

CONTENTS

Chapter 1 Review of literature	1
1.1 Bacterial endophytes and their diversity.....	2
1.3 Factors influencing the colonization of endophytes in various plants.....	5
1.2.1 Environmental factors.....	5
1.2.2 Role of quorum sensing in endophytic lifestyle	6
1.2.3 Effect of root exudates in colonization	7
1.3 Mechanism of endophytic colonization	7
1.3.1 Attachment to root surfaces	7
1.3.2 Bacterial entry inside the plant tissue	8
1.4 Plant growth promotion by endophytic bacteria.....	9
1.4.1 Nitrogen fixation by endophytes.....	10
1.4.2 Biocontrol activity of endophytes.....	13
1.5 Other plant growth promoting traits in endophytes	16
1.5.1 Indole acetic acid production (IAA)	16
1.5.2 Siderophore production.....	17
1.6.3 Phosphate solubilization	17
1.7 Modulation of plant immune response by pathogens and plant growth promoting bacteria.....	18
1.6.1 Endophyte induced priming of defense in plants.....	21
1.7 Genomics and metagenomics of endophytes	23
1.8 Genetic modification of endophytes	25
Scope of the Thesis	27
Objectives	28
Chapter 2 Isolation and characterization of diazotrophic endophytic plant growth promoting bacteria from indigenous Poaceae plants	31
2.1 Introduction.....	32
2.2 Materials and Methods.....	33
2.2.1 Community DNA extraction.....	33
2.2.2 PCR amplification of 16S rRNA gene fragments.....	34
2.2.3 DGGE analysis.....	35

2.2.4 Isolation of diazotrophic endophytic bacteria.....	36
2.2.5 Bacterial identification.....	36
2.2.5.1 Biochemical characterization.....	36
2.2.5.2 16S rRNA gene sequence analysis	37
2.2.6 Characterization of plant growth promoting (PGP) traits.....	37
2.2.6.1 Amplification of nifH gene	37
2.2.6.2 Phosphate solubilization	38
2.2.6.3 Indole Acetic Acid (IAA) production	38
2.2.6.4 Siderophore production.....	38
2.2.6.5 HCN production.....	39
2.2.6.6 Dual culture test for antifungal activity	39
2.2.6.7 Lytic enzyme activity.....	39
2.2.7 Transformation of plasmid pHC60	41
2.2.7.1 Plasmid transformation of <i>E. coli</i> by competent cell preparation.....	41
2.2.7.2 Plasmid transfer into endophytes by biparental mating by conjugation	41
2.2.8 Detection of endophytic presence of isolates by confocal laser scanning microscopy	42
2.2.9 Pot inoculation experiments.....	43
2.2.9.1 Chlorophyll estimation.....	44
2.2.9.2 Determination of total nitrogen content in plants	44
2.2.10 Data analysis	45
2.3 Results.....	45
2.3.1 Total and diazotrophic endophytic bacterial community profiling of <i>Poaceae</i> plants by DGGE	45
2.3.2 Isolation, characterization and identification of diazotrophic endophytic bacteria	50
2.3.3 Plant Growth-promoting traits of endophytic diazotrophic bacterial isolates	52
2.3.3.1 Detection of nif genes in endophytic bacterial isolates	52
2.3.3.2 Antifungal activity of endophytic bacterial isolates	53
2.2.3.3 HCN production by endophytic bacteria	53
2.3.3.4 Phosphate solubilization by endophytic bacterial isolates.....	53
2.3.3.4 IAA production by endophytic bacterial isolates.....	53
2.3.3.5 Siderophore production by endophytic bacterial isolates	54

2.3.3.6 Hydrolytic enzyme activities of endophytic bacterial isolates	54
2.3.4 Endophytic colonization by <i>gfp</i> tagged bacterial isolates in wheat	56
2.3.5 Effect of endophytic bacterial colonization on wheat plants	57
2.3.6 PCA analysis of microbial traits contributing to plant growth promotion.....	60
2.4 Discussion.....	61
Chapter 3 Plant growth promotion and disease suppression by antibiotic producing diazotrophic endophytic <i>Streptomyces</i> spp.	66
3.1 Introduction.....	67
3.2 Materials and Methods.....	69
3.2.1 Microbial strains and culture conditions.....	69
3.2.2 Antifungal activity assay.....	70
3.3.3 Extraction and identification of antifungal metabolite using GC-MS analysis	71
3.2.4 Detection of polyketide synthase gene in <i>Streptomyces</i> spp.	71
3.2.5 EGFP tagging of <i>Streptomyces</i> spp.....	71
3.2.6 Plant inoculation and visualization of colonization.....	72
3.2.7 Plant growth promotion and plant protection assays	72
3.2.8 Study of defense related gene expression in rice	73
3.2.9 Data analysis	74
3.3 Results.....	74
3.3.1 Inhibition of phytopathogenic fungi by diazotrophic endophytic <i>Streptomyces</i> spp....	74
3.3.2 Identification of antifungal metabolites produced by <i>Streptomyces</i> spp.	75
3.3.3 Analysis of polyketide gene in diazotrophic endophytic <i>Streptomyces</i> spp.....	76
3.3.4 Evaluation of endophytic colonization by diazotrophic endophytic <i>Streptomyces</i> spp	76
3.3.5 Plant growth promotion by diazotrophic endophytic <i>Streptomyces</i> spp.....	78
3.3.6 Plant protection by endophytic <i>Streptomyces</i> spp. upon phytopathogen challenge	78
3.3.7 Effect of endophytic colonization by <i>Streptomyces</i> spp. on defense gene expression in rice plants against blast pathogen	83
3.4 Discussion.....	85
Chapter 4 Engineered production of 2,4-Diacetylphloroglucinol in diazotrophic endophytic bacteria and its beneficial effect in multiple plant-pathogen systems.....	91
4.1 Introduction.....	92
4.2 Materials and Methods.....	94

4.2.1. Bacterial and fungal strains and plasmids used in this study.....	94
4.2.2 <i>phlD</i> and <i>phlACB</i> gene amplification	97
4.2.3 DNA manipulation and plasmid transformation.....	98
4.2.4 Phloroglucinol sensitivity test.....	99
4.2.5 Phloroglucinol Production	99
4.2.6 2,4-DAPG production, extraction and quantification.....	100
4.2.7 Quantification of 2,4- DAPG using HPLC	100
4.2.8 Antagonistic activity of ethyl acetate extracts	101
4.2.9 Surface sterilization and bacterization of seedlings.....	101
4.2.10 Plant growth promotion and disease protection by recombinant endophytic bacteria.....	101
4.2.11 Defense gene expression analysis in rice plants	101
4.2.12 Statistical analysis	102
4.3 Results.....	102
4.3.1 Construction of plasmids containing <i>phlD</i> and <i>phlACB</i> genes.....	102
4.3.2 Phloroglucinol tolerance	104
4.3.3 2,4-DAPG produced by diazotrophic endophytic <i>Pseudomonas</i> sp. WS5 transformants.....	105
4.3.4 Heterologous 2,4-DAPG production in diazotrophic endophytic <i>Pseudomonas</i> sp. WS5	108
4.3.5 Antifungal activity of DAPG against fungal pathogen.....	109
4.2.6 Colonization of endophytic bacterium <i>Pseudomonas</i> sp. WS5 in various plants.....	109
4.3.7 Plant growth promotion by 2,4-DAPG producing transformants of <i>Pseudomonas</i> sp. WS5 in cereals	110
4.3.8 Plant protection upon pathogen challenge	111
4.3.9 Defense gene expression in rice upon bacterization with 2,4-DAPG producing recombinant strains of <i>Pseudomonas</i> sp. WS5	116
4.4 Discussion.....	118
Summary and conclusion.....	122
Appendices	126
References	147
Publications and Presentations	170
