
LIST OF TABLES

<i>Table No.</i>	<i>Table Caption</i>	<i>Page No.</i>
<u>CHAPTER 1</u>		
Table 1.1	Sequence homology/ conservation of polyphosphate metabolism genes across different strains of <i>Ralstonia solanacearum</i> (BlastN)	14
<u>CHAPTER 2</u>		
Table 2.1	Bacterial strains used in this study	26-27
<u>CHAPTER 4</u>		
Table 4.1	List of plasmids and bacterial strains used in this study	80-81
Table 4.2	List of primers used in the characterization study of PolyP homologues	84-85
Table 4.3	List of primers used in the expression study of PolyP homologues	98
Table 4.4	Conservation of polyphosphate gene homologues in <i>R. solanacearum</i> F1C1	115

LIST OF FIGURES

<i>Figure No.</i>	<i>Figure Caption</i>	<i>Page No.</i>
<u>CHAPTER 1</u>		
Fig. 1.1	Schematic representation of <i>Ralstonia solanacearum</i> infection cycle.	3
Fig. 1.2	Photographs of wilted eggplants from field, near Tezpur University, Assam, India.	10
Fig. 1.3	Schematic representation of polyphosphate molecule and its metabolism.	13
<u>CHAPTER 2</u>		
Fig. 2.1	Picture depicting different steps of the pathogenicity assay of <i>R. solanacearum</i> in eggplant seedlings by the leaf clip inoculation method.	29
Fig. 2.2	Representative picture showing pathogenicity assay set up used for comparative pathogenicity study between eggplant and tomato seedlings.	30
Fig. 2.3	Agarose gel showing confirmation of insertion mutation in <i>hrpG</i> of F1C1.	33
Fig. 2.4	Hypersensitive response assay in tobacco leaf.	34
Fig. 2.5	Colony phenotype of <i>phcA</i> mutant of <i>R. solanacearum</i> F1C1.	35
Fig. 2.6	Schematic diagram depicting bacterial load count experiment.	37
Fig. 2.7	Representative picture depicting pathogenicity of <i>R. solanacearum</i> F1C1 in eggplant seedlings inoculated by the leaf clip method.	38
Fig. 2.8	Pathogenicity study of <i>R. solanacearum</i> F1C1 and non-pathogenic bacteria in eggplant seedlings by the leaf clip inoculation.	38
Fig. 2.9	Pathogenicity of <i>R. solanacearum</i> F1C1 in different cultivars of eggplant.	39
Fig. 2.10	Pathogenicity assay with different concentration of F1C1 in eggplant seedlings.	40
Fig. 2.11a	GUS staining of infected eggplant seedlings inoculated by the leaf clip method.	40

Fig. 2.11b	Fluorescence staining of eggplant seedlings inoculated with mCherry tagged F1C1.	41
Fig. 2.12a	Virulence of <i>R. solanacearum</i> F1C1 wild type, <i>hrpB</i> , <i>hrpG</i> and <i>phcA</i> mutants in eggplant seedlings.	42
Fig. 2.12b	Kaplan–Meier survival probability [S(t)] curve of eggplant seedlings inoculated with wild type F1C1 and derivative mutants.	42
Fig. 2.13a	A representative picture showing differential pathogenicity of <i>R. solanacearum</i> between eggplant and tomato seedlings.	43
Fig. 2.13b	Comparative virulence of <i>R. solanacearum</i> F1C1 wild type, <i>hrpB</i> , <i>hrpG</i> and <i>phcA</i> mutants between eggplant and tomato seedlings.	44
Fig. 2.13c	Kaplan–Meier survival probability [S(t)] of eggplant and tomato seedlings inoculated with wild type F1C1, <i>hrpB</i> , <i>hrpG</i> and <i>phcA</i> mutants of F1C1 by the leaf clip method.	45
Fig. 2.14	Comparative virulence of <i>R. solanacearum</i> F1C1 between eggplant and tomato seedlings in lower concentration.	46
Fig. 2.15a	Fluorescence staining of eggplant seedlings inoculated with F1C1 and derivative mutants	47
Fig. 2.15b	Fluorescence staining of tomato seedlings inoculated with F1C1 and derivative mutants.	48
Fig. 2.15c	Fluorescence staining of seedlings co-inoculated with F1C1 and <i>hrpB</i> mutant.	49
Fig. 2.16	Representative picture showing bacterial colonies in plates in infected eggplant and tomato seedlings inoculated with <i>R. solanacearum</i> .	49
Fig. 2.17	Comparative bacterial population in infected eggplant and tomato seedlings inoculated with <i>R. solanacearum</i> .	50

CHAPTER 3

Fig. 3.1	Picture showing escapee tomato seedlings inoculated with <i>R. solanacearum</i> after ten days of inoculation.	60
Fig. 3.2	Schematic representation depicting pathogenicity assay set up in tomato seedlings by the leaf clip method.	62
Fig. 3.3	Schematic representation depicting steps followed to count bacterial load in infected seedlings.	64
Fig. 3.4a	Pathogenicity of <i>R. solanacearum</i> F1C1 in tomato seedlings inoculated in one leaf and two leaves by clip inoculation method.	65

Fig. 3.4b	Kaplan–Meier survival probability [S(t)] curve of tomato seedlings inoculated in one leaf and two leaves by clip inoculation method.	65
Fig. 3.5	Pathogenicity of <i>R. solanacearum</i> F1C1 at lower concentrations inoculated in one leaf vs two leaves of tomato seedlings.	66
Fig. 3.6	Fluorescence staining of healthy looking and infected tomato seedlings.	68
Fig. 3.7	Representative picture showing bacterial colonies isolated from leaves of healthy looking and infected tomato seedlings inoculated with <i>R. solanacearum</i> .	70
Fig.3.8	Comparative bacterial population in leaves of infected and healthy looking tomato seedlings inoculated with <i>R. solanacearum</i> .	71
<u>CHAPTER 4</u>		
Fig. 4.1	Arrangement of polyphosphate metabolism genes in <i>R. solanacearum</i> GMI1000.	79
Fig. 4.2	Schematic representation of <i>lacZ</i> reporter gene fusion strategy by single homologous recombination event used to construct insertion mutagenesis.	83
Fig. 4.3	Agarose gel showing partial amplification of <i>ppk1</i> homologue in F1C1 genome.	86
Fig. 4.4	Agarose gel showing cloning of <i>ppk1</i> gene fragment in pGEM-T-easy vector.	86
Fig. 4.5	Agarose gel showing cloning of <i>ppk1</i> gene fragment in pCZ367 vector.	87
Fig. 4.6	Agarose gel showing insertion mutation in <i>ppk1</i> homologue of F1C1.	88
Fig. 4.7	Agarose gel showing partial amplification of <i>ppk2</i> homolog in F1C1 genome.	89
Fig. 4.8	Agarose gel showing cloning of <i>ppk2</i> gene fragment in pTZ57R/T vector.	89
Fig. 4.9	Agarose gel showing cloning of <i>ppk2</i> gene fragment in pCZ367 vector.	90
Fig. 4.10	Agarose gel showing insertion mutation in <i>ppk2</i> homologue of F1C1.	90

Fig. 4.11	Agarose gel showing partial amplification of <i>ppx</i> homologue in F1C1 genome.	91
Fig. 4.12	Agarose gel showing cloning of <i>ppx</i> gene fragment in pTZ57R/T vector.	92
Fig. 4.13	Agarose gel showing cloning of <i>ppx</i> gene fragment in pCZ367 vector.	92
Fig. 4.14	Agarose gel showing insertion mutation in <i>ppx</i> homologue of F1C1.	93
Fig. 4.15	Agarose gel showing partial amplification of <i>ppnk</i> homologue in F1C1 genome.	94
Fig. 4.16	Agarose gel showing cloning of <i>ppnk</i> gene fragment in pGEM-T-easy vector.	94
Fig. 4.17	Agarose gel showing cloning of <i>ppnk</i> gene fragment in pCZ367 vector	95
Fig. 4.18	Agarose gel showing insertion mutation in <i>ppnk</i> homologue of F1C1.	95
Fig. 4.19	X-gal assay in polyphosphate homologue mutants of F1C1.	96
Fig. 4.20	Schematic representation of Ω cassette fusion strategy by double homologous recombination event used to construct insertion/deletion mutagenesis.	100
Fig. 4.21	Agarose gel showing partial amplification of <i>ppk1</i> homologue in F1C1 genome.	101
Fig. 4.22	Agarose gel showing cloning confirmation of <i>ppk1</i> gene fragment in pGEM-T vector used for deletion mutation.	101
Fig. 4.23	Agarose gel showing cloning confirmation of pTP004 recombinant harboring Ω Spc cassette.	102
Fig. 4.24	Agarose gel showing confirmation of deletion mutation in <i>ppk1</i> homologue of F1C1.	102
Fig. 4.25	Agarose gel showing confirmation of deletion mutation in <i>ppk1</i> homologue of F1C1.	103
Fig. 4.26	Map of pNP267 vector used for complementation study.	108
Fig. 4.27	Agarose gel showing confirmation of pNP267 vector.	108
Fig. 4.28	Agarose gel showing amplification of full length <i>ppk1</i> homologue along with promoter region in F1C1 genome.	110
Fig. 4.29	Agarose gel showing amplification of full length <i>ppnk</i> homologue along with promoter region in F1C1 genome.	110

Fig. 4.30	Agarose gel showing cloning confirmation of <i>ppk1</i> in pNP267 vector used for complementation.	111
Fig. 4.31	Agarose gel showing cloning confirmation of <i>ppnk</i> in pNP267 vector used for complementation study.	111
Fig. 4.32	Agarose gel showing cloning confirmation of pTP012 plasmid.	112
Fig. 4.33	Agarose gel showing cloning confirmation of pTP014 plasmid.	112
Fig. 4.34	Agarose gel showing confirmation of double homologous recombination event in <i>ppk1</i> complemented strain.	113
Fig. 4.35	Agarose gel showing confirmation of double homologous recombination event in <i>ppnk</i> complemented strain.	113
Fig. 4.36	Agarose gel showing confirmation of full length <i>ppk1</i> insertion in TRS1024 genome.	114
Fig. 4.37	Agarose gel showing confirmation of full length <i>ppnk</i> insertion in TRS1025 genome.	114
Fig. 4.38	Schematic representation of mutation sites in polyphosphate metabolism homologues.	116
Fig. 4.39	Schematic representation of insertion/deletion mutation site in <i>ppk1</i> homologue.	116
Fig. 4.40	Expression pattern of polyphosphate metabolism genes.	117
Fig. 4.41	Expression pattern of polyphosphate metabolism genes by qRT-PCR.	118
Fig. 4.42	Swimming motility of polyphosphate metabolism mutants.	119
Fig. 4.43	Swarming motility of polyphosphate metabolism mutants.	120
Fig. 4.44	Twitching motility on BG agar plate.	120
Fig. 4.45	Growth curve of wild type and PolyP mutant strains.	121
Fig. 4.46	HR assay of polyphosphate metabolism mutants.	122
Fig. 4.47	Cellulase assay on CMC agar plates.	123
Fig. 4.48	Determination of hydrogen peroxide sensitivity of PolyP mutant strains.	123
Fig. 4.49	Virulence data analysis of <i>ppk1</i> insertion mutant by root inoculation.	125
Fig. 4.50	Virulence data analysis of <i>ppk1</i> insertion mutant by leaf clip inoculation.	125

Fig. 4.51	Virulence data analysis of <i>ppk1</i> deletion mutant by root inoculation.	126
Fig. 4.52	Virulence data analysis of <i>ppk2</i> mutant by root inoculation.	127
Fig. 4.53	Virulence data analysis of <i>ppk2</i> mutant by leaf clip inoculation.	127
Fig. 4.54	Virulence data analysis of <i>ppx</i> mutant by root inoculation.	128
Fig. 4.55	Virulence data analysis of <i>ppx</i> mutant by leaf clip inoculation.	128
Fig. 4.56	Virulence data analysis of <i>ppnk</i> mutant by root inoculation.	129
Fig. 4.57	Virulence data analysis of <i>ppnk</i> mutant by leaf clip inoculation.	129
Fig. 4.58	Virulence data analysis of <i>ppk1::ppk2</i> double mutant by root dip inoculation.	130
Fig. 4.59	Detection of PolyP accumulation in wild type F1C1 and PolyP mutants of F1C1.	131
Fig. 4.60	Swimming motility assay of <i>ppk1</i> complemented strain.	132
Fig. 4.61	Swimming motility assay of <i>ppnk</i> complemented strain.	132
Fig. 4.62	Virulence data analysis of <i>ppk1</i> complemented strain by root dip inoculation.	133
Fig. 4.63	Virulence data analysis of <i>ppnk</i> complemented strain by root dip inoculation.	133

LIST OF ABBREVIATIONS AND SYMBOLS

Abbreviation/Symbol	Name
%	: Percent
µg	: Microgram
µl	: Microlitre
µM	: Micromolar
°C	: Degree Celsius
Amp	: Ampicillin
BLAST	: Basic Local Alignment Search Tool
bp	: basepair
cDNA	: Complementary DNA
CFU	: Colony Forming Units
Conc.	: Concentration
C _T	: Threshold Cycle
DAPI	: 4',6-diamidino-2-phenylindole
DEPC	: Diethylpyrocarbonate
DMF	: Dimethylformamide
DMSO	: Dimethylsulfoxide
DNA	: Deoxy ribonucleic Acid
DPI	: Days post inoculation
EtBr	: Ethidium bromide
Fig	: Figure
g/l	: Gram per litre
Gen	: Gentamycin
hr	: hour
HR	: Hypersensitive Response
kb	: Kilo basepair
LB	: Luria Bertani
M	: Molar
mA	: Mili Ampere
mg	: miligram
min	: Minutes

ml	: Mililitre
mM	: Mili molar
MM	: Minimal Medium
NCBI	: National Center for Biotechnology Information
NCM	: Nitrocellulose membrane
nm	: Nanometer
ONPG	: ortho-Nitrophenyl- β -galactoside
PCR	: Polymerase Chain Reaction
qPCR	: Quantitative PCR
Rif	: Rifampicin
Rif ^r	: Rifampicin resistant
RNA	: Ribonucleic Acid
rpm	: Rotation per minute
RSSC	: <i>Ralstonia solanacearum</i> Species Complex
RT	: Room temperature
RT-PCR	: Real-Time PCR
SDS	: Sodium dodecyl sulphate
sec	: Second
Spc	: Spectinomycin
Spc ^r	: Spectinomycin resistant
spp.	: Species
T2SS	: Type II Secretion System
T3SS	: Type III Secretion System
TAE	: Tris-Cl, Acetic Acid and EDTA
TZC	: 2, 3, 5 - triphenyl-tetrazolium chloride
U/ μ l	: Units per microlitre
V	: Volt
X-gal	: 5-bromo-4-chloro-3-indolyl- β -D-galactosidase
X-gluc	: 5-Bromo-4-chloro-3-indolyl- β -D-glucuronidase
Ω -Spc	: Omega spectinomycin cassette