

TABLE OF CONTENTS

CONTENTS	PAGE NO.
DECLARATION OF RESEARCHER	
CERTIFICATION OF SUPERVISOR	
ANTI-PLAGIARISM REPORT	
DRC CERTIFICATE	
ABSTRACT	i
PREFACE	iv
LIST OF FIGURES	xiii
LIST OF TABLES	xvi
1. Chapter 1. Introduction	1-4
2. Chapter 2. Review of literature	5-24
2.1. Importance of phosphorus in plant growth	6
2.2. Phosphorus availability in soil	7
2.3. Phosphate solubilizing microorganisms	8
2.4. Contributions of phosphate solubilizing microorganisms	9
2.5. Mechanism of phosphate solubilization	
2.5.1. Lowering soil pH	10
2.5.2. Chelation	11
2.5.3. Mineralization	11
2.6. Mechanism of solubilization of inorganic phosphate by PSM	12
2.7. Mechanism of mineralization of organic phosphate by PSM	13
2.8. Mycorrhizae	14
2.9. Factors influencing phosphate solubilization by microbes	15
2.10. Role of microbial exopolysaccharides in phosphate solubilization	16
2.11. Screening and isolation of PSMs	16
2.12. Mode of plant growth promotion by PSM	17
2.13. Contribution of phosphate solubilizing microorganisms as inoculants	18
2.14. How are phosphate solubilizing microorganisms applied	20
2.15. Factors affecting viability of PSM used as inoculants	21
2.16. Why do phosphate solubilizing microorganism inoculations fail	21
2.17. Emerging tolerance of the phosphate solubilizing microorganisms	22
2.18. Molecular studies and genetic engineering of the PSMs	23
3. Chapter 3. Materials and methods	25-46
3.1. Collection of soil samples	25
3.2. Soil physicochemical analysis	25

CONTENTS	PAGE NO.
3.2.1. Moisture content	25
3.2.2. pH	25
3.2.3. Electrical conductivity	25
3.2.4. Organic carbon and organic matter	26
3.2.5. Nitrogen	26
3.2.6. Phosphorus	27
3.3. Isolation and selection of phosphate solubilizing microbial consortium from soil samples	27
3.3.1. Isolation of phosphate solubilizing microbial consortium from soil samples	27
3.3.2. Authentication and selection of phosphate solubilizing microbial consortium from soil samples	28
3.4. Tolerance studies of PSM consortia	28
3.4.1. Pesticide tolerance	28
3.4.2. Heavy metal tolerance	28
3.4.3. Antibiotic tolerance	29
3.4.4. Antifungal tolerance	29
3.5. Study of isolated pure bacterial culture from phosphate solubilizing microbial consortia	29
3.5.1. Isolation of pure bacterial culture	29
3.5.2. Confirmation of pure bacterial culture	30
3.5.3. Estimation of phosphate solubilizing efficiency of the pure bacterial cultures	30
3.5.4. Characterization of pure cultures	30
3.5.4.1. Gram staining	30
3.5.4.2. Sulphide and indole production and motility test	30
3.5.4.3. Catalase test	31
3.5.4.4. Coagulase test	31
3.5.4.5. Citrate utilization test	31
3.5.4.6. Urease test	31
3.5.4.7. Starch hydrolysis test	32
3.5.4.8. Gelatin hydrolysis test	32
3.5.4.9. Triple sugar iron test	32
3.5.4.10. Methyl red Voges-Proskauer (MR-VP) test	32
3.5.4.11. Nitrate reduction test	33
3.5.4.12. Blood agar test	33

CONTENTS	PAGE NO.
3.5.4.13. Growth study	34
3.5.5. Statistical analysis	34
3.6. Molecular study of pure culture isolates	34
3.6.1. Isolation of DNA	34
3.6.2. Sequencing of 16S rRNA region of DNA	37
3.6.3. Identification of pure cultures	37
3.6.4. Submission of sequence in GenBank	37
3.6.5. Phylogeny analysis	37
3.7. Tolerance assessment of isolated Phosphate Solubilizing Bacteria	37
3.7.1. Pesticide tolerance of Phosphate Solubilizing Bacteria	37
3.7.2. Determination of Minimum Inhibition Concentration (MIC) against pesticides of the Phosphate Solubilizing Bacteria	38
3.7.3. Heavy metal tolerance of Phosphate Solubilizing Bacteria	39
3.7.4. Determination of Maximum Tolerance Concentration of Phosphate Solubilizing Bacteria against Cadmium and Mercury	39
3.7.5. Antibiotic tolerance of Phosphate Solubilizing Bacteria	39
3.8. Study of plant growth promoting (PGP) ability	41
3.8.1. <i>In vitro</i> study	41
3.8.1.1. Indole-3-acetic acid production	41
3.8.1.2. Ammonia production	41
3.8.1.3. Hydrogen cyanide production	41
3.8.1.4. Siderophore production	42
3.8.1.5. Seed germination	42
3.8.2. Pot experiment	42
3.9. Complex hydrocarbon utilization test	43
3.9.1. Diesel degradation and utilization	43
3.9.2. Petrol degradation and utilization	43
3.10. Metabolomic study of pure culture PSB isolates	43
3.10.1. Cultivation of isolates for GC-MS analysis	43
3.10.2. Preparation of secondary metabolite extraction (methanolic) for GC-MS analysis	43
3.10.3. Gas Chromatography Mass Spectrometry procedure	44
3.10.4. GC-MS analysis	44
3.10.5. Correlation study	45
3.11. Whole genome characterization and analysis	45
3.11.1. DNA isolation and sequencing	45

CONTENTS	PAGE NO.
3.11.2. Whole genome annotation analysis	45
3.12. Encapsulation and dissemination of Phosphate Solubilizing Bacteria	45
3.12.1. Encapsulation	45
3.12.2. Administration of encapsulated PSB in soil (<i>in vitro</i>) and quantification of available phosphate	45
4. Chapter 4. Results and discussion	47-154
4.1. Collection of soil sample	47
4.2. Soil physicochemical analysis	49
4.2.1. Moisture content	49
4.2.2. pH	50
4.2.3. Electrical conductivity	51
4.2.4. Organic carbon and organic matter	52
4.2.5. Nitrogen	53
4.2.6. Phosphorus	54
4.3. Isolation and selection of phosphate solubilizing microbial consortium from soil samples	55
4.4. Tolerance studies of PSM consortia	56
4.4.1. Pesticide tolerance	56
4.4.2. Heavy metal tolerance	67
4.4.3. Antibiotic tolerance	78
4.4.4. Antifungal tolerance	82
4.5. Study of isolated pure bacterial culture from phosphate solubilizing microbial consortia	84
4.5.1. Isolation of pure bacterial culture	84
4.5.2. Confirmation of pure bacterial culture	85
4.5.3. Estimation of phosphate solubilizing efficiency of the pure bacterial cultures	86
4.5.4. Characterization of pure cultures	86
4.5.4.1. Gram staining	86
4.5.4.2. Sulphide and indole production and motility test	87
4.5.4.3. Catalase test	88
4.5.4.4. Coagulase test	88
4.5.4.5. Citrate utilization test	89
4.5.4.6. Urease test	90
4.5.4.7. Starch hydrolysis test	90
4.5.4.8. Gelatin hydrolysis test	91

CONTENTS	PAGE NO.
4.5.4.9. Triple sugar iron test	91
4.5.4.10. Methyl red Voges-Proskauer (MR-VP) test	92
4.5.4.11. Nitrate reduction test	93
4.5.4.12. Blood agar test	94
4.5.4.13. Growth study	95
4.5.5. Statistical analysis	98
4.6. Molecular study of pure culture isolates	99
4.6.1. Isolation of DNA	99
4.6.2. Sequencing of 16S rRNA region of DNA	99
4.6.3. Identification of pure cultures	100
4.6.4. Submission of sequence in GenBank	100
4.6.5. Phylogeny analysis	102
4.7. Tolerance assessment of isolated Phosphate Solubilizing Bacteria	104
4.7.1. Pesticide tolerance of Phosphate Solubilizing Bacteria	104
4.7.2. Determination of Minimum Inhibition Concentration (MIC) against pesticides of the Phosphate Solubilizing Bacteria	107
4.7.3. Heavy metal tolerance of Phosphate Solubilizing Bacteria	110
4.7.4. Determination of Maximum Tolerance Concentration of Phosphate Solubilizing Bacteria against Cadmium and Mercury	117
4.7.5. Antibiotic tolerance of Phosphate Solubilizing Bacteria	118
4.8. Study of plant growth promoting (PGP) ability	126
4.8.1. <i>In vitro</i> study	126
4.8.1.1. Indole-3-acetic acid production	126
4.8.1.2. Ammonia production	127
4.8.1.3. Hydrogen cyanide production	127
4.8.1.4. Siderophore production	128
4.8.1.5. Seed germination	129
4.8.2. Pot experiment	130
4.9. Complex hydrocarbon utilization test	132
4.9.1. Diesel degradation and utilization	132
4.9.2. Petrol degradation and utilization	133
4.10. Metabolomic study of pure culture PSB isolates	135
4.10.1. Cultivation of isolates for GC-MS analysis	135
4.10.2. GC-MS analysis	136
4.10.2.1. Metabolites of interest	136
4.10.2.2. Role of metabolites in plant growth promoting activity	139

CONTENTS	PAGE NO.
4.10.2.3. Bacterial metabolic pathways in biosynthesis of PGP active metabolites	145
4.10.3. Metabolomics correlation	149
4.11. Whole genome characterization and analysis	150
4.11.1. Whole genome annotation analysis	150
4.12. Encapsulation and dissemination of Phosphate Solubilizing Bacteria	152
4.12.1. Encapsulation	152
4.12.2. Administration of encapsulated PSB in soil (<i>in vitro</i>) and quantification of available phosphate	153
CONCLUSION	155-159
APPENDIX	160-167
A. LIST OF PUBLICATIONS	160
B. PARTIAL GENOMES	162
C. CULTURE MEDIA	163
D. SOFTWARE & DATABASES	165
E. ABBREVIATIONS	166
INDEX	168-169
BIBLIOGRAPHY	170-198