CHAPTER 6
SUMMARY

A total of 750 fluorescent pseudomonad strains were isolated from rice rhizosphere, out of which 25 strains (3.3%) exhibited antifungal activity towards the phytopathogenic fungi. These antagonistic strains were characterized genetically and functionally by comparing their primary characteristics, carbon assimilation patterns, fatty acid methyl ester (FAME) profiles, extracellular fungal cell wall degrading enzymes, antifungal metabolites, plant growth promoting hormones and enzymes, quorum sensing molecules, genes that encode specific functions (antibiotic production and denitrification), 16S rRNA, DNA gyrase B subunit (gyrB) and RNA polymerase sigma factor 70 (rpoD) genes.

Three major phenons were identified on the basis of phenotypic characteristics such as fluorescence, arginine dihydrolase, oxidase, gelatin hydrolysis, levan, citrate utilization, nitrate reduction, growth at 4°C and 42°C and assimilation of carbons.

FAME profiling identified the antagonistic fluorescent bacteria as fluorescent pseudomonad species of rRNA homology group I (Pseudomonas pseudoalcaligenes, P. plecoglossicida, P. fluorescens, P. aeruginosa, P. putida) and V (Stenotrophomonas maltophilia, formerly P. maltophilia). On the basis of major and differentiating fatty acids, strains were grouped into five major FAME groups viz., group I (P. pseudoalcaligenes) group II (P. plecoglossicida), group III (P. fluorescens), group IV (P.
aeruginosa) and group V (P. putida). Characteristic presence of high relative proportions (0.7% to 13.95%) of cyclopropane fatty acid (CFA C\textsubscript{17:0}) was observed in all antagonistic fluorescent pseudomonad species strains with the exception of P. pseudoalcaligenes. Therefore, CFA C\textsubscript{17:0} can be used as a FAME marker for rapid screening of antagonistic fluorescent pseudomonads as reported (Ellis et al. 2000).

To study the functional diversity of antagonistic strains, production of fungal cell wall degrading enzymes (protease, cellulase, pectinase and chitinase), phytohormone, indole-3-acetic acid (IAA), plant growth promoting enzymes, phosphatase and aminocyclopropane-1-carboxylate (ACC) deaminase and quorum sensing molecule, N-acyl homoserine lactone (AHL) was determined. Strains were further evaluated for the presence of genes that specify functions such as production of antibiotics, 2,4-diacetylphloroglucinol (DAPG), phenazine-1-carboxylic acid (PCA) phenazine-1-carboxamide (PCN), pyoluteorin (PLT), pyrroline (PRN) and as well as for denitrification genes, nitrate reductase (\textit{narG}) and nitrous oxide reductase (\textit{nosZ}).

Putative antibiotic producing strains were grown in the fermentation media and the production of DAPG by strains FP2, FP3, FP5, FP7, FP8, FP10, FP14, FP18 FP21, PCA by strains FP3, FP7, FP15, PRN by strains FP2, FP3, FP5, FP7, FP8, FP14, FP15, FP18, FP19, FP21, FP22 and PLT by strains FP2, FP3, FP5, FP7, FP8, FP9, FP14, FP18, FP19, FP21, FP22 was confirmed by TLC and HPLC. The Rf values were 0.77 for DAPG, 0.53 for PCA, 0.80 for PRN, 0.50 for PLT as determined by co-migration with
pure standards. DAPG (270 nm) and PCA (257 nm) were detected by HPLC with retention time 10.77 min and 4.94 min respectively.

The 16S rRNA, gyrB and rpoD sequence analyses further confirmed the taxonomic affiliation of antagonistic fluorescent pseudomonad strains. Strains FP2, FP3, FP5, FP7, FP8, FP9, FP14, FP16, FP17, FP18, FP19, FP21, FP22, and FP23 were identified as *P. fluorescens* and strains FP12, FP15, FP24, and FP25 as *P. putida*. Strains FP10, FP11 and FP13 were identified as *P. aeruginosa* and strains FP1 and FP4 as *P. pseudoalcaligenes*. Strain FP20 and FP6 were identified as *P. plecoglossicida* and *S. maltophilia* (formerly, *P. maltophilia*) respectively.

Phylogenetic analyses of 16S rRNA gene of antagonistic fluorescent pseudomonad strains revealed 3 major clusters viz., cluster I with *P. putida*, *P. pseudoalcaligenes* and *P. plecoglossicida*, cluster II with *P. aeruginosa* and cluster III with *P. fluorescens*.

Phylogenetic analyses of gyrB gene of antagonistic fluorescent pseudomonad strains were resulted into 3 major clusters viz., cluster I with *P. fluorescens*, cluster II with *P. putida* and *P. plecoglossicida* and cluster III with *P. aeruginosa* and *P. pseudoalcaligenes*.
Five major clusters were identified on the basis of rpoD-based phylogenetic analysis. Cluster I and II consisted of P. fluorescens. Cluster III contained P. putida and P. plecoglossicida. Cluster IV consisted of P. pseudoalcaligenes and cluster V contained P. aeruginosa.

Phylogenetic analysis based on the combination of gyrB and rpoD gene sequences improved the reliability of phylogenetic tree and yielded better resolution than 16S rRNA. Based on this new approach, antagonistic fluorescent pseudomonad strains were grouped into four major clusters viz., cluster I (P. fluorescens), cluster II (P. putida, P. plecoglossicida), cluster III (P. pseudoalcaligenes) and cluster IV (P. aeruginosa). The non-pseudomonad bacterium, S. maltophilia formed an out group in the phylogenetic tree. Clustering based on the combination of gyrB and rpoD analyses had good agreement with FAME groups. The lack of congruence between clusters from phenotypic (primary characteristics and carbon assimilation profiles) and genetic data was due to the high diversity among antagonistic fluorescent pseudomonads associated with rice rhizosphere.

Considering the diversity of strains reported in the present investigation, biocontrol and plant growth promoting abilities appear to be the general and genetically dispersed traits and not selective traits to a specialized, genetically restricted group of fluorescent pseudomonads. Results achieved in this investigation may provide enormous resources for biological control of crop diseases and other biotechnological applications.