Anti-pathogenic Potential of Coral Associated Bacteria Isolated from Gulf of Mannar Against *Pseudomonas aeruginosa*

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Abstract Infections of *Pseudomonas aeruginosa* are of great concern because of its increasing resistance towards conventional antibiotics. Quorum sensing system of *P. aeruginosa* acts as a global regulator of almost all the virulence factors and majorly its biofilm formation. In the present study, quenching of QS system of *P. aeruginosa* has been explained with bioactives from bacteria associated with the coral *Acropora digitifera*. Isolated bioactives inhibited the expression of various virulence traits of *P. aeruginosa* like biofilm formation, and the production of extracellular enzymes like protease and elastase. This study also emphasises the potential of coral associated bacteria in producing bioactive agents with anti-pathogenic properties.

Keywords Quorum sensing · *Pseudomonas aeruginosa* · Coral associated bacteria · Biofilm · Anti-QS · Quorum sensing inhibitor

Density dependent cell to cell communication between bacterial population known as quorum sensing (QS) is of great interest, since virulence and resistance being its major outcome in bacteria. It has been reported that QS controls the regulation of virulence and biofilm formation that are 1000-fold more resistant to antibiotics [1].

Pseudomonas aeruginosa, an opportunistic pathogen causes infections in cystic fibrosis (CF), burns, wounds and pneumonia patients [1]. QS plays a major role in controlling the virulence of *P. aeruginosa* by regulating various virulence traits viz. LasA protease, LasB elastase, superoxide

dismutase, alkaline protease, hydrogen cyanide, pyocyanin, pyoverdine, rhamnolipid and biofilm formation [1].

QS being the major factor concerning enhanced virulence and broad spectrum resistance in *P. aeruginosa*, its inhibition will be of importance to treat infections caused by pathogenic drug resistant *P. aeruginosa*. Compounds with the ability to interfere the QS system of bacteria are called as QS inhibitors (QSI) or anti-pathogenic agents [2].

In recent past, many sources were explored for QSI, and few with the potential to inhibit various pathogens were reported from plants, synthetic chemicals and microbes [3–7]. Because of the increasing rate of rediscovery of known drugs from terrestrial sources, marine microorganisms have gained much attention, since many remain unexplored [8]. In this study, coral associated bacteria (CAB) were screened for antibiofilm and anti-QS properties, since they play a defensive role in coral reefs in controlling the pathogens of the host [9].

Out of 41 CAB screened, nine have shown anti-QS activity against the bioreporter strain *Chromobacterium violaceum* ATCC 12472 (Fig. 1a) [10]. CAB23 and 41 which showed around 90 % anti-QS activity against *C. violaceum* were studied further. Biofilm inhibitory concentration (BIC)—lowest concentration required to inhibit the biofilm formation—of the extracts was assessed against *P. aeruginosa* PAO1 with varying concentrations (5–2,500 $\mu\text{g mL}^{-1}$) and found to be 1.75 mg mL^{-1} . Further, the activity of CAB extracts on the growth of *P. aeruginosa* PAO1 was studied and found no significant reduction in growth of CAB treated cultures which further prove the potential of CAB extracts as a candidate for QSI.

Inhibition of biofilm formation by the CAB extracts was studied as earlier [11]. Both the light microscopic and confocal images have shown the reduction in surface area covered by the biofilms in slides treated with extracts

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Fig. 1 a Screening of CAB isolates for anti-QS activity by agar overlay method using *C. violaceum* ATCC 12472 as an indicator organism. **b** Confocal microscopy images showing the reduction in surface area covered by biofilms treated with CAB23 and 41 extracts

(Fig. 1b). Further, the Z-stack analysis of the 3D confocal images have revealed the reduction (60 %) in biofilm thickness.

The effect of CAB23 and 41 extracts on the QS system of *P. aeruginosa* PAO1 (LasA protease and LasB elastase) was studied as earlier [3]. Treatment of *P. aeruginosa* PAO1 with CAB extracts significantly inhibited the QS controlled production of LasA protease and LasB elastase. The inhibition percentage of elastase production has been determined for CAB23 and 41 as 75 and 30 %.

Since, cell surface hydrophobicity (CSH) plays an important role in biofilm formation and adhesion [12], effect of CAB extracts on CSH has been measured and found to have no action, that proves the inhibition of biofilm formation is not merely because of biofilm disruption as exhibited by detergents and surfactants, but because of QS inhibition that controls the formation of biofilm in *P. aeruginosa*. CAB23 and 41 which showed anti-QS activity were identified as *Delftia tsuruhatensis* (JN248445) and *Stenotrophomonas maltophilia* (JN248442) respectively by 16S ribosomal RNA gene sequencing and the sequences were submitted in GenBank.

In summary, inhibition of QS is the superior way to combat drug resistant pathogens, since the conventional antibiotics which target the growth of bacteria further increases the chance of resistance development. QS inhibition has no effect on the growth other than attenuating the bacteria, facilitating its clearance by host's immune system.

Further studies about such novel ecosystems will reveal bacteria with numerous bioactivities that could be explored to address the problems regarding human diseases.

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