the cell membranes due to small size or are actively transported out of the cell. There are various types of Autoinducers like Acyl homoserine lactones, Autoinducers-2 etc (Juan E *et.al* ;2006). AHLs produced by Gram negative bacteria are synthesized by homologues from the LuxI family of AHL synthases and mediate transcription of various target genes through an interaction with a homologue of the LuxR protein (Manefield M *et.al* ; 2002).

Autoinducing peptides- AIP mediated cell-to-cell signalling is found exclusively in Gram-positive bacteria and are based on the prototypic *agr* system.

Autoinducer Synthases - LuxI is the enzyme responsible for the synthesis of AHLs in the quorum-sensing system. The LuxI synthase specifically catalyzes the amide bond formation between *S*-adenosyl methionine (SAM) and a fatty acyl-acyl carrier protein of a specific chain length. It mediates the formation of the acyl homoserine lactones from the acyl-SAM intermediate (Juan E et.al;2006). The specificity depends upon the bacterial strain from which it is extracted. LuxS synthase is responsible for the production of the AI-2 signal molecule which is an *S*-ribosylhomocysteinase that catalyzes the cleavage of the thio-ether linkage of *S*-ribosylhomocysteine to produce L-homocysteine required for QS pathway.

QS Regulators – Lux R type Regulator is a transcriptional activator of Quorum Sensing for Gram negative bacteria and LuxP/Q-type proteins,a type of periplasmic receptor mediates AI-2-type quorum sensing.

3.Gram-negative Bacteria use N-acyl-homoserine lactones (AHL) as Signal Molecules –

The *lux*-type system of communication in Gram negative species consist of 2 components-

An auto inducer synthase (e.g., LuxI) that synthesizes AHLs from homocysteine moiety of *S*-adenyosyl methionine.

A transcriptional regulator (e.g., LuxR). AHL, because of their small size and lipophilic character can freely diffuses across cell membranes. As the population density increases, intracellular AHL binds the functionally linked (cognate) LuxR-like receptor upon reaching a sufficient concentration within the cytoplasm to induce differential gene expression of the pathogenic traits (Costi D. Sifri; 2008). The Signal specificity is usually bestowed by the length and the nature of the substitution at C-3 position of the acyl side-chain of *N*-acyl homoserine lactones (Cha C *et.al* ;1998).
Interestingly, the LuxR-AHL complex also induces expression of LuxI and hence "Auto-Inducing".

Gram-positive Bacteria use Modified Oligopeptides as Signal Molecules

Rather than using AHL as signaling molecules, cell communication in gram-positive bacteria is based on the production and detection of modified oligopeptides called Auto inducing peptides (AIPs) (Cha C *et.al* ;1998) as signals and "two component-type" membrane-bound sensor histidine kinases as receptors. Peptide signals are not diffusible across the membrane due to their large size, hence signal release is usually mediated by dedicated oligopeptide exporters (Christopher M *et.al*; 2005). The mechanism of signal transduction is via a conserved phosphorylation/ dephosphorylation mechanism (Stephan Schauder *et.al* ; 2001).

Although the biochemistry underlying these events is poorly defined, a hypothesis that most peptide quorum-sensing signals are cleaved from larger precursor peptides, which then are modified to contain lactones and lanthionines, thiolactone rings and isoprenyl groups is generally accepted for the Gram-positive QS signal molecules (Ansaldi M *et.al* ;2001). The QS mechanism is encoded by the accessory gene regulator *(agr)* locus that has a complex relationship with biofilm formation and virulence traits having significant clinical implications.

The *agr* gene locus consists of 2 divergent transcripts:-
RNAII which is a polycystronic transcript of 4genes i.e *agrB, agrD, agrC,* and *agrA.*

RNAIII which enhances exoprotein secretion in response to high cell density and contains the d-hemolysin gene *hld* (Cha C *et.al* ;1998).

The 4 genes encoded by RNAII are involved in the production and sensing of the AIP signals. The 46-residue propeptide *agrD* encodes the precursor of the auto inducing signal peptide, whereas the integral membrane protein AgrB is involved in its processing and secretion as a thiolactone-modified cyclic oligopeptide. AgrA and AgrC constitute a two-component histidine-kinase receptor response regulator pair that responds to the extracellular accumulation of the AIP. Activation of AgrA-AgrC induces the transcription of RNAII completing the auto inducing circuit.

Synthesis of the precursor peptide is followed by subsequent processesing and modification into AIP which binds to AgrC leading to phosphorylation of AgrA which induces the expression of a RNAIII, which represses expression of cell adhesion factors while inducing expression of secreted factors responsible for virulence (Novick RP *et.al*;1995).

Quorum Inhibition or Quorum Quenching

Quorum Quenching (QQ) is the Quorum Sensing inhibition or interference with bacterial cell-cell communication to prevent colonization by pathogenic bacteria that use QS to coordinate virulence (Stephan Schauder *et.al* ; 2001).

Potential strategies for barring bacterial invasion includes:

Inhibiting the receptor synthesis or function.
Reducing further production or release of functional auto inducer signal molecules.
Stimulating auto inducer degradation.
Inhibiting auto inducer- receptor binding.

The marine macroalga *Delisea pulchra* produces a range of lactones specifically halogenated furanones that inhibit QS (Manefield M *et.al*; 2002) by binding to Lux R and proteolytic degradation, thereby reducing the amount of protein available to interact with AHL and act as transcriptional regulator (Manefield M *et.al*; 2002).

AHL degrading enzymes like AHL-lactonases, one of the widely used QS inhibitors is a metalloprotein that contains two zinc ions in the active site. Many *Bacillus* species secrete an enzyme, AiiA, that cleaves the lactones rings from the acyl moieties of AHLs and delivers inactivity to the AHL molecules in signal transduction (Dong YH *et.al*; 2000).

The substrate's carbonyl carbon is attacked by a nucleophilic water/hydroxide bridging the two Zn ions. Following this, the lactone ring and carbonyl oxygen of AHL interact with Zn ion resulting in enhanced polarization of the carbonyl bond, which makes it more susceptible to a nucleophilic attack. This attack on the substrate's carbonyl carbon results in formation of a negatively charged intermediate that is stabilized primarily by the interactions with Zn ion. The C–O bond of the lactone ring of AHL then breaks to yield the ring-opened product, thereby degrading the lactone ring (Yi-Hu *et.al,* 2007). AHL-acylase and paraoxonase enzymes (PON's) from humans, mouse, rabbits etc also show an inhibitory effect by degrading the Qs signaling molecules.

Comparative Analysis with other Techniques

Traditional Methods of Bacterial Pathogen inhibition usually focuses on antibiotics that evolves rapid growth of bacterial populations with -
(a) High ability to degrade antibacterial compounds;
(b) Decreased permeability;
(c) Decrease in affinity for the antibiotic;
(d) Increased efflux of many different antibiotics (Lewis K ; 2001).
Comparative analysis and studies on the two mice administered with antibiotics and QS inhibitors separately resulted in better clearance of the symptoms of respective bacterial infections in the mice administered with QS inhibitors.

Recent Applications and Future Prospects

Inhibition of QS by addition of QS inhibitors to dispatch water borne bacteria as a part of waste water processing step has provided a way far better option for water treatment in waste water treatment plant. Some natural compounds extracted from plants like Vanillin (4-hydroxy, 3-methoxybenzaldehyde) from vanilla beans show substantial inhibition of AHL molecules and is non-toxic, hence can be used in pharmaceuticals and various comestible products due to their non-toxicity and stability (Stephan Schauder *et.al* ;2001).
Biotechnological approaches designed to exploit beneficial QS processes can further be used to improve industrial antibiotic production. Creation of transgenic plants by cloning AHL synthases or lactonases holds assurance for preventing plant diseases in future. Studies on the presence of QSI compounds in natural foods are staggeringly interesting and one such ingredient is garlic due to its anti-fungal, anticancer and antimicrobial activities can be a part of QS Inhibition.

QS mechanism is central virulence regulator and is responsible for various bacterial infections. Since co-ordinated attack on the host is only made when the bacterial population reaches a high population density, QS mechanism adopted by the pathogens enhances the survival aspects of the clinically relevant pathogenic bacteria. Biotechnological research now concentrates on the development of molecules that are either structurally related to the signalling auto inducers or molecules that inhibit the QS mechanism. Such molecules have high potency to be used as antimicrobial drugs that produces a long pertaining effect on the bacterial virulence and can provide mankind with a whole new era of treatment of bacterial infections.

Figure.1 Production and release of AHLs by Gram-negative bacteria(Cha C *et.al* ;1998)

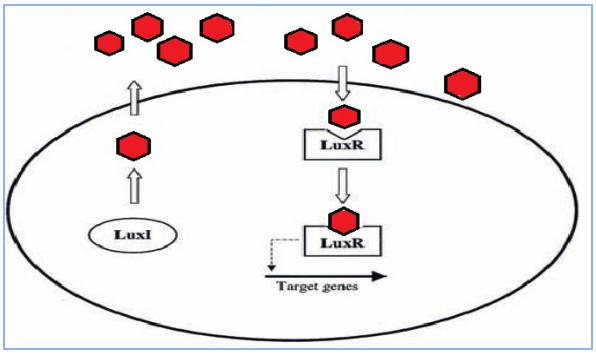


Figure.2 Production& release of AIPs in Gram-positive bacteria (Cha C *et.al* ;1998)

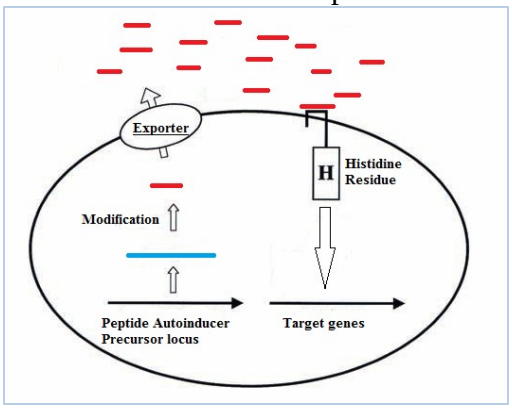
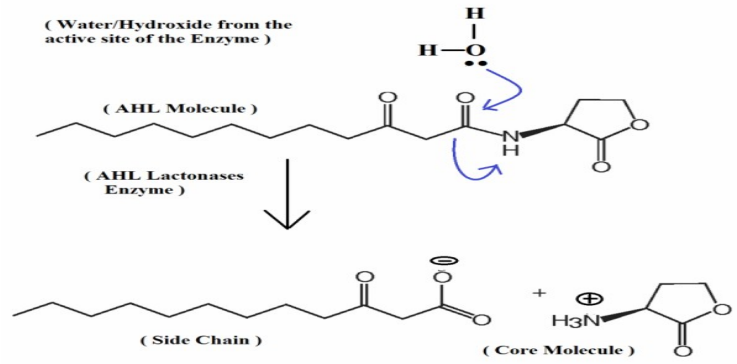


Figure.3 Nucleophilic attack of AHL-Lactonases on AHL Signal molecule.



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