# Abstract

Interference in quorum sensing of plant pathogens leads to the attenuation of their virulence. Quorum sensing is acyl homoserine lactones (AHL) mediated cell-to-cell communication mechanism by which many pathogenic Gram negative bacteria sense their population density and control pathogenesis by regulating the production of major virulence factors. In *Pectobacterium carotovorum* subsp. *carotovorum* (*Pcc*) the virulence factors are secretory plant cell wall degrading enzymes that macerate plant tissues and produce the soft rot phenotype. Therefore, disruption of quorum sensing could be an effective strategy for its biocontrol, especially in the light of rising antimicrobial resistance. Enzymatic degradation of AHLs by lactonases, known as quorum quenching, is thus a potential strategy for attenuating quorum sensing regulated bacterial infections. For this purpose, 97 isolates from root and rhizospheric soils were screened for quorum quenching phenotype. Out of which, four isolates viz. *B. firmus* As30, *B. subtilis* Gs42, *Lysinibacillus* sp. Gs50 and *B. thuringiensis* Gs52 were selected on the basis of ARDRA and biocontrol ability against *Pcc*BR1. These isolates degraded AHL produced by *Pcc*BR1 and could decrease the enzyme activities of polygalacturonase and pectin lyase which are responsible for soft rot. The selected isolates could significantly attenuate soft rot caused by *Pcc*BR1, both on the slices of host vegetables (potato, carrot and cucumber) and *in planta* on the mung bean sprout. *Bacillus* isolates also demonstrated preventive as well as curative biocontrol ability. Among four isolates *Lysinibacillus* sp. Gs50 showed the best quorum quenching based biocontrol ability, therefore its quorum quenching mechanism was characterised in detail. The AHL degrading enzyme of *Lysinibacillus* sp. Gs50 showed broad specificity and was found located intracellularly. Heterologous expression of cloned gene for putative hydrolase (792 bp) designated *adeH* from *Lysinibacillus* sp. Gs50 produced a ~29 kDa protein which degraded AHLs of varying chain length. Further, Mass spectrometry analysis of AdeH enzymatic reaction product revealed that AdeH hydrolyses the lactone ring of AHL and hence is an AHL lactonase. Moreover, multiple sequence alignment of the amino acid sequence of AdeH showed that it belongs to the metallo-β- lactamase superfamily, has the conserved “HXHXDH” motif typical of AHL lactonases. AdeH has not been reported from *Lysinibacillus* genus so far and has < 40% identity with known AHL lactonases. To address the limitation of spatial interactions between the extracellular AHLs produced by quorum sensing pathogen and the AHL degrading bacterium with an intracellular enzyme AdeH, an infection model of another well studied quorum sensing pathogen *Pseudomonas aeruginosa* with mung bean plant was developed. Using confocal scanning laser microscopy it was demonstrated that degradation of the pathogen made AHLs by the biocontrol agent required co-localisation and close

proximity. Thus, this study demonstrated the efficacy of using an AHL lactonase producing soil isolate *Lysinibacillus* sp. Gs50 in quorum quenching based attenuation of virulence of two different quorum sensing pathogens *Pectobacterium carotovorum* subsp. *carotovorum* and *Pseudomonas aeruginosa*.