Abstract

Quorum sensing is N-acyl homoserine lactones (AHL) mediated cell-to-cell communication mechanism by which many pathogenic Bacteria sense their population density and control pathogenesis by regulating the production of major virulence factors. Phytopathogen such as *Pectobacterium carotovorum* subsp. *carotovorum* (*Pcc*) termed brute force pathogen uses Quorum sensing as a mechanism for regulation of its virulence. In *Pectobacterium carotovorum* subsp. *carotovorum* (*Pcc*) the virulence factors are secretory Plant Cell Wall Degrading Enzymes (PCWDEs) that macerate plant tissues and cause blackleg and soft rot diseases. Any process that inhibits quorum sensing without harming the bacteria is known as Quorum Quenching (QQ). Quorum quenching can be enzymatic or non-enzymatic depending on if AHL stability is affected or AHL production is controlled. A total of 79 bacterial isolates enriched and isolated from various sources were screened AHL degradation which is a requisite for quorum quenching. Two isolates viz. *Glutamicibacter nicotianae* AI5a and *Rhodococcus pyridinivorans* AI4 from the 79 were selected on basis of their biocontrol activity against *Pcc*BR1. An additional isolate *Rhodococcus erythropolis* CRD13.3C was used in the studies. The three isolates degraded AHL produced by *Pcc*BR1, decreased the enzyme activity of PCWDEs (PNL, PL and PGA) and attenuated and prevented soft rot in an *in vitro* assay with potato and cucumber as hosts. The QQ enzyme of *G. nicotianae* AI5a operated over wide temperature and pH range of 20°C to 37°C and 6.5 to 9, respectively. *G. nicotianae* AI5a did not grow on AHL as sole source of carbon in minimal media, the degraded AHL restored back to its original form on acidification and the C6-AHL showed 2 distinct product peaks when degraded upon HPLC analysis which pointed towards the enzyme being a putative lactonase.

A cucumber *in planta* infection model was developed for phytopathogen *Pcc*. All 3 isolates *G. nicotianae* AI5a, *R. pyridinivorans* AI4 or *R. erythropolis* CRD13.3C reduced the pathogenicity of *Pcc*BR1 in *in planta* experiments. Further *in planta* study was undertaken with potato plant as host where three different types of infections were carried out: I) Soil inoculation II) Stem inoculation and III) Leaf and lateral stem inoculation. *G. nicotianae* AI5a was able to stop the pathogenicity of *Pcc*BR1 in all the three cases. Quantitative Real Time PCR was used to quantify *G. nicotianae* AI5a and *Pcc*BR1 pHC60. *G. nicotianae* AI5a was able to reduce soft rot symptoms emphasised the quorum quenching mechanism of biocontrol under storage conditions for various hosts such as potato, tomato, capsicum and brinjal without killing or

affecting the growth of *Pcc*BR1, thus confirming its QQ ability. In a Mung bean *in planta* experiment, population of *G. nicotianae* AI5a were maintained and it was able to prevent the pathogenicity of *Pcc*BR1 without killing the pathogen. Similar trend was observed by qPCR in storage experiments.

Phytochemicals are also reported to perform quorum quenching. Usually, they perform non-enzymatic quorum quenching which is also referred to as Quorum sensing inhibition (QSI). This mechanism hampers the production of AHL in a QS mediated pathogen. Twelve phytochemicals were screened for their AHL degradation ability at their respective sub-lethal concentrations against *Pcc*BR1. Three selected phytochemicals viz. Eugenol, Carvacrol and Salicylic acid were able to reduce the virulent traits of *Pcc*BR1 such as AHL production, motility, PCWDEs and biofilm formation ability at sub-lethal concentrations. The three phytochemicals were also efficient in reducing maceration in potato and cucumber host in *in vitro* soft rot attenuation assay. Under storage conditions, Eugenol, Carvacrol and Salicylic acid reduced the soft rot in potato host without killing *Pcc*BR1 which is a prerequisite for quorum quenching. An *in planta* experiment using mung bean as host under gnotobiotic conditions also showed that Eugenol, Carvacrol and Salicylic acid decreased the pathogenicity of *Pcc*BR1 without reducing the amount of *Pcc*BR1 in the plant. Thus, this study demonstrates the various aspects of Quorum quenching strategies using AHL degrading Actinomycetotal isolates *G. nicotianae* AI5a, *R. pyridinivorans* AI4 or *R. erythropolis* CRD13.3C and phytochemicals at sub-lethal concentrations to attenuate the virulence of quorum sensing dependent phytopathogen *Pectobacterium carotovorum* subsp. *carotovorum* and highlights their biocontrol potentials for further scale up usage.