Abstract

Bacterial blight (BB) is one of the major diseases of rice caused by a host specific pathogen *Xanthomonas oryzae* pv. oryzae (Xoo) incurring huge economic loss. It inhabits xylem vessels and deploys variety of virulence factors to cause pathogenesis in the host plant. New variants of pathovars emerge owing to resistance development towards the deployed methods like chemicals, biocontrol agents and resistance genes in host used to prevent the occurrence of the BB disease. An approach wherein multiple mechanisms are used to control the pathogenesis of Xoo could be more effective and might delay the emergence of resistance. Thus, current study used a strategy of combining antibiotic and virulence attenuating small molecule compounds to study the control of pathogenesis of Xoo.

Isolation and screening by Cz/Cs ratio gave *Bacillus altitudinis* S2 as the best antagonist of Xoo BXO43 amongst the 90 isolates, which showed significant protection of rice leaves from disease symptoms by detached leaf assay. Antimicrobial activity of the isolate against different bacteria and fungi indicated no inhibition of the tested *Bacillus* and *Pseudomonas* species or fungi demonstrating lower risk of off-target inhibition. It was found sensitive against *S. aureus* in addition to Xoo indicating its activity against both Gram positive as well Gram negative bacteria. For characterization of the mechanism of antagonism various assays for its production, extraction and other physicochemical assays were conducted. The results revealed that the bioactive metabolite (antibiotic S2) was secretory in nature and its production was induced in the presence of biotic and abiotic stress. The synthetic minimal media, Sucrose Bushnell Haas medium for the production and isopropanol as a solvent for extraction, were found to be most suitable. The antibiotic S2 was stable over a wide range of temperature and pH and resistant towards various lytic enzymes indicating its suitability for the application in the diverse and unpredictable field conditions. Purification by preparative TLC, semi-preparative HPLC and characterization by mass spectrometry, TLC-Direct Bioautography, Tricine SDS-PAGE established the non-proteinaceous nature of the antibiotic S2. The Mass spectrometry results showed that the antibiotic molecule might have repetitive units. The presence *pks* and *pmt* genes in *Bacillus altitudinis* S2, coding for polyketide synthase and phospholipid methyltransferase, respectively involved in type III polyketide antibiotic synthesis pointed towards the possibility that it produces polyketide class of antibiotics. Quantification of

antibiotic S2 by MIC and MBC and bactericidal nature was concluded from Live-Dead staining and time-kill assay. Further, antibiotic S2 was found to affect the total protein of Xoo BXO43 as indicated by SDS-PAGE and no effect on surface morphology as observed by SEM, thereby implying that its mode of action is by affecting protein synthesis.

Small molecule compounds of plant origin like phenolic acids and few other synthetic chemical agents like acibenzolar S methyl and indole acrylic acid at their sub-inhibitory concentration (as determined by microdilution assay), were used to assess their effect on different virulence factors of Xoo BXO43. A transcriptional reporter system was constructed using a promoter region of one of the effector gene *xopQ,* known to be secreted by Type III Secretion Sytem (TTSS) of Xoo and effect of small molecule compounds was assayed in terms of GUS activity (the transcriptional reporter phenotype). Results indicated that cinnamic acid and its derivatives caffeic acid and ferulic acid were able to significantly reduce the GUS activity indicating their effect on TTSS of Xoo. No effect was observed on other virulence factors tested like secretion of exoenzymes, EPS production and motility tested. Same phenolic acids exhibited a significant reduction of disease symptoms when tested by detached leaf assay, confirming their potential as virulence attenuating agents of Xoo BXO43. Thus, these phenolic acids could protect the disease symptoms on plant by affecting TTSS of the pathogen.

Caffeic acid exhibited synergy with antibiotic S2 and other conventional antibiotics like Streptomycin and Ofloxacin, as calculated by FIC index of checkerboard assay. EtBr efflux assay showed its potential to reduce the efflux of EtBr demonstrating its potential to act as Efflux pump Inhibitor (EPI). Further to test the involvement of efflux pump in the mechanism of synergy, transcription analysis of six representative genes of efflux pumps found in Xoo genome was carried out. Expression of only *norM* gene was found to be upregulated in the presence of antibiotic S2, caffeic acid and the two combined, indicating the involvement of NorM in the synergy between antibiotic S2 and caffeic acid. However, the mechanism by which caffeic acid interacts with the efflux pump NorM needs further investigation. Using combination of antibiotic S2 and caffeic acid like phenolic acids, multiple targets were affected thus affecting overall

pathogenesis of Xoo. This could be an effective strategy to tackle Xoo pathogenesis that could also delay the emergence of resistance in Xoo.