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Int J Pharm Bio Sci 2013 Apr; 4(2): (B) 850 - 861

Review Article **Biotechnology**

International Journal of Pharma and Bio Sciences

ISSN 0975-6299

BACTERIAL QUORUM SENSING AND ITS APPLICATION IN BIOTECHNOLOGY

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ABSTRACT

Many bacteria use small diffusible signalling molecules called Autoinducers to communicate each other termed as Quorum Sensing. The signalling molecules in gram-positive bacteria are oligopeptides, in gram- negative bacteria are N- acyl homoserine lactone and a family of auto inducers known as auto inducer- 2 in both gram- positive and gram- negative bacteria. These molecules are internalized in the cell and activate a particular set of genes in all bacterial population, the bacterial virulence which is also controlled by quorum sensing. The detection and identification of quorum sensing molecules include cell based assays using specific bacteria biosensors, Thin- layer chromatography, Liquid chromatography and Mass spectrometry. Paper strip whole cell biosensors are a novel, inexpensive and portable filter paper based strip biosensor for the detection of bacterial quorum sensing signalling molecules, N- acyl homoserine lactones. Quorum sensing in bacteria has a wide application in Biotechnology like pathogen diagnostics and therapeutics. As many of the human and plant bacterial pathogens employ the acyl homoserine lactone based quorum sensing mechanism for regulation of pathogenicity determinant synthesis or biofilm formation. The application of quorum quenching strategy may be an alternative approach for fighting these microorganisms. The acyl homoserine lactone degrading enzymes together with quorum sensing inhibitors may be successfully used to disrupt bacterial cell to cell communication and to control bacterial infections.

KEYWORDS:Quorum Sensing, Biosensors, Autoinducers, Quorum Quenching.

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INTRODUCTION

"Quorum Sensing is a cell-cell communication mechanism that enables bacteria to sense and respond to changes in the density of the bacteria in a given environment". Bacteria can easily adapt to various new environmental conditions, and they have thrived on earth ubiquitously with a much longer history and wider occupation than humans. The discovery of the cell by Robert Hooke in the late 1600s marked the beginning of a revolution in biology that continues to this day as scientists labour to fully understand the inner workings of living organisms. The Dutch scientist Anton van Leeuwenhoek in 1676, observed the microbial world using specialized microscopes, and his discoveries represent the roots of microbiology. For nearly three centuries after van Leeuwenhoek's work, scientists believed that bacteria existed solely as single cell organisms and were incapable of any form of intercellular communication. This view of bacterial behaviour dramatically changed ~40 years ago.Recently, researchers discovered that bacteria do not live as a non-communicating single cell, but they are organized like a society that shows unique social and cooperative traits[1]. They can use a so called ''Quorum Sensing'' (QS) mechanism to 'talk to' and 'listen to' each other, and hence they can coordinate population behaviours like ''multicellular'' species[2]. Bacteria release various types of signalling molecules produced within the cell and are released into the environment called as Autoinducers (AI), these molecules are mediators of quorum sensing. When concentration of these signalling molecules exceed a particular threshold value, these molecules are internalized in the cell and activate particular set of genes in all bacterial population, such as genes responsible for virulence, competence, stationary phase etc[3]. A variety of different molecules can be used as signals. Common classes of signalling molecules are oligopeptides in gram-positive bacteria, N- Acyl Homoserine Lactone (AHL) in Gram- negative bacteria, and a family of autoinducers known as autoinducer- 2 (AI-2) in both gram-negative and gram-positive bacteria[4]. Quorum sensing was originally described in the marine luminescent bacterium *Vibrio fischeri*, where it functions as the control mechanism of light production and numerous other traits[5]. For years, it was thought that this phenomenon was limited to a few marine organisms but it is now widely recognized that many bacterial species utilize quorum sensing as part of their regulatory machinery[3,6,7]. Of interest, we now know that bacterial virulence is in many cases controlled by quorum sensing[3].

Quorum sensing in Gram-negative Bacteria

AHL-mediated QS is widespread in gram negative bacteria. It is comprised of an AHL synthase (LuxI-type family protein) and an AHL receptor (LuxR-type family transcription regulator). AHL-mediated QS was first described in the bioluminescent bacterium *Vibrio fischeri*, in which the LuxI and LuxR proteins controlled the expression of luxCDABE operon (a luciferase)[8]. LuxI is responsible for the synthesis of the AHL signalling molecule 3OC6HSL. LuxR is the receptor of 3OC6HSL and the transcription activator of Lux- controlled genes. Once the 3OC6HSL is produced, it can freely diffuse into the environment and accumulate with increasing cell densities. When the concentration of 3OC6HSL reaches its threshold, it will interact with the LuxR protein to form the LuxR-HSL complex and then activate the transcription of luxCDABE[2]. If the quorum is reached, the AHL synthesis will be accelerated and the expression of QS controlled targets will be rapidly enhanced. Homologous LuxI/LuxR systems have been identified in many gram-negative bacteria, each capable of producing specific AHLs. In the opportunistic pathogens, such as *P. aeruginosa* and *Serratia marcescens*, these signaling mechanisms control the expression of the virulence factors. *Pseudomonas aeruginosa* contains 2 systems homologous to LuxI/LuxR. LasI/LasR has been shown to control biofilm formation and the production of extracellular enzymes, as well as transcription of another quorum-sensing system, RhlI/RhlR, adding an additional level of control through AHL signaling[9].

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Quorum sensing in Gram-positive bacteria

In Gram-positive bacteria, the QS system is usually comprised of modified oligopeptides (Autoinducing peptides- AIP) as signalling molecules. These peptide signals interact with the sensor element of a histidine kinase two-component signal transduction system. Besides using different types of signalling molecules, there are two main differences between AIP mediated QS and AHL-mediated QS. First, AIP is a modified oligopeptides and cannot freely diffuse across the cell membrane; it should be exported with the aid of exporters. Second, the signalling process for AIP-mediated QS is relayed by two component histidine kinase. The most typical example of AIP-mediated QS is the *agr* (accessory gene regulator) system in *Staphylococcus aureus*[10,11]. The *agr* QS system is encoded by *agrBDCA* operon. Gene *agrD* encodes the precursor of the signaling oligopeptides (AIP), and AgrB protein is responsible for the oligopeptide exporting and modification. AgrC and AgrA consist of a two- component histidine kinase-response regulator. AgrC is a transmembrane protein that can bind with AIP. Once AgrC binds with AIP, the intracellular AgrA will be phosphorylated by a two step phosphorelay. This phosphorylation can activate AgrA to induce the transcription of target genes. Similar to LuxI/R type QS, the phospho-AgrA can also induce the expression of the *agrBDCA* operon to form a positive feed-back loop.

Quorum sensing signal AI-2

Many Gram-negative and Gram-positive bacteria possess quorum-sensing systems that detect an extracellular signal named AI-2. Both of AHL- and AIP-mediated QS are involved in intraspecies cell–cell communication; the AIs and their cognate receptors are species specific and are only effective in intraspecies communication. In contrast, AI-2 can be synthesized in a remarkably wide variety of bacterial species and can be recognized by other species besides the producer[12,13]. Hence, AI-2 mediated QS are an interspecies cell–cell communication system, and AI-2 is considered to be a universal language in bacterial world. AI-2 is a furanosyl borate diester (3A- ethyl-5,6-dihydrofuro [2,3-D] [1,3,2] dioxa-borole-2,2,6,6A tetraol in *Vibrio harveyi*) synthesized by LuxS from S-adenosylmethionine (SAM)[14,15]. The signalling process relied on several phosphorylation-relay steps[16]. In *V. harveyi*, the signal-relay system is comprised of LuxPQ, LuxU, and LuxO. At low cell densities, in the absence of AI-2, LuxQ serves as a kinase and is autophosphorylated. The phosphate was transduced to LuxU and then transferred to LuxO. Phospho–LuxO (LuxO–P) then activates the transcription of Qrr1-5 (genes encoding five small regulatory RNAs (sRNAs)). Qrr sRNAs are master regulators controlling the transcription of a variety of genes [15,16]. At high cell densities, once AI-2 reaches its critical concentration, it can be detected by the periplasmic proteins LuxP and LuxQ complex. Binding with AI-2 will switch the LuxQ from kinase to phosphatase and result in the dephosphorylation of LuxO–P. Therefore, the Qrr sRNAs are not transcribed and the response is the switch-off of the Qrr sRNAs regulation (Ng and Bassler, 2009).

Detection of Quorum sensing molecules

A number of techniques for detection and identification of quorum sensing molecules or for monitoring the activity of these compounds have been described [17]. Approaches used for detection and identification of AHLs include cell- based assays using AHL-specific bacteria biosensors, thin-layer chromatography, gas chromatography/ mass spectrometry, and liquid chromatography coupled with electrospray ionization and a hybrid quadrupole linear ion trap and Fourier-transform ion-cyclotron-resonance mass spectrometry [18]. Steindler and Venturi (2007) described the available bacterial biosensors used to detect various AHL signal molecules. The very large number of AHL QS systems identified has been rendered possible mainly *via* the use of bacterial biosensors that are able to detect the presence of AHLs. These biosensors do not produce AHLs and contain a functional LuxR-family protein cloned together with a cognate target promoter (usually the promoter of the cognate luxI synthase), which positively regulates the transcription of a reporter gene (e.g. bioluminescence, β-galactosidase, green-fluorescent protein and violacein pigment production).

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The first step in determining whether a bacterial strain contains a LuxI/R QS system is to test for the production of AHL signal molecules. Each AHL biosensor relies on a particular LuxR family protein, thus displaying specificity towards the cognate AHL and in some cases to closely related AHLs. As many biosensors detect a narrow range of AHLs, it is essential, when testing a bacterium for AHL production, to use several biosensors, each responding to AHLs with different structural features. AHL biosensor strains can be used in different ways; firstly, the tester strain can be streaked and grown on solid media close to the biosensor to form a 'T' and the phenotypic change associated with the presence of exogenous AHLs will be observed as a gradient with most response observed at the meeting point of the two strains. Secondly, AHLs can be extracted from spent supernatants of late exponential phase cultures [19,20], and partial characterization can be carried out by TLC on C18 reversed phase plates. This organic extraction increases many-fold the sensitivity of biosensors; AHLs generally partition into the organic phase, and the solvent is removed by drying. The TLC plates are loaded with the sample extracts and with different standards and, after chromatography, overlaid with a soft-agar suspension of the AHL biosensor strain [19]. Each AHL migrates with a characteristic mobility and results in a spot shape of response detected in a way depending on the reporter of the biosensor strain. The 3-oxo-AHLs produce tear-shaped spots, whereas alkanoyl- AHLs and 3-OH-AHLs migrate and form well-defined circles. It is sometimes advantageous that before TLC, the sample extracts are further purified by C18-reverse phase HPLC and the resulting fractions tested for activity against AHL-biosensors either directly or via TLC [20]. Separation by TLC coupled with detection by AHL biosensors gives a rapid and direct visual index of the AHL(s) produced by the tester bacteria. AHLs cannot be unambiguously identified using TLC. However, their chromatographic properties can be used to assign tentative structures, as Rfs calculated for the samples can be compared with Rfs of AHL standards. AHL structures are unequivocally determined on the basis of spectroscopic properties [20] including MS and nuclear magnetic resonance spectroscopy (NMR). Several of the AHL biosensors can also be used for quantifying AHLs by measuring the activity of the reporter system present in the biosensor bacterial strain. This is useful for studying regulation of AHL synthesis and for identifying strain-level differences in AHL production. In order to quantify accurately one must determine, using the synthetic AHL, the minimal amount of AHL required for a response as well as the amount necessary for a saturated response in order to plot the linear dose response.

Agrobacterium tumefaciens AHL biosensors based on the TraI/R QS system detect a broad range of AHLs and also display the greatest sensitivity towards these compounds [21,22]. AHL biosensor *A. tumefaciens* NT1 (pZLR4) consists of strain NT1 cured of the Ti plasmid and thus unable to produce AHLs, and plasmid pZLR4. The plasmid contains the traR gene and one of the tra operons, responsible for Ti plasmid conjugal transfer, containing a traG::lacZ reporter fusion, the transcription of which is known to be regulated by the TraI/R AHL QS system [21]. This β-galactosidase-based biosensor is particularly well suited for TLC analysis. It is so sensitive to many AHLs that it requires only small volumes of AHL extracts from spent supernatants [21.] Paper strip whole cell biosensors are a novel, inexpensive, and portable filter-paper-based strip biosensor for the detection of bacterial quorum sensing signalling molecules, AHLs. First, a bacterial cell-based sensing system employing components of AHL-mediated QS regulatory system as recognition elements and *β*-galactosidase as the reporter protein was designed and developed. The bacterial-sensing cells were then liquid dried in strips of filter paper. *β*-Galactosidase as the reporter allows for the visual monitoring of the analyte induced signal when a colorimetric method of detection is applied. The paper strip biosensor was able to detect low AHL concentrations down to 1×10^{-8} M. Furthermore, it was successfully applied to the detection of AHLs in physiological samples, such as saliva. The filter-paper-based sensing strips could provide reproducible results upon storage at 4 °C for at

least 3 months. A filter-paper-based strip biosensor developed allows visual, fast, and convenient detection of AHLs in a dose-dependent manner in a test sample. In addition, it does not require expensive equipment or trained personnel and allows ease of transportation and storage. Therefore, this biosensor will serve as a simple and economical portable field kit for on-site monitoring of AHL in a variety of clinical and environmental samples [23].

Determination of the presence of AI-2, a well-known QS reporter kit (designed for detection of QS molecule AI-2 in Vibrio, ATCC # BAA 1116–1121) was developed by Bassler *et al*. [*V. harveyi* BB170 (luxN::tn5Kan) and *V. harveyi* MM32 (luxN::cm, luxS::tn5Kan)] is commonly used[24]. Because the AI-2 QS system regulates the luciferase operon in *V. harveyi*, *V. harveyi* should be a suitable host for biosensor construction by rewiring the bioluminescence with exogenous AI-2. In *V. harveyi*, there is another QS system that used AI-1 (N-(3-hydroxybutanoyl)-L-homoserine lactone) as a signaling molecule interfering with the AI-2 signalling process [10]. Therefore, both of the AI-2 biosensors, BB170 and MM32, had an insertion mutation on the LuxN receptor needed for AI-1 detection [24]. They only responded to exogenous AI-2 by producing induced luminescence. Because its original LuxS is intact in BB170, BB170 can synthesize AI-2 and produce low base-level bioluminescence without exogenous AI-2. It is also widely used as a simple tool for a qualitative test for the production of AI-2 in various bacteria [25]. However, for BB170 detection, supernatants from *V. harveyi* BB152 (luxLM::Tn5; AI-1-; AI-2+) and MM77 (luxLM::Tn5, luxS::Tn5; AI-1-; AI-2-) are usually required to serve as positive and negative controls [26]. By mutation on AI- 2 synthase LuxS and AI-1 receptor LuxN, the strain MM32 thus produced no endogenous AI-2 and only responded to exogenous AI-2; it showed better performance than BB170 with lower base-level luminescence. However, it should be noted that these biosensors were sensitive to growth conditions (such as pH and glucose) and bioluminescence could be inhibited by a high concentration of AI-2 [27].

The AIP sensing system in Gram-positive bacteria is highly specific [28,29]. For example, *S. aureus* has been divided into four groups based on the specific interactions between AIP and ArgC [30]. Each group produced the structurally distinct AIP (different amino acid sequences and lengths), and only activated the agr response in itself and in the same group members, but showed intra-group inhibition on agr response [29,30]. Therefore, a series of AIP biosensors have been constructed for application in different species and groups. The most common AIP biosensors are constructed in the AIP gene mutant (e.g., agrD mutant of *S. aureus*, comQ mutant of Bacillus species) of itself or the same group members. The reporter genes (gfp, yfp, lacZ, lux, etc., optimized for expression in Gram-positive bacteria) are usually harbored on plasmids and transcriptional fused reporter genes with the AIP QS controlled promoters (e.g., P3 promoter in agr system in *S. aureus*, srfA promoter in com system in Bacillus). These biosensors use the host-expressed AIP receptors (e.g., AgrC in *S. aureus*) to detect the exogenous AIP, process the signaling based on the AIP QS system in the host, and then activate the reporters by the inducible promoters. This kind of biosensor can only specifically respond to the AIP of itself or the same group. However, by heterogeneously expressing another group AgrC in agrC and agrD double mutant, the strain of *S. aureus* could be converted to detect other groups of AIP[31]. Similarly, the ComX pheromones in Bacillus are defined as four different groups [32]. Biosensors for different groups of ComX were constructed in a Bacillus substilis mutant (comQ::Kan, ComQ is responsible for the maturation of ComX precursor) by replacing the original comXP genes with another comXP that belongs to other groups [33].

Interference with quorum sensing

As the QS mechanism, among others, controls virulence factor synthesis in many pathogenic bacteria, it is now generally accepted that the ability to inactivate autoinducers and suppress QS signal generation and/or response might be useful in controlling infection development and persistence of human, animal and plant bacterial pathogens [34,35]. Interference in the

QS mechanism can be achieved in a variety of ways. First, many natural substances can disturb the signal perception by imitating AHLs structure. The AHL analogues block the AHL receptor (regulator) protein and therefore prevent activation of the target gene expression (Manefield et al., 1999). Halogenated furanones produced by red alga *Delisea pulchra* were able to inhibit AHL-dependent carbapenem antibiotic synthesis and extracellular plant cell wall degrading enzyme production in *P. carotovorum* subsp. *Carotovorum* [36]. Many studies showed that also higher plants produce and secrete secondary metabolites that interfere with the microbial QS systems [37,38]. During the plant development, different compound that mimic bacterial signal molecules are secreted through the plant root system affecting significantly the rhizosphere bacterial gene expression. Those active plants compound have not been identified yet, so the mechanism of their interference with bacterial QS remians unknown. Recently, Adonizio et al. (2008)[39] reported an inhibitory effect of aqueous extracts of plants *Conocarpus erectus*, *Callistemon viminalis* and *Bucida buceras*, on *P. aeruginosa* QS resulting in the inhibition of virulence factor production. Also here, the mode of action of active compounds is not known, however, their bacteriocidal or bacteriostatic activity has been excluded.

The growing potential of synthetic QS inhibitors is well-documented, too[40]. Synthetic analogues of *N*- acyl homoserine lactones, such as *N*-acyl-3-amino-5*H*-furanone effectively block LuxR protein preventing cognate signal molecules binding. A review concerning the influence of natural and synthetic analogues of AHLs on QS of Gram-negative bacteria has been recently presented by Geske et al. (2008)[41]. Any factor that interferes with the QS of pathogenic bacteria by inactivating of signal molecules and affecting the expression of the target genes might be an excellent tool for the development of antibacterial therapies. Since the low molecular weight compounds mentioned above enables inhibition of the QS mechanism, an alternative strategy is offered by AHL-degrading enzymes, which seems to be one of the most potent approaches for silencing QS.

Application of Quorum sensing in Biotechnology

Pathogen diagnostics and therapeutics

Most of the whole cell QS biosensors that have been described so far recognize gram-negative AHLs [18,42]. A typical AHL biosensor contains an AHL-responsive transcriptional regulator and a cognate promoter, which directs the transcription of a reporter gene. It has been suggested that QS signals alone can be used as markers for the presence of pathogenic bacteria in clinical and environmental samples. However, QS-deficient mutants often develop after the host has been successfully colonized [43]. Consequently, QS signals should not be employed as the only inputs for microbial biosensors. Nevertheless, QS-based amplification circuits can still be used to engineer biosensing circuits to detect the presence of pathogenic microbes in contaminated groundwater, produce, dairy, and meat products. For example, the pathogen EHEC (*E. coli* O157:H7) is estimated to cause more than 70,000 illnesses and 60 fatalities each year in the US alone [44]. Genetic circuits could be designed to recognize EHEC cell surface antigens (such as somatic O antigen O157 and the flagella antigen H7) or the shiga toxin as input. This information could then be interfaced with a QS-like signal amplification module and finally transformed into a readable output. An alternative approach would be to apply directed evolution to modify existing QS receptors to recognize the surface antigens of pathogenic bacteria.

Future design directions include the creation of ingestible whole-cell biosensors by introducing QS-based biosensing devices into GRAS organisms like lactic acid bacteria (LABs) [45]. Such diagnostic biosensors would be very useful in detecting the presence of pathogens in the gut microflora. Previously, LABs and other commensal bacteria have been engineered to deliver therapeutic compounds and vaccines to the mucosa [46]. Taken together, these results bring up the interesting possibility that future QS-based microbial biosensors may not only detect pathogens, but also mount a concerted response against them. The *P. aeruginosa* QS signal 3-oxo-C12- HSL inhibits proliferation and induces apoptosis in human breast cancer cell

lines[47]. However, its direct application in anti-cancer therapy is prevented by the possibility that it may incite *P. aeruginosa* virulence in immune-compromised individuals. Nevertheless, 3-oxo- C12-HSL is a good starting point for developing synthetic AHL homologs that retain anti-cancer toxicity while losing the ability to activate QS[48]. Microbial whole-cell biosensors and gene delivery vehicles are of great interest in cancer therapy. Interestingly, intravenous administration of *E. coli*, *Bifidobacterium longum*, and attenuated strains of *V. cholerae*, *Salmonella typhimurium*, and *L. monocytogenes* results in their selective localization to, and proliferation in, mouse solid tumours and metastases [49,50]. Targeted microbial localization has been demonstrated for cancers in murine bladder, breast, and brain. Their selective accumulation in tumours is likely influenced by the advantageous conditions in tumour microenvironment, such as poor immune surveillance and hypoxia. Microbial aggregation in tumour sites in vivo can be exploited to create biosensors that target cancer cells. Anderson et al. (2006) have provided an excellent example of how bacteria may be engineered to use their own cell density as a decision point for invasion of cancer cells [51]. The authors created in *E. coli* a genetic network in which the Yersinia pestis invasin gene was regulated by the *V. Fischeri* LuxR/I system. Expression of invasin allows *E. coli* to bind and invade mammalian cells displaying β1-integrin cell surface receptors. High AHL concentrations triggered the expression of invasin and allowed *E. coli* to penetrate cocultured human cancer-derived cells. Taken together, these studies suggest that microbial biosensors can be designed to recognize cancer cell aggregations *in vivo*. A key requirement of such biosensors would be specificity, i.e. clear differentiation between tumour and healthy cells. This can be achieved by designing synthetic genetic circuits to recognize multiple inputs such as cancer cell surface antigens, cancer microenvironment-specific conditions such as hypoxia, and local cell density of the biosensor (the latter through QS). Combining these inputs through an AND gate would provide higher specificity and tighter control. One concern in designing cancer biosensors would be to ensure that the engineered microbe itself does not trigger off a host immune response or cause other harm to the host.

Quorum Quenching Application

The biotechnological applications of AHL degradation as a new, promising method for fighting detrimental bacteria have focused researchers' attention. As many of the human and plant bacterial pathogens employ the AHL-based QS mechanism for regulation of the pathogenicity determinant synthesis or biofilm formation, the application of Quorum Quenching (QQ) strategy may be an alternative approach for fighting these microorganisms. Fighting bacterial plant pathogens remains difficult and ineffective. The most extensive studies of QQ application have been performed on the plant pathogenic bacteria from the former pectolytic *Erwinia* genus. These bacteria, which nowadays are classified into three different species (*Pectobacterium atrosepticum, P. carotovorum* subsp. *carotovorum* and *Dickeya* sp.) are the causative agents of soft rot diseases of many economically important plants. They are also responsible for the blackleg disease of potato plants in temperate climate. As the AHL-dependent QS mechanism is involved in bacterial pathogenesis [52], QS interference strategies are proposed as a new, alternative approach to the attenuation of bacterial infection development in plants. The first strategy relies on introduction of a gene coding AHL synthase directly to the plant cells and its stable expression in the plant tissue [53]; the second one employs AHL-degrading bacteria for plant protection against *Pectobacteria* [54]. Yet another approach involves heterologous expression of genes encoding AHL-degrading enzymes in pathogen cells or in plant tissue [55]. The success of preliminary research has enabled a new and alternative strategy for controlling bacterial infections.

As bacterial populations use signal molecules to sense cell density and coordinate their own behavior, the artificially increased level of AHLs (due to expression of AHL-synthase gene in the plant tissue) makes bacteria to misinterprete the population size. Such misinterpretation leads to the production of virulence determinants long before the

pathogen population is large enough to sustain infection in the plant which in turns would enable the pathogens invasion to be overcome[56]. However, Toth et al. (2004) reported that AHL production by transgenic plants not only can induce bacterial infection development but also makes the plants more sensitive and susceptible to infection caused by pectolytic *Erwinia* (*Pectobacteria*)[57]. This is in contradiction to earlier results obtained by [56]. Application of bacterial cells producing AHL degrading enzymes prevents maceration of plant tissue by the tested pathogens [58]. This strategy gives the opportunity for direct application of AHL degrading bacteria as biological control agents of plant bacterial diseases. Transgenic potato and tobacco plants expressing the gene encoding AiiA lactonase manifested strong resistance against infection by *P. Carotovorum* subsp. *carotovorum* [59]. This was the first example of purposeful usage of the AHL degrading enzyme for attenuation of infection symptoms development in plants, however, this strategy requires the genetic modification of higher organisms which is not universally accepted.

Introduction of genes encoding AHL degrading enzymes resulted in a decrease of the AHL level in the surrounding environment and hampered the ability of *P. carotovorum* to prolong infection [55]. Heterologous expression of the *aiiA* gene encoding the AiiA lactonase from *Bacillus* sp. in *P. carotovorum* cells, impedes production of exoenzymes, and disease symptoms development. A similar approach was used for two other important plant pathogens *Burkholderia thailandensis* and *Erwinia amylovora* where AHL lactonase encoded by *aiiA* gene homologues was expressed [60,61]. The same approach was employed to study the silencing of the hierarchical QS system of *P. aeruginosa.* In this case, it resulted in reduction of signal amount of the first QS system (3-oxo-C12-HSL) and in consequence prevented accumulation of the second QS system signal molecule (C4-HSL) [62]. The pathogenicity factor synthesis and swarming motility were markedly reduced. However, the fading of the signal did not influence the bacterial cell adhesion capacity and did not interfere with surface colonization by these bacteria. Park et al. (2005) demonstrated that addition of purified AhlM protein to the growth medium of *P. aeruginosa* eliminated 3-oxo-C12-HSL and reduced and delayed C4-HSL accumulation and strongly decreased virulence factor synthesis [63]. The extracts from *Prosopis juliflora, Nerium oleander, Eucalyptus globulus* and *Catharanthus roseus* showed significant reduction on biofilm formation[64]. Also the south Indian folk plants extract of *Termanalia chebula Awsonia inermis, Solanum torvum, Termanalia arjuna* has the Quorum Quenching property [65]. The above examples show that, the AHL degrading enzymes together with QS inhibitors may be successfully used to disrupt bacterial cell to cell communication and to control bacterial infections.

CONCLUSION

Most of the bacteria control the variety of microbial cell activities like biofilm formation and virulence by quorum sensing, which significantly impact human health, agriculture and food industries. The detection of the quorum sensing molecules using biosensors has been developed which is able to detect most of the QS molecules. The paper strip whole cell biosensor developed novel filter-paper strip biosensor provides a fast and convenient method for the detection of AHLs, which could be employed for first-level screening of a variety of environmentally and clinically relevant samples. The sensing system could also be utilized for evaluating the presence of molecules able to interfere with QS, including agonists and antagonists. A filter-paper-based strip sensor is amenable to high-throughput analysis, provides easy transportation and storage, and does not require instrumentation or trained personnel; therefore, it could be a component of a simple and inexpensive field kit. Numerous reports show that bacteria become resistant to a great variety of antibiotics, which results in the reduction in the potential use of pharmaceuticals and their availability for humans. Such observations lead straightforwardly to the conclusion that new therapies, alternative to antibiotic usage, ought to be invented in the near future. QS inhibition by enzymatic degradation of signal molecules

is one such option. It has been reported that most, if not all bacteria, both gram negative and gram positive, use a sort of QS for controlling population-dependent behaviour. Therefore even if the signal molecules exploited in these processes have many distinct structures, it is usually possible to find an enzyme which could destroy any one of them.

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