
CONTENTS

<i>Subject</i>	<i>Page No.</i>
Abstract	i-ii
Declaration by the Candidate	
Certificate of the Supervisor	
Certificate of the External Examiner and ODEC	
Acknowledgement	iii-iv
Contents	v-ix
List of Tables	x
List of Figures	xi-xvi
List of Abbreviations and Symbols	xvii-xviii
CHAPTER 1 Introduction and Review of Literature	1-22
1.1 Introduction	1-7
1.2 Objectives	7-8
1.3 Review of Literature	8-14
1.3.1 <i>R. solanacearum</i> pathogenicity study in host plants	8
1.3.2 Escapees: susceptible plants evading the bacterial wilt symptom after <i>R. solanacearum</i> inoculation	10
1.3.3 Polyphosphate metabolism	11
<i>References</i>	14-22
CHAPTER 2 Comparative Pathogenicity Study of <i>Ralstonia solanacearum</i> F1C1 between Eggplant and Tomato Seedlings by The Leaf Clip Inoculation Method	23-57
2.1 Abstract	23
2.2 Introduction	23-25
2.3 Material and Methods	25-37
2.3.1 Media, chemicals and lab wares	25
2.3.2 Bacterial strains and growth conditions	25
2.3.3 Germination process of eggplant seeds	27
2.3.4 Preparation of bacterial inoculums	28

2.3.5 Pathogenicity studies by the leaf clip method	28
2.3.5.1 Pathogenicity Assay	28
2.3.5.2 Inoculation of different of eggplant cultivars	29
2.3.5.3 Inoculation of non pathogenic bacteria	29
2.3.6 GUS staining of eggplant seedlings	30
2.3.7 Inoculation of eggplant and tomato seedlings within a single microfuge tube	30
2.3.8 Inoculation of eggplant seedlings with different concentration of <i>R. solanacearum</i> inoculums	31
2.3.9 Transformation in <i>R. solanacearum</i> F1C1	31
2.3.10 Creation of <i>hrpB</i> and <i>phcA</i> mutant of <i>R. solanacearum</i> F1C1	31
2.3.11 Creation of <i>hrpG</i> insertion mutant of <i>R. solanacearum</i> F1C1	32
2.3.12 Confirmation of <i>hrpB</i> and <i>phcA</i> mutant of <i>R. solanacearum</i> F1C1	33
2.3.12.1 Hypersensitive response (HR) assay of <i>hrpB</i> and <i>hrpG</i> mutants of F1C1	33
2.3.12.2 Swimming motility assay of <i>phcA</i> mutant of F1C1	34
2.3.13 Creation of mCherry tagged <i>R. solanacearum</i> F1C1, <i>hrpB</i> and <i>phcA</i> mutant of F1C1 and their colonization study in eggplant and tomato seedlings	35
2.3.14 Isolation of bacteria from the inoculated seedlings at different DPI	36
2.4 Results	37-50
2.4.1. <i>R. solanacearum</i> F1C1 causes wilt in eggplant seedlings inoculated by the leaf clip method	37
2.4.2 <i>R. solanacearum</i> F1C1 is more aggressive in eggplant seedlings than tomato seedlings inoculated by the leaf clip method	43
2.5 Discussion	50-53
<i>References</i>	53-57
CHAPTER 3 A Comparative Study of the Pathogen Load in Wilted and Healthy looking Tomato Seedlings Inoculated by the Leaf Clip Method	58-76

3.1 Abstract	58
3.2 Introduction	58-60
3.3 Material and Methods	60-63
3.3.1 Bacterial strains and growth conditions	60
3.3.2 <i>R. solanacearum</i> pathogenicity study in tomato seedlings by the leaf clip method	61
3.3.3 <i>R. solanacearum</i> colonization study in tomato seedlings	62
3.3.4 Bacterial population count in inoculated tomato seedlings	63
3.4 Results	64-71
3.4.1 Inoculation of <i>R. solanacearum</i> in two cotyledon leaves has a higher chance of wilting occurrence than inoculation in one cotyledon leaf of a tomato seedling	64
3.4.2 Pathogen occurrence in the escapees like the wilted plants	67
3.4.3 Similar bacterial load in wilted and healthy looking seedlings	69
3.5 Discussion	71-74
<i>References</i>	74-76
CHAPTER 4 Characterization of Polyphosphate Metabolism Homologues of <i>Ralstonia solanacearum</i> F1C1 with regard to their role in Virulence	77-139
4.1 Abstract	77
4.2 Introduction	77-79
4.3 Material and methods	79-115
4.3.1 Media and chemicals	79
4.3.2 Bacterial strains, growth media and culture conditions	80
4.3.3 Competent cell preparation and transformation	82
4.3.4 Identification of polyphosphate metabolism homologues in <i>R. solanacearum</i> F1C1 strain	82
4.3.5 Creation of insertion mutagenesis in polyphosphate metabolism homologues of <i>R. solanacearum</i> F1C1	82
4.3.5.1 Creation of <i>ppk1</i> insertion mutant	85
4.3.5.2 Creation of <i>ppk2</i> insertion mutant	88

4.3.5.3 Creation of <i>ppx</i> insertion mutant	91
4.3.5.4 Creation of <i>ppnk</i> insertion mutant	93
4.3.5.5 Assay for <i>lacZ</i> gene insertion in polyphosphate homologue mutants	95
4.3.6 Expression study of polyphosphate metabolism homologues	96
4.3.6.1 <i>lacZ</i> expression assay:	96
4.3.6.2 Real time expression assay	97
4.3.7 Creation of a deletion mutation in <i>ppk1</i> homologue of <i>R. solanacearum</i> F1C1	99
4.3.8 Creation of a <i>ppk1</i> and <i>ppk2</i> double mutant of <i>R. solanacearum</i> F1C1 strain	103
4.3.9 Characterization of virulence phenotypes in polyphosphate metabolism mutants	104
4.3.9.1 Swimming motility assay	104
4.3.9.2 Swarming motility assay	104
4.3.9.3 Twitching motility assay	104
4.3.9.4 Growth comparison of wild type and mutant strains	105
4.3.9.5 Hypersensitive Response (HR) assay	105
4.3.9.6 Cellulase assay	105
4.3.9.7 Oxidative stress assay	105
4.3.9.8 Virulence study in tomato seedlings	106
4.3.10 Detection of PolyP accumulation in wild type F1C1 and PolyP mutants	107
4.3.11 Creation of <i>ppk1</i> and <i>ppnk</i> complemented strain	107
4.3.11.1 Confirmation of vector used for complemented study	107
4.3.11.2 Cloning of full length <i>ppk1</i> and <i>ppnk</i> homologues in pNP267 vector	109
4.3.11.3 Insertion of Spectinomycin cassette (Ω Spc) into pNP267:: <i>ppk1</i> and pNP267:: <i>ppnk</i> construct	111
4.3.11.4 Transformation of pTP012 and pTP014 into <i>ppk1</i> and <i>ppnk</i> insertion mutant strains of F1C1	112
4.4 Results	115-131
4.4.1. Polyphosphate metabolism genes are conserved in <i>R. solanacearum</i> F1C1	115

4.4.2 Creation of <i>ppk1</i> , <i>ppk2</i> , <i>ppx</i> and <i>ppnk</i> insertion mutants	115
4.4.3 Creation of <i>ppk1</i> deletion mutant	116
4.4.4 Creation of <i>ppk1</i> and <i>ppk2</i> double mutant	116
4.4.5 Polyphosphate metabolism genes showed higher expression in minimal medium	117
4.4.6 <i>ppk1</i> , <i>ppk2</i> , <i>ppnk</i> mutants were swimming motility deficient	118
4.4.7 The <i>ppk1</i> mutant showed reduced swarming motility	119
4.4.8 Polyphosphate mutants are twitching motility proficient	120
4.4.9 Polyphosphate metabolism mutants were growth proficient like the wild type	121
4.4.10 Polyphosphate metabolism mutants were proficient for Hypersensitive Response (HR)	121
4.4.11 Polyphosphate metabolism mutants were cellulase proficient	122
4.4.12 PolyP mutants exhibited tolerance to oxidative stress	123
4.4.13 <i>ppk1</i> and <i>ppnk</i> mutants were virulence deficient in tomato seedlings	124
4.4.14 PolyP accumulation was detected in <i>ppx</i> mutant of F1C1	130
4.4.15 Complementation study of <i>ppk1</i> and <i>ppnk</i> mutants	131
4.5 Discussion	133-135
<i>References</i>	135-139
CHAPTER 5 Conclusion And Future Aspects	140-142
<i>Appendix I</i>	143-147
<i>Appendix II</i>	148-150
<i>Appendix III</i>	151-153
<i>Appendix IV</i>	154-159
<i>List of Publication</i>	160-161