29th ECCMID Amsterdam, Netherlands 13 – 16 April 2019

The congress of ESCMID

O1138 Application of quorum quenching enzymes from endophytic bacteria to control biofilm formation and virulence factor production in *Pseudomonas aeruginosa* PA01

Ravishankar Rai Vittal*[1]

[1] Department of Studies in Microbiology, University of Mysore, India

Background: Quorum sensing mechanism regulates expression of virulence factor and biofilm formation in pathogenic bacteria. Quorum quenching is part of the mechanisms to control the QS-regulated phenotype expression and involves degradation of QS signal molecules. In the present study, quorum quenching (QQ) enzymes isolated from endophytes were used to control the QS-regulated virulence expression and biofilm formation in the pathogen, *Pseudomonas aeruginosa* PA01.

Materials/methods: The endophytic bacteria (224 isolates) from medicinal plants, *Ventilago madraspatana* Gaertn., *Pterocarpus santalinus* Linn., and *Coscinium fenestratum* Gaertn. were screened for quorum quenching activity in the biosensor *Chromobacterium violaceum*. The cell free lysates of the bacterial endophytes were tested for QQ enzymes and the percentage of AHL hydrolysis was quantified by HPLC. The aiiA homologous genes in these isolates were identified and sequenced. The production of AHL-lactonase by *Enterobacter* sp. CS66 was optimized using RSM. The AHL-lactonase was produced and purified using AKTA pure chromatography to achieve maximum purity of the enzyme. The AHL-lactonase was tested for its ability to inhibit virulence factors expression and biofilm formation in *Pseudomonas aeruginosa* PAO1. The aiiE gene from *Enterobacter* sp. CS66 was cloned into pMAL-c2X and pET-19m plasmid expressed in *E. coli* JM109 and Rosetta DE3, respectively, to obtain pure AHL-lactonase.

Results: Cell-free lysates of endophytic bacteria, *Bacillus firmus*, *B. cereus* and *Enterobacter* species showed AHL degrading ability by inhibiting violacein production by 80 % in *C. violaceum*. The identification of the aiiA homologous gene confirmed the presence of AHL-lactonase in cell-free lysate. Under optimized culture condition, AHL-lactonase isolated from *E. aerogenes* VT66 had a molecular weight of 30 k Daltons. In *P. aeruginosa* PA01, the AHL-lactonase significantly inhibited biofilm formation and virulence expression such as pyocyanin production by more than 80%, rhamnolipid production by more than 70% and protease production by 80% ($P<0.001$). Cloning of novel aiiE gene from *Enterobacter* sp. CS66 into pMAL-c2X and pET-19m led to one step purification method in AHL-lactonase production.

Conclusions: The study shows the potential of AHLs degradation by AHL-lactonase, a QQ enzyme from endophytic bacteria and inhibition of quorum sensing regulated virulence and biofilm formation in *P. aeruginosa* PAO1.