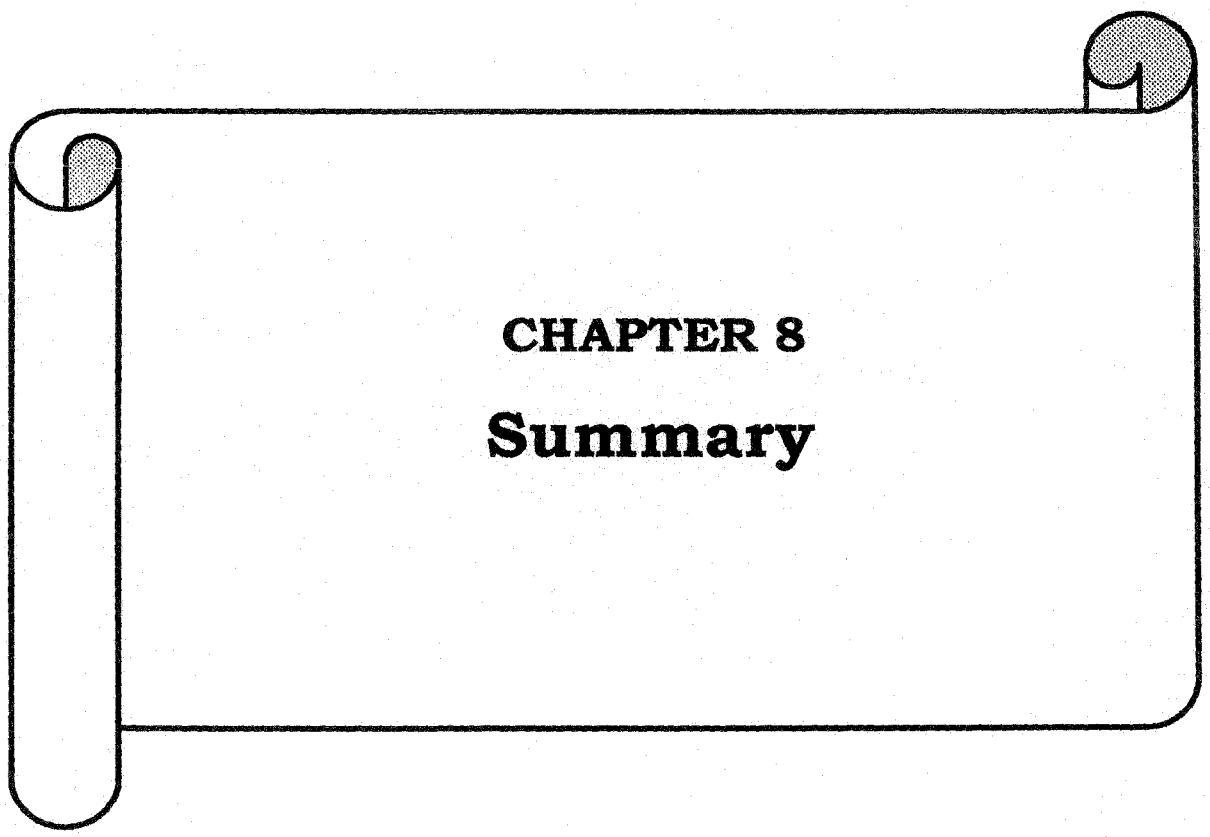

CHAPTER 8

Summary

SUMMARY

- The present study deals with "Studies on Soil-Inhabiting Siderophore-Producing Bacteria and Their Role in Suppression of Plant Root Pathogens".
- After a short introduction to the work, a brief review of literature on siderophore has been presented. This section deals with the findings of the previous workers with respect to the present line of research. This study includes the chemistry, biosynthesis and transport of siderophore in microorganisms. Extensive study has been done on the siderophores produced by the fluorescent pseudomonads, the pyoverdins. Literature review was also done on the biological control of plant diseases. The common mechanisms of antagonisms are described in brief which included the production of antibiotics; signal interference; parasitism and production of extracellular enzymes; induced systemic resistance; competition for ferric iron ions; root colonization and PGPR traits. The literature review was extended to describe the role of Gram negative and Gram positive rhizosphere bacteria in suppression of plant diseases which are reported as biocontrol agents.
- Basic objectives of the present study were: (i) To isolate siderophore-producing bacteria from soil; (ii) To study the antifungal activity of the isolated siderophore producing strains in suppressing some plant root pathogens *in vitro*; (iii) To characterize the selected siderophore-producing and antagonistic strains and their identification; (iv) To partially purify and chemically characterize the siderophores produced by the selected strains; (v) To study the efficiency of siderophore-producing bacteria in suppressing plant root pathogens *in vivo*.
- The experimental study is depicted in four chapters: (i) Isolation of siderophores producing antagonistic bacteria from soil and their characterization, (ii) Mechanism of action of siderophore producing rhizobacteria showing antagonistic activity against plant pathogenic fungi, (iii) Characterization and purification of siderophores, (iv) *In vivo* evaluation of *Pseudomonas putida* strains AS01 and AS04 as biocontrol agents against wilt in brinjal.

- A detailed description of different experimental procedures and techniques used during the present study are explained in the section of materials and methods of each chapter.
- Chapter 3 deals with collection of rhizosphere soil samples from 9 different locations of Darjeeling and Jalpaiguri districts of West Bengal, India, to isolate different bacterial strains. A total of 208 bacterial isolates were obtained which were then checked for siderophore production on CAS agar. Altogether 68 siderophore producing strains were obtained.
- *In vitro* study of antagonistic activity of 68 siderophore producing bacterial isolates were performed against seven pathogens, namely *Fusarium equiseti, Lasiodiplodia theobromae, Rhizoctonia solani, Colletotrichum gloeosporioides, Alternaria alternata, Fusarium solani,* and *Fusarium graminearum.* Twenty isolates were found to exhibit antagonistic activity towards all the test pathogens.
- The characterization of the antagonistic bacterial isolates was done. Both the morphological and phylogenetic characterizations were performed. For identification of the isolates, 31 biochemical tests were performed followed by the genetic study. The 16S rRNA gene of each bacterium was amplified, cloned and sequenced. Identification was done by similarity searches of the sequences using the BLAST function of GenBank. The results of the phenotypic tests and 16S rRNA gene sequencing revealed that the strains belonged to the genera *Pseudomonas, Klebsiella, Serratia, Enterobacter, Bacillus, Alcaligenes* and *Citrobacter.* The sequences were deposited in NCBI GenBank through BankIt and Accession Numbers were provided for each of the strains (Accession numbers: EU661864, EU661866, JX535385, JX960418, KC109315-28, KC117153-4). Phylogenetic relationship was studied with the seven antagonistic *Pseudomonas* spp. with other 50 species of *Pseudomonas* obtained in GenBank using MEGA 4.0.
- Chapter 4 deals with a study on the mechanism of inhibition of the antagonistic isolates against the plant pathogens. Production of different antimicrobial metabolites by the antagonistic isolates was

observed in specific media. Siderophore production was estimated by the CAS shuttle assay which showed that AS04 was the highest producer. The antimicrobial metabolites produced by different strains included chitinase, amylase, lipase and protease. Moreover, some PGPR traits were also tested which showed that all strains were producers of IAA and one strain, namely AS04 produced phosphatase.

- The interaction of the *Pseudomonas putida* strains AS01 and AS04 with fungal pathogens *Fusarium equiseti* and *F. solani* was studied by Scanning Electron Microscopy. Severe deformities of the fungal mycelia and hyphal lysis were observed.
- The siderophores of each of the 20 isolates were characterized (chapter no. 5) which revealed that 15 strains produced hydroxamate type and 5 produced catecholate type but none of them produced carboxylate type of siderophore.
- The media and growth parameters were optimized for maximum amount of siderophore production by the strain *Pseudomonas putida* AS04. Media supplements, temperature and incubation time were optimized which showed that Fiss glucose minimal media supplemented with 1% sucrose and 0.1% $(NH_4)_2SO_4$ was the best media for siderophore production. Siderophore production began after 8 hours of incubation and reached maximum after 30 hours. The optimum temperature for siderophore production was recorded to be 30°C.
- For partial purification of siderophore culture supernatants were passed through Amberlite XAD-2 column and the siderophore was eluted with methanol. All fractions tested positive for siderophore were combined and concentrated and the concentrate was passed through sephadex LH20 column. Fractions eluted with methanol were monitored for presence of siderophore by TLC using chromogenic spray with $FeCl_3$ in HCl. A brown spot indicated hydroxamate siderophore on TLC plate.
- The partially purified siderophore was studied spectrophotometrically in 300-700 nm visible range and a peak was obtained at 430 nm which confirmed that the sample contained a trihydroxamate type siderophore.

- In the final chapter (chapter no. 6) *in vivo* studies were performed to evaluate the efficacy of *P. putida* strains AS01 and AS04 in suppressing wilt in brinjal caused by *Fusarium solani*. Initially the pathogenicity of *Fusarium solani* was confirmed through verification of Koch's postulates. For this, six-week old potted brinjal seedlings of three different varieties (PPL, Lalita and a locally cultivated variety) were used as host plant and disease index was evaluated.
- The *Pseudomonas putida* strains AS01 and AS04 were grown in liquid culture (PDB) in presence of the pathogen *Fusarium solani* in order to assess the antagonistic activity in liquid media. The percentage reduction of biomass of *F. solani* after 5 days was found to be 75.72% in AS01 co-inoculated cultures and 71.67% in cultures co-inoculated with AS04.
- The isolates AS01 and AS04 were selected for *in vivo* studies for management of *Fusarium* wilt in brinjal seedlings. The brinjal seedlings (var. PPL) were used for this experiment. The disease control efficacy exhibited by strain AS04 was higher than that showed by AS01. AS04 strain exhibited 73.5% disease inhibition while AS01 showed 64.7% inhibition in sterilized soil.
- The findings of the present study have been discussed in detail and compared with the results of other prominent works in each chapter.
- A generalized discussion on the entire work is presented in chapter 7.
- In conclusion, the present study found out some potential soil inhabiting antagonistic bacteria which produced siderophore and several other bioactive principles such as hydrolytic enzymes and IAA. The findings of the study have suggested a possible means to biologically control fungal phytopathogens in an eco-friendly way for a more sustainable agricultural system.