

TABLE OF CONTENTS

Biochemical and Molecular Approaches for Developing Phosphate Solubilizing Plant Growth Promoting Rhizobacteria (PGPR)		
Chapters	TITLE	Page No.
Chapter 1	Review of Literature and Introduction	
1.1	Soil Phosphorus	1
1.2	Plant Phosphate Availability In Soil	2
1.3	Need For Biofertilizers In Plant Phosphate Nutrition	4
1.4	Nature Of Phosphate Biofertilizers	5
1.4.1	Mycorrhizae	5
1.4.2	Mineral Phosphate Solubilizing Microorganisms	6
1.4.3	Organic Phosphate Mineralizing Microorganisms	7
1.5	Plant Growth Promoting Rhizobacteria (PGPR) As Phosphate Solubilizers	11
1.6	Mechanism Of Phosphate Solubilization	13
1.6.1	Acidification	15
1.6.2	Chelation	17
1.6.3	Ligand Exchange Reactions	18
1.6.4	H ⁺ Excretion	18
1.7	Mineralization Of Organic Phosphate	19
1.8	Biotechnological Approaches For Development Of Phosphate Solubilizing Bacteria	19
1.9	Beneficial Composite Inoculations For Microbial Phosphate Solubilization	21
1.9.1	Efficacy Of Coinoculation Of Phosphate Solubilizing And Nitrogen Fixing Bacteria On Plant Growth	22

Chapters	TITLE	Page No.
1.9.2	Efficacy Of Coinoculation Of Phosphate Solubilizing Microorganisms And Arbuscular Mycorrhizal Fungi On Plant Growth	24
1.9.3	Efficacy Of Coinoculation Of Phosphate Solubilizing Microorganisms, Nitrogen Fixing Bacteria And Arbuscular Mycorrhizal Fungi On Plant Growth	25
1.10	Factors Affecting The Survival Of Phosphate Solubilizing Microorganisms In Field Conditions	25
1.11	Strategies For Developing Efficient Phosphate Solubilizing Rhizobacteria	27
1.12	Objectives Of The Present Study	28
Chapter 2	Biochemical Characterization of Variation in the Nature of Organic Acid Secretion and Rock Phosphate Solubilization by Rhizobacteria, <i>Citrobacter</i> sp. DHRSS on Various Carbon Sources	
2.1	Introduction	29
2.2	Materials And Methods	30
2.2.1	Screening Of Phosphate Solubilizing Bacterium (PSB) Using Buffered Media	30
2.2.2	Identification Of PSB By 16S rRNA Gene Sequence Analysis	30
2.2.3	Characterization Of mps Ability Of The Isolate	31
2.2.4	Measurement Of GDH Activity	31
2.2.5	Invertase Assay	32
2.2.6	Analytical Methods	32
2.3	Results	33
2.3.1	Isolation And Identification Of PSB Using Sucrose Containing Buffered Minimal Media	33
2.3.2	Characterization Of The mps Ability Of <i>Citrobacter</i> sp. DHRSS	37

Chapters	TITLE	Page No.
2.3.3	Determination Of The Nature Of Organic Acids	39
2.4	Discussion	39
Chapter 3	Repression of Mineral Phosphate Solubilizing (mps) Phenotype in the Presence of Weak Organic Acids in Plant Growth Promoting Fluorescent <i>Pseudomonads</i>	
3.1	Introduction	43
3.2	Materials And Methods	45
3.2.1	Bacterial Strains Used And Growth Conditions	45
3.2.2	Characterization Of mps Ability Of <i>P. aeruginosa</i> M3 and SP1	45
3.2.3	Identification Of The Rhizospheric Fluorescent <i>Pseudomonads</i> M3 And SP1 By 16S rDNA Gene Sequence Analysis	46
3.2.4	Carbon Catabolite Repression Studies With M3 And SP1	46
3.2.5	Enzyme Assays	47
3.2.6	Analytical Methods	47
3.3	Results	48
3.3.1	Characterization Of mps Ability Of <i>P. aeruginosa</i> M3 And SP1	48
3.3.2	Identification Of The Isolates M3 And SP1	48
3.3.3	Carbon Catabolite Repression Studies With <i>P. aeruginosa</i> M3 and SP1	53
3.3.4	Effect Of Organic Acids On GDH And GAD Enzyme Activities Of <i>P. aeruginosa</i> M3 And SP1	57
3.4	Discussion	61
Chapter 4	Screening of <i>Arabidopsis thaliana</i> cDNA Library for Mineral Phosphate Solubilizing (mps) Ability	
4.1	Introduction	63

Chapters	TITLE	Page No.
4.2	Materials And Methods	66
4.2.1	<i>Arabidopsis thaliana</i> cDNA Library	66
4.2.2	Preparation Of Plating Bacteria For Phage Infection	69
4.2.3	Determination Of Titre Of <i>A. thaliana</i> cDNA Library	69
4.2.4	Preparation Of Lambda Stocks From Plaques For Library Amplification	69
4.2.5	Construction Of λ kc Lysogen	70
4.2.6	Infection Of <i>E. coli</i> With <i>A. thaliana</i> λ YES cDNA Library	70
4.2.7	Screening <i>A. thaliana</i> cDNA Library In <i>E. coli</i>	71
4.2.7.1	Determining The Minimal Tris Cl (pH 8.0) Buffering Conditions In <i>E. coli</i> For Screening The cDNA Library	71
4.2.7.2	Screening of <i>A. thaliana</i> cDNA Library in <i>E. coli</i> For mps Genes	71
4.2.8	Screening <i>A. thaliana</i> cDNA Library In <i>Pseudomonas fluorescens</i>	71
4.2.8.1	Preparation And Extraction Of <i>A. thaliana</i> Plasmid (pYES) cDNA Library For Screening In <i>P. fluorescens</i>	71
4.2.8.2	Screening Of <i>A. thaliana</i> Plasmid (pYES) cDNA Library In <i>P. fluorescens</i> For mps Genes	72
4.2.8.3	Characterization Of mps Ability Of <i>P. fluorescens</i> pYES cDNA Transformants	72
4.2.9	Analytical Methods	72
4.2.10	PCR Amplification Of cDNA Inserts From The pYES Clones	73
4.3	Results	73
4.3.1	Determination Of Titre Of <i>A. thaliana</i> Phage (λ YES) cDNA Library	73
4.3.2	Construction Of <i>E. coli</i> λ kc Lysogen And Its Confirmation	74
4.3.3	Determining The Minimal Tris Cl (pH 8.0) Buffering Conditions In <i>E. coli</i> For Screening The cDNA Library	75

Chapters	TITLE	Page No.
4.3.4	Infection Of <i>E. coli</i> With <i>A. thaliana</i> λ YES cDNA Library	76
4.3.5	Screening Of <i>A. thaliana</i> Phage (λ YES) cDNA Library In <i>E. coli</i> For mps Genes	77
4.3.6	Preparation Of Plasmid (pYES) From Phage (λ YES) cDNA Library For Screening In <i>P. fluorescens</i>	81
4.3.7	Screening Of <i>A. thaliana</i> Plasmid (pYES) cDNA Library In <i>P.</i> <i>fluorescens</i> For mps Genes	81
4.3.8	Characterization Of mps Ability Of <i>P. fluorescens</i> pYES cDNA Transformants	81
4.4	Discussion	87
Bibliography		90
Summary		114
Posters and Publications		117