Herbal Based Quorum Identifying Inhibitors of Pseudomonas Aeruginosa

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ABSTRACT - *Phytochemical research has enlarged a lot of momentum in the therapeutic field for the discovery of new, safe, and operative remedies. In the context ofantimicrobial research, various plant foundations have been exposed with the potential to disrupt bacterial quorum sensing (QS), which plays a keyrole in the parameter of virulence in many Gram positive and Gram negative bacteria. Pseudomonas aeruginosa, a Gram negative bacterium, isknown to crop multiple QS systems that control the expression of virulence elements and biofilm expansion in this pathogen. Hence, theinhibition of QS has been pursued as aauspicious therapy for treatment of drug resistant Pseudomonas infections. Aninclusive review of theresearch data available for plant products as QS inhibitors of the organism has been accessible here, while further suggesting the future prospectsfor these inhibitors.*

Keywords: *Pseudomonas aeruginosa, Phytochemicals, Quorum sensing, Anti-quorum sensing, Anti-virulence.*

I. INTRODUCTION

Quorum sensing (QS), the cell-to–cell signaling system in bacteria,has now been well predictable as an antimicrobial target. It isunstated that inhibition of QS, also called as quorum quenching(QQ), results in the succeeding reduction of bacterial virulence, sothat the contamination is controlled and the host immune system canfurther clear out the bacteria. It also offerings an advantage of lowerrisks for resistance expansion owing to the absence of selectivepressure, since bacterial growth is not exaggerated. The QS pathwaymonitors the populace density of bacteria in a confinedenvironment by the production of small signals called asauto inducers. At the recognizing of a quoratepopulation level, it inducesexpression of specific genes accompanying with secondary phenotypesin bacteria, such as bioluminescence, conjugation, antibioticproduction, virulence, biofilm maturation, sporulation, pigmentproduction, etc. Dissimilar types of signal molecules are fashionedin Gram positive and Gram negative bacteria. The most commonsignalingparticles include acyl homoserine lactones (AHLs), autoinducing peptides (AIPs) and AI-2. Other less categorized signalmolecules have been detected in some bacteria, such as the cyclicdipeptides, diffusible signal factor, AI-3, etc., which appear toregulate motility, virulence and biofilm formation.

1.1 AHL – LuxR/I system in Gram negative bacteria:

AHLs are the autoinducers frequently produced in Gram negativebacteria and commonly follow the LuxR/I mechanism of thebioluminescent bacterium, Vibrio fischeri. Each bacterial cellharvests a small amount of AHL, which is catalyzed by the synthaseprotein, LuxI. The AHLs diffuse in and out of the cell. With increasein bacterial number, the AHL amasses in the environment. At athreshold concentration of AHLs, it binds andstimulates transcriptional regulator, LuxR. The AHL-LuxR complexfurther binds to detailed promoter regions, regulating the countenanceof QS controlled genes, including the LuxI gene. The LuxR/Ihomologous proteins have been pragmatic in many Gram negativebacteria, such as Aeromonas hydrophila, Acinetobacter baumannii,Agrobacterium tumefaciens, Chromobacterium violaceum, Pseudomonasaeruginosa, Serratia marcescens, etc. An added AHL

system hasbeen identified in V. harveyi, called as the LuxM/N system, whichadjusts the production of luminescence in this marine bacterium.

1.2 AIP system in Gram positive bacteria:

The peptide pheromones or auto inducing peptides shaped in Gram positive bacteria, are small peptides (5-34 aa long), that aretwisted from the post-translational alteration of largeprecursor peptides (40-65 amino acids (aa) long). The AIPsformed by each cell are secreted into the atmosphere by an ATPbindingcassette (ABC) exporter. At a threshold attentiveness, theAIPs bind to the external domain of a cognate membrane boundsensor (histidine kinase), subsequent in the phosphorylation of itscytoplasmic domain. This stimulated sensor further phosphorylatesand activates another cytoplasmic response regulator protein, which is directly accountable for regulating the appearance of QScontrolled genes. The Gram positive bacteria known to produceAIPs, include Staphylococcus aureus, Staphylococcus epidermidis, Bacillus subtilis, Enterococcus faecalis, Streptococcus pneuomoniae,etc.

1.3 LuxS/AI-2 system in Gram positive and Gram negative bacteria:

AI-2 forms the collectiveauto inducercreated by both Gramnegative and Gram positive bacteria. It was initially discovered in *V.harveyi*, where it normalizes the production of bioluminescence. AI-2,in general, is a furanosyl borate diester and the modifiablemechanism involves a two component signaling system. Thespecific AI-2 produced by *V. harveyi* is a boron diester of (2*R*,4*S*)-2-methyl-2,3,3,4-tetrahydroxytetrahydrofuran (S-THMF), which ismanufactured by the enzyme, LuxS. At a low cell density, a smallamount of AI-2 is produced, which gathers in the environmentwith an increase in cell density. At a threshold concentration, itbinds to the periplasmic receptor protein LuxP. This protein thenactivates the sensor kinase LuxQ which further regulates expressionof luciferase genes in *V. harveyi*.

II. HUMAN PATHOGEN, PSEUDOMONAS AERUGINOSA

The Gram negative bacterium, *P. aeruginosa* is an aerobic bacillusthat is able to survive easily in trifling nutrient condition, andexists profusely in the environment. The organism is able to causeinfection in humans, animals, as well as plants. In individuals, it acts asaresourceful pathogen, commonly associated withimmunocompromised patients, burn victims, ICU patients, andpatients on lengthier antibiotic treatment. It is commonly discovered inthe sputum of CF individuals who are normallyexaggerated by thechronic pulmonary infection of *P*. The decreased mucusclearance in the lungs of CF patients provides a suitable niche for thegrowth of many bacteria, and further the CF specific alteration in thereceptor for the adhesins on pili of *P*. aeruginosa, facilitates Pseudomonasinfection in CF lung. It has been one of the mostmutual nosocomial pathogens, prominently causing ventilatoraccompanying pneumonia, surgical site infections or wound infections,catheter associated urinary tract infections and bloodstreaminfections. Almost all the clinical strains of *P*. aeruginosa. Haveevolved as multidrug resistant, and have been commonly connectedwith low susceptibility to the fluoroquinolones, cephalosporins andcarbapenems.

The competence to form biofilms and its adaptive nature, allows the creature to thriveon the medical devices for longer periods, while being unscathedwith the detergents and disinfectants, and such devices often formsthe source of entry into the host. The organism launches itsinfection by numerous virulence factors that are answerable forentering the host tissue, inducing inflammation, damaging hosttissue, inactivating host defense components, consequently leadingto tissue obliteration and further broadcasting to other tissues byinvading the blood vessels. The cell accompanying virulence factors suchas pili, flagella, lectins, etc. assist in bacterial adherence to the hosttissue, facilitating bacterial colonization within the host. Then,establishment may proceed to acute infection or chronic infection, bythe aid of its extracellular virulence factors. Chronic infection isconsidered by a low production of

virulence factors, biofilmformation and tissue damage, mainly caused by chronicinflammation. Acute infections involve extensive tissue impairment,bloodstream invasion and dissemination, realized by theproduction of several extracellular virulence factors such aselastases, exotoxin A, alkaline protease, exoenzymeS, rhamnolipids, phospholipase, pyocyanin, etc. In the currentscenario, very few treatment options are available for themanagement of Pseudomonasinfections, and hence, there is acrucial need to develop new drugs with stronger efficiency and lowerrisks of resistance. Besides the development of new antibiotics orantibacterial drugs, medical research has been besieged towards thediscovery of QQ compounds as anti-virulence drugs to fight theinfections caused by P. aeruginosa.

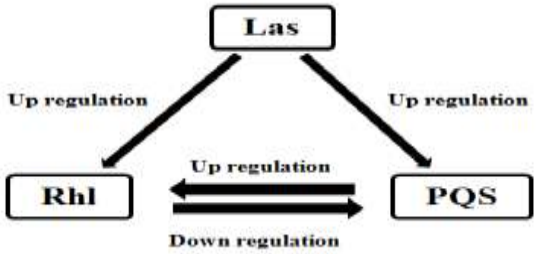
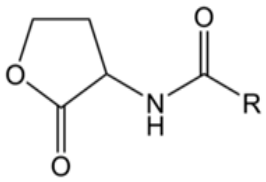


Fig. 1: The regulatory functions of Las, Rhl and PQS systems of *P.*



Homoserine lactone **Acyl chain**

Short chain: R = C4-C8

Long chain: R = C10-C18

Fig. 2: General structure of AHL molecule

The second mechanism of AHL inhibition involves disorderly itssynthesis, which can be accomplished either by affecting the expressionof LuxI, or by interfering with the normalfunctioning of enzyme catalysis. In a study by Tateda *et al*, thesub-MIC concentration of azithromycin was found to disrupt QS in *P.aeruginosa*, by distressing the expression of LasI, and it was furthercapable of attenuating the organism's virulence *in vitro* as well as *invivo*. Moreover, azithromycin was also found to be effective in arandomized clinical trial as a preventive measure against ventilatorassociated pneumonia [19]. An AHL analog, called as J8-C8, exhibitedinhibition of AHL synthesis by the LuxI homolog, TofI synthase of thebacterium, Burkholderia glumae. *In* silico studies were applied tounderstand the mode of action, wherein J8-C8 was found to bindstrongly at the acyl chain binding site of TofI, suggesting acompetitive inhibition of the enzyme.

Most of the QQ studies have engrossed on the inhibition of LuxR, as anoperative means to disrupt the QS system in bacterial pathogens.LuxR is the transcriptional regulator protein, which gets activated bythe binding of AHLs. It possesses separate binding sites for the AHL molecule, and the promoter sequences in the bacterial DNA [21].Inhibition of LuxR can be achieved by preventing the binding of itscognate AHL, or by affecting gene expression. The halogenatedfuranone, the first natural QQ compound to be discovered, has beenfound to operate by the competitive inhibition of the LuxR protein [22]. Various synthetic furanones, AHL analogs or other

dissimilarcompounds such as triphenyl compounds, salicylic acid,nifuroxazide, etc. have been identified as identified as LuxRinhibitors, and have been found to attenuate QS and its regulatedphenotypes in Gram negative bacteria such as A. tumefaciens, P. aeruginosa, V. fischeri, etc.

2.1 Plant products as anti-microbial and anti-QS agents:

It is very well known that plants have been traditionally used inancient civilization for the treatment of various human diseases, andthey also form the major principle of current medicinal practices,such as Ayurveda, Homeopathy, Naturopathy, etc. Plant powders, decoctions, extracts are commercially available and have beeneffective not only for specific diseased states, but also for the generalwellbeing of human health. Furthermore, plants have firm stand inthe pharmaceutical sector since many phytochemicals have guidedthe development of successful and effective drugs. The well-knownexamples of plant derived drugs include the pain killer, aspirinwhich is a simplified form of the compound, salicin (*Salix alba*), the
Anti-malarial quinine, anti-cancer drugpaclitaxel etc. Plants are the sources of variedbioactive metabolites such as the non-polar hydrocarbons and theirderivatives, such as terpenes, aromatic compounds such asphenolics and nitrogen containing alkaloids. Many of these havebeen identified with potential anti-microbial properties, suggestingtheir prospective future in infection therapy. Common phenoliccompounds such as catechol, eugenol, caffeic acid, catechin, phloretin, warfarin, etc. have been reported with antimicrobialpotential. The essential oils (monoterpenoids) occurring in plantspecies such as S. Alba, Valeriana officinalis, Rosmarinus officinalis, Cinnamomum verum, exhibited potential antimicrobialactivity.

III. CONCLUSION

The organism, *P.* aeruginosa, is emergent as a dreadful nosocomialpathogen, with its embryonic drug resistance and the amazingadaptability to diverse environments. Hence, it is indispensable todevelop new drugs, not only to regulator the infections, but also tocounteract the resistance phenomena. It can be observed that anenormous resource of active metabolites has been contained in thehuge diversity of plants on earth and only a small portion has beenexposed with the quorum quenching potential. Not only is itessential to explore more plant sources for the anti-QS activity, it isalso very important toward promote the known phytochemical QSinhibitors as anti-pathogenic drugs, before the pathogen evolves to
Defeat all the available antibiotics.

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