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A review article: Anti-quorum sensing agents as a potential replacement for antibiotics in Phytobacteriology

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Abstract
Quorum sensing is a bacterial cell to cell communication, which helps bacteria to mount population-density-dependent infection to overcome the defence responses from the host. In this mechanism some diffusible chemical signalling compounds are involved, known as autoinducers, which are directly proportional to the population cell density. The main role of quorum sensing is to coordinate the expression of several collective traits, including biofilm formation, bioluminescence, epiphytic fitness, Production of virulence factors, secondary metabolites with antimicrobial activity, pigments, siderophores, plasmid transfer and motility. Due to the growing bacterial resistance to the antibiotics that have been overused, it has become necessary to search for alternative antimicrobial therapies. One of them is anti-quorum sensing agents/anti-biofilm agents/quorum sensing inhibitors that disrupts the bacterial communication. This review article discusses the various quorum sensing-disrupting mechanisms used by anti-quorum sensing agents such as, inhibition of Autoinducer synthesis inhibition of transport of Autoinducers, degradation of autoinducers using enzymes, sequestration using monoclonal antibodies, signal competition, as well as the different techniques applied artificially to inhibit the quorum sensing pathways in bacteria and thus, protecting plant from bacterial diseases.

Keywords: Anti-quorum sensing agents, autoinducers, quorum sensing, quorum quenching

Introduction
'Quorum' is a Latin word which means "number of members of a group required to be present to carry out an activity legally". Quorum sensing (QS) was 1st reported by Nealson *et al.* (1970) [31] in *Vibrio fischeri* and *Vibrio harveyi*, a luminous marine gram negative bacterium. QS is the regulation of gene expression in response to fluctuation in cell population density (Miller *et al.*, 2002) [28]. This allows them to carry out colony wide function and help them to survive, compete, and persist in nature or to colonize a particular host.

QS involves the exchange of low molecular weight, diffusible signal molecules between members of a localized population, known as autoinducers (AIs), which are directly proportional to the population cell density. Three major AIs involved in QS are N-Acylhomoserine lactones (AHLs) in gram negative bacteria, Oligopepetides in gram positive bacteria, Autoinducers 2 (AI-2) in both gram positive and gram negative bacteria. These signal molecules are secreted by bacteria extracellularly and after reaching some threshold level it diffuses inside the cell and binds to receptor protein. The main role of QS is to coordinate the expression of several collective traits, including the production of antibiotics (Bainton *et al.*, 1992) [6], bioluminescence (Nealson and Hastings, 1979) [32], virulence factors (Barber *et al.*, 1997) [8], swarming (Eberl *et al.*, 1996) [18], plasmid conjugal transfer (Fuqua and Winans, 1994) [20] and exopolysaccharide biosynthesis (Beck and Farrand, 1995) [9]. The plant pathogenic bacterium *Erwinia carotovora* causes soft-rot in potato and other vegetables by secreting cell wall degrading enzymes such as cellulase and pectinase and the production of these virulence factors are coordinated by QS (Fig. 1). A cognate pair of ExpI/ExpR (LuxI/LuxR homologues) is involved in extra-cellular enzyme secretion (Hinton *et al.*, 1989; Loh *et al.*, 2002) [21, 24]. ExpI produces primary AHL, 3-oxoC6HL whereas, ExpR encodes for ExpR regulator protein. Mutants defective in *ExpI* do not produce extracellular enzymes and fail to secrete harpin. Therefore, they are completely non-pathogenic (Chatterjee *et al.*, 1995; Cui *et al.*, 1996) [12, 16].

At high AHL density, 3-oxoC6HL binds with regulator protein and forms active complex which triggers the expression of target genes encoding for cellulase, pectinase and polygalacturonase.

In this review article, we have concentrated on different mechanisms used by (or can be used) anti quorum sensing (AQS) agents against agriculturally important bacteria, its limitations over antibiotics and future thrust.

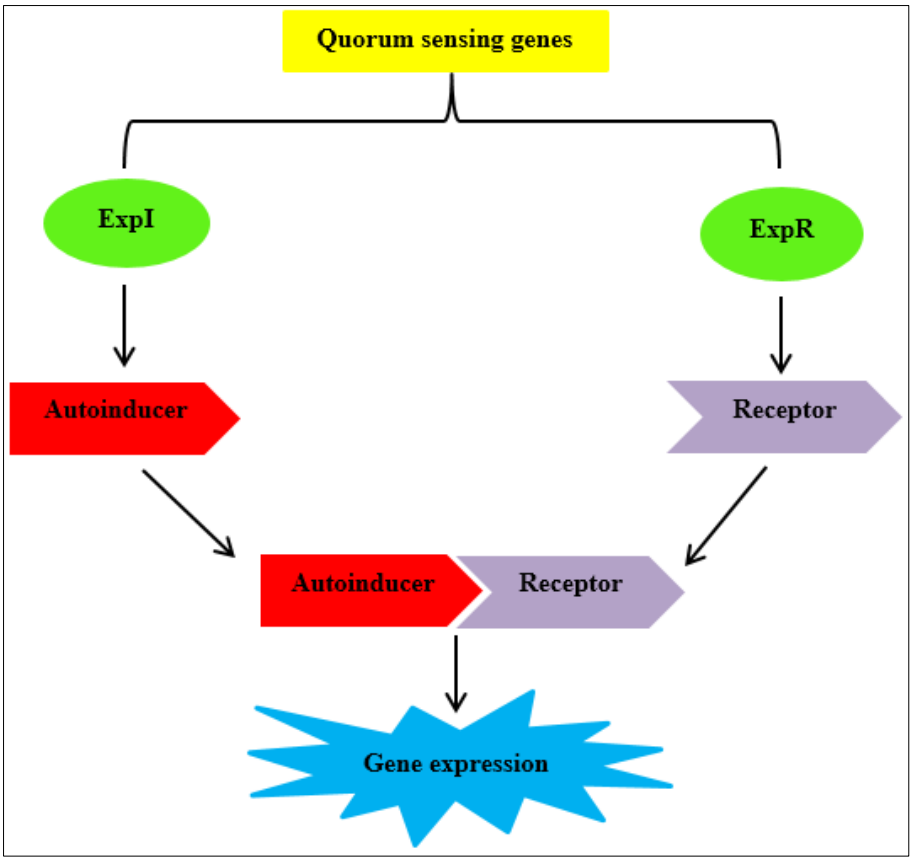

Fig 1: ExpIR mediated signalling in *Erwinia carotovora*

Anti-quorum sensing agents

Inactivation and disruption of quorum sensing signalling is known as quorum quenching (QQ) and the agents involved are known as anti-quorum sensing agents/anti-biofilm agents/quorum sensing inhibitors. The ideal AQS agents should be chemically stable, low molecular weight and it should not posses any toxic side effects on the bacteria, posses high degree of specificity for the QS receptor protein, (Asfour, 2018) [5]. Givskov *et al.* (1996) [18] identified halogenated furanone as first AQS compound, produced by the benthic marine Australian macro-alga, *Delisea pulchra* which inhibited the QS-regulated behaviours in *Serratia liquefaciens* (opportunistic human pathogen) by competitively bind with the SwrR (LuxR type proteins).

Working mechanism of anti-quorum sensing agents

Various mechanisms are used by by AQS for quenching the communication in bacteria. Some of the important mechanisms are (1.) Inhibition of AIs synthesis (2.) Inhibition of AIs transport (3.) The degradation of AIs using enzymes (4.) Sequestration of AIs using antibodies (5.) QS signal competition (QS mimicry).

Inhibition of AIs synthesis

AQS agents working under this mechanism targets the precursors of AHL synthesis such as acyl-ACP and SAM (S-adenosyl-methionine). Analogues of SAM, namely sinefungin (an SAM like antibiotic), competitively binds with AHL synthase thus, inhibit the synthesis of AHL. Triclosan is another good example of AHL synthesis inhibitor which targets the enoyl-ACP reductase activity (Hoang and Schweizer, 1999) [22]. Chung *et al.* (2011) [14] identified another AHL antagonists (named J8-C8), which is an acyl-ACP carrier competitive inhibitor.

Precursors involved in autoinducing peptide (AIP) signal synthesis in gram-positive bacteria are also good targets but till now no inhibitors targeting these proteins have been reported (Brackman & Coenye, 2014) [10].

Inhibition of AI transport

In *Escherichia coli* QS is mediated by the signal generation, secretion, and uptake of autoinducer- 2 via ABC transporter (ATP Binding Cassette protein). Inside the cell AI-2 gets phosphorylated in to phospho-AI-2 in the presence of LsrK (AI-2 kinase), which triggers gene expression. Phospho-AI-2 degrades overnight to 2-phosphoglycolic acid (PG). Roy *et al.* (2010) [35] added LsrK and ATP outside the cell which phosphorylated AI-2 into phospho-AI-2 which apparently prevented from being transported inside cells, in this way QS mechanism was quenched.

The degradation of AIs using enzymes

QS signals can be enzymatically degraded by using AHL lactonases and AHL acylases, which hydrolyze the homo-serine lactone ring and amide bonds of AHL molecule, respectively (Fig.2). Whereas, AHL oxidases and AHL reductases do not degrade the AHL molecule instead they modify it by reducing carbonyl or hydroxyl groups

(Brackman & Coenye, 2014) [10]. Bacterial species such as *Agrobacterium tumefaciens*, *Arthrobacter*, *Acinetobacter* spp., *Bacillus* spp., *Bosea* spp., *Delftia acidovorans*, *Pseudomonas. Aeruginosa*, *Sphingomonas* spp., have been reported to produce enzymes which are capable of degrading AHLs (Uroz *et al*., 2009) [36]. Apart from these eukaryotes like plants and root associated fungi including *Hordeum vulgare*, *Lotus corniculatus* and *Pachyrhizus erosus* can degrade AHLs (Uroz & Heinonsalo, 2008) [37]. To date no AIP or AI-2 QS signal specific degrading enzyme have been described.

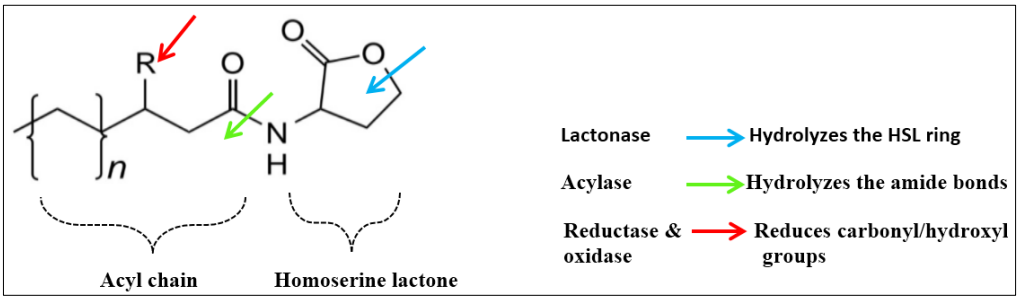

Fig 2: Sites of action of various degrading enzymes on AHL

There are different ways to expose phyto-pathpathogenic bacteria to AIs degrading enzymes, one of them is by biotization. Biotization is the process by which non-native microbes (AHLs degrading enzymes producing microbes) are introduced inside plant. These microbes increases plant immunity against phytopathogens by helping them to obtain more transition metals by producing siderophores (Fones and Preston, 2013) [19]. Apart from producing AHL-degrading enzymes these QQ microbes will occupy most of the intercellular space thus leaving very few spaces for later-invading phyto-pathogenic bacteria (Alagarasan *et al*., 2017) [1] (Table 1). Another way is by Mutagenesis, in *A. tumefaciens*, production of the AHL lactonase is encoded by attM which in normal condition gets suppressed by the negative transcription factor attJ. Zhang *et al*. (2003) [39] knocked attJ out by transposon (Tn5) mutagenesis which resulted in biosynthesis of AHL lactonase, which degraded AHL and thus, QS-dependent conjugal transfer of Ti plasmid in plants was inhibited. Plants can also be genetically transformed by engineering it with aiiA gene (autoinducer inactivation gene) from *Bacillus spp*. which encodes for lactonase enzymes (Table 2).

Sequestration of AIs using antibodies: Anti-AHL monoclonal antibodies that sequester the AHL signal molecules was first time used against *P. aeruginosa* (Park *et al*., 2007) [31]. Marin *et al*. (2007) [27] have made further efforts on the synthesis of QQ catalytic antibodies which bear analogy to the transition-state structure of AHL-ring hydrolysis thus effecting QS process.

QS Signal competition (QS mimicry)

In this mechanism signal analogs compete with AHL signal molecules and competitively bind with the receptor protein which leads to the conformational change in the protein. Rasmussen *et al*. (2000) [34] used halogenated furanone compounds (AHL analog) produced by the Australian marine macro-alga *Delisea pulchra*, which inhibited AHL-regulated processes, especially extracellular enzyme production, which is virulence factor in *E. carotovora*. Biofilm formation of *Serratia marcescens* and *P. aeruginosa* was drastically affected when bacteria was treated with AHL analogs in which the HSL ring was replaced by a cyclopentyl or a cyclohexanone ring (Morohoshi *et al*., 2007; Ishida *et al*., 2007) [30, 23] whereas, when the amide function in AHL was replaced by a triazolyldihydrofuranone, affected biofilm formation in *B. cenocepacia* and *P. aeruginosa* (Brackman *et al*., 2012) [11].

Table 1: Quorum sensing inhibiting endophytes that have been identified

Host plant	Endophytic organisms	Disrupts QS of pathogens	References
Potato & tomato	*Bacillus* sp. A24, **P. fluorescens* P3/pME6863 strain	*Pe. carotovorum* and *A. tumefaciens*	Molina *et al*., 2003 [29]
Tobacco	*Bacillus* sp., *Lysinibacillus* sp., *Acinetobacter* sp., *Serratia* sp.	Tobacco pathogens	Ma *et al*., 2013 [25]
Rice	**Burkholderia* sp. KJ006–engineered with aii A gene of *Bacillus thurungiensis*	*Burkholderia glumae*	Cho *et al*., 2007 [13]

* Genetically engineered

Table 2: Genetically engineered plants producing AIs degrading enzymes

Genetically engineered host plant	Aii A gene donor	Pathogen	References
Nicotiana tabacum and Solanum tuberosum	*Bacillus* sp. 240B1	*E. caratovora*	Dong *et al*., 2001 [17]
Amorphophallus konjac	*Bacillus thuringiensis*	*Erwinia carotovora subsp. Carotovora* (Ecc) SCG1	Ban *et al*., 2009 [7]
Carica papaya	*Bacillus cereus* strain CHB37	*Erwinia mallotivora*	Amin *et al*., 2016 [3]

Challenges

Major challenge is the specificity of AQS agents. The AQS agents are highly specific but if we are using anti-QS agents targeting AI-2 i.e. interspecific type signal molecule may affect non-target bacteria also. There are various reports suggesting that the deletion of *luxS* (Δ*luxS*) increased the

pathogenicity features in *Helicobacter pylori* (Cole *et al*., 2004; Anderson *et al*., 2015) [15, 4], *Vibrio cholerae* (Ali and Benitez, 2009) [2], and *Haemophilus parasuis* (Zhang *et al*., 2019) [38]. Maeda *et al*. (2012) [26] reported, *P. aeruginosa* could develop resistance to against furanones by mutating genes encoding efflux pumps, which are proteins responsible for the removal of harmful substances from cells.

Conclusion

Antibiotics kill or slow down the growth of bacteria and therefore, are more likely to yield resistant phenotype in bacteria but AQS do not threaten bacteria with life-or-death situations instead they attenuate bacterial virulence and therefore are less likely to yield resistant phenotype. Quorum-quenching mechanisms act by targeting key steps of quorum sensing by blocking signal generation, signal degradation, signal competition, signal transportation and signal sequestration. They have promising potential in basic research as well as biotechnological applications. There is a novel possibility of exploiting the QQ endophytes as a systematic and sustainable tool for plant disease management.

Future Thrust

With respect to QQ strategies, a number of problems remain unsolved like targeting and delivery of the enzymes or molecule, evaluation of the cytotoxicity in plants, adverse effect of QQ enzymes and agents at organism, population, cellular & subcellular levels. The development of new sophisticated assay techniques combined with new analytical imaging systems in future will help us in understanding bacterial behavioural and developmental strategies.

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