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Thesis title

Identification and characterization of *PhlD* gene for the synthesis of polyketide antibiotic, 2, 4-DAPG from *Pseudomonas fluorescens* strains of Kashmir.

ABSTRACT

Pseudomonas spp. that can colonize the roots of crop plants and produce antifungal metabolites represent a real alternative to the application of chemical pesticides. Presently, much research is aimed at understanding, at the molecular level, the mechanisms that enable *Pseudomonas* strains to act as efficient biological control agents .This approach is facilitating the development of novel strains with modified traits for enhanced biocontrol efficacy. The objective of this research study was to isolate efficient wild isolates of *Pseudomonas fluorescens* having the biocontrol activity against *Fusarium oxysporum* and *Aspergillus* species. In order to start up with this research study one hundred and thirty six wheat rhizosphere soil samples were collected from three different locations of Kashmir valley and subjected to isolation of *Pseudomonas fluorescens.* The samples were cultured on King's B agar, which was supplemented with different concentrations of Ampicillin, Chloramphenicol and Kanamycin.

All the 136 isolates showed fluorescence on King's B agar under UV light but the intensity of fluorescence varied between the isolates. After initial screening and biochemical identification, 52 isolates were tentatively selected as *Pseudomonas fluorescens*. All the 52 isolates were studied for their colony morphology. The results indicated existence of variation among the isolates. All the 52 isolates were also tested for siderophore production. Interestingly, all of the

52 isolates produced Siderophore on CAS (Chrome Azurol S) blue agar except SKW2 and MSV5, they changed the colour of the CAS medium(Chrome Azurol S) from blue to brownish red.

Out of 52 isolates, only 7 isolates of *Pseudomonas fluorescens* showed antifungal activity against *Fusarium oxysporum* and *Aspergillus* species. Four isolates from Bandipora due to different soil chemistry (Bandi6, Bandi11, Bandi24 & Bandipora63) demonstrated varied antifungal activity against both the fungi.

In our study PCR based analysis of 2, 4-DAPG was carried out. Detection of *PhlD* gene fragment corresponding to 745bp DNA fragment in four of the seven isolates along with one reference strain i.e. SKW1, BG6, Bandi6, Bandi24 and Pf-5 (Reference strain) corroborated well with previously reported *PhlD* gene. Amplified DNA products were cloned into pGEMT-Easy vector and at least two clones of each *PhlD* gene were sequenced to ensure that variation was not due to misincorporation of bases by the Taq DNA polymerase. The TLC plates showed an identical Rf value of 0.54 for DAPG extracted from 4 isolates. Our HPLC analyses showed 2, 4-DAPG peak with the retention time of 8.740 min in BG6 and Bandi24.

By focusing on the Phylogenetic aspects, we also identified that BG6 and Bandi24 showed maximum homology with the other reported DAPG producing *Pseudomonas fluorescens* strains.

These results go on to emphasize that these isolates of *Pseudomonas fluorescens* may play an important role in controlling the growth and occurrence of pathogenic microorganism in the arable fields of J&k.