

CONTENTS

| | Page No |
|---|---------|
| 1. Introduction | 01-05 |
| 2. Literature Review | 06-49 |
| 3. Material and Methods | 50-74 |
| 3.1. Collection of soil samples | 50-50 |
| 3.2. Isolation of bacteria from rhizosphere | 50-50 |
| 3.3. Characterization and identification of selected isolates | 50-58 |
| 3.3.1. Gram staining | |
| 3.3.2. Endospore staining | |
| 3.3.3. SEM studies of bacterial isolates | |
| 3.3.4. Biochemical tests | |
| 3.3.4.1. Catalase test | |
| 3.3.4.2. Oxidase activity | |
| 3.3.4.3. Voges-Proskauer reaction | |
| 3.3.4.4. Urea digestion | |
| 3.3.4.5. Casein hydrolysis | |
| 3.3.4.6. Starch hydrolysis | |
| 3.3.4.7. Indole test | |
| 3.3.4.8. Nitrate reduction | |
| 3.3.4.9. Gelatin hydrolysis | |
| 3.3.4.10. Utilization of carbon sources | |
| 3.3.5. Protein pattern analysis | |
| 3.3.5.1. SDS-PAGE analysis of protein | |
| 3.3.6. Molecular Identification | |
| 3.3.6.1. Extraction of DNA | |
| 3.3.6.2. Quantification | |
| 3.3.6.3. Agarose gel electrophoresis | |
| 3.3.6.4. Amplification of 16S rDNA by PCR | |
| 3.3.6.5. 16S rDNA sequencing and phylogenetic Analyses | |
| 3.4. Fungal culture | 58-60 |
| 3.4.1. Source and maintenance | |
| 3.4.2. Assessment of mycelial growth | |

| | |
|---|-------|
| 3.4.2.1. Solid media | |
| 3.4.2.2. Liquid media | |
| 3.5. <i>In vitro</i> characterization of PGPR activity of selected bacterial isolates | 60-62 |
| 3.5.1. IAA production | |
| 3.5.2. Phosphate solubilisation | |
| 3.5.3. Siderophore production | |
| 3.5.4. HCN production | |
| 3.5.5. Volatile production | |
| 3.5.6. Ammonia production | |
| 3.6. Extracellular hydrolytic enzyme production | 62-65 |
| 3.6.1. Chitinase | |
| 3.6.1.1. Qualitative assay | |
| 3.6.1.2. Quantitative assay | |
| 3.6.2. Protease | |
| 3.6.2.1. Qualitative assay | |
| 3.6.2.2. Quantitative assay | |
| 3.6.2.3. Optimization of protease production | |
| 3.6.2.3.1. Effect of incubation period | |
| 3.6.2.3.2. Effect of incubation temperature | |
| 3.6.2.3.3. Effect of pH on protease production | |
| 3.6.2.3.4. Effect of carbon source on production of protease | |
| 3.6.2.3.5. Effect of nitrogen sources on production of protease | |
| 3.6.2.3.6. Effect of metal ions on production of protease | |
| 3.6.3. Amylase | |
| 3.6.4. β -1-4-glucanase | |
| 3.7. <i>In vitro</i> testing of antagonism | 66-67 |
| 3.7.1. Solid medium | |
| 3.7.2. Liquid medium | |
| 3.7.3. SEM analyses | |
| 3.8. Extraction of antifungal compounds from bacteria | 67-67 |
| 3.8.1. Preparation of Cell free culture filtrate | |
| 3.9. Partial characterization of active principle | 67-68 |

| | |
|---|--------|
| 3.9.1. UV-spectrophotometry | |
| 3.9.2. GC-MS analysis of crude cell free extract | |
| 3.10. Plant materials | 68-68 |
| 3.10.1. Source of seeds | |
| 3.10.2. Growth of plants | |
| 3.11. Soil analysis | 68-69 |
| 3.12. Application of bacteria | 69-69 |
| 3.13. Inoculation of pathogen | 69-69 |
| 3.13.1. Preparation of pathogen inoculum | |
| 3.13.2. Inoculation to healthy jute plants | |
| 3.14. Disease assessment | 70-70 |
| 3.15. Determination of plant growth promoting activity | 70-70 |
| 3.16. Biochemical analyses | 70-74 |
| 3.16.1. Extraction and estimation of biochemical components | |
| 3.16.1.1. Extraction and estimation of chlorophyll | |
| 3.16.1.2. Extraction of carbohydrate | |
| 3.16.1.2.1. Estimation of total sugar | |
| 3.16.1.2.2. Estimation of reducing sugar | |
| 3.16.1.3. Extraction and estimation of protein | |
| 3.16.1.4. Extraction of phenol | |
| 3.16.1.4.1. Estimation of total phenol | |
| 3.16.1.4.2. Estimation of ortho-Phenol | |
| 3.16.2. Extraction and assay of enzyme activities | |
| 3.16.2.1. Chitinase | |
| 3.16.2.2. Phenylalanine ammonia lyase | |
| 3.16.2.3. β -1, 3-Glucanase | |
| 3.16.2.4. Peroxidase | |
| 4. Results | 74-189 |
| 4.1. Isolation of microorganisms from soil, screening and selection | 74-78 |
| 4.2. Characterization and identification of selected bacterial isolates | 78-97 |
| 4.2.1. Gram staining | |
| 4.2.2. Endospore staining | |
| 4.2.3. Biochemical test | |

| | |
|--|---------|
| 4.2.4. 16S rDNA sequencing, identification and diversity analysis | |
| 4.2.5. SEM studies of bacterial isolates | |
| 4.2.6. Carbon source utilization | |
| 4.2.7. Protein pattern analysis | |
| 4.3. Cultural condition affecting the growth of the selected bacterial isolates | 98-100 |
| 4.3.1. Incubation period | |
| 4.3.2. Temperature | |
| 4.3.3. pH | |
| 4.4. Cultural condition affecting the growth of the pathogen | 100-104 |
| 4.4.1. Media | |
| 4.4.2. Incubation period | |
| 4.4.3. pH | |
| 4.4.4. Carbon source | |
| 4.4.5. Nitrogen source | |
| 4.5. <i>In vitro</i> PGPR test of selected bacterial isolates | 104-105 |
| 4.5.1. IAA production | |
| 4.5.2. Phosphate solubilization | |
| 4.5.3. Siderophore production | |
| 4.5.4. HCN production | |
| 4.5.5. Volatile production | |
| 4.5.6. Ammonia production | |
| 4.6. Extracellular hydrolytic enzyme production | 105-112 |
| 4.6.1. Chitinase production | |
| 4.6.1.1. Qualitative assay | |
| 4.6.1.2. Quantitative assay | |
| 4.6.2. Protease production | |
| 4.6.2.1. Qualitative assay | |
| 4.6.2.2. Optimization of protease production | |
| 4.6.2.2.1. Effect of incubation period | |
| 4.6.2.2.2. Effect of incubation temperature | |
| 4.6.2.2.3. Effect of initial pH of medium | |

| | |
|---|--------------------------------|
| 4.6.2.2.4. Effect of carbon source | |
| 4.6.2.2.5. Effect of nitrogen source | |
| 4.6.2.2.6. Effect of metal ions | |
| 4.6.3. Amylase | |
| 4.6.4. β -1-4-glucanase | |
| 4.7. Effect of bacterial antagonist on growth of fungal pathogen | 112-129 |
| 4.7.1. Solid medium | |
| 4.7.1.1. Radial growth inhibition | |
| 4.7.2. Liquid medium | |
| 4.7.3. Changes in the hyphal structure | |
| 4.7.4. Partial characterization of active principles from <i>B. amyloliquefaciens</i> | |
| 4.8. Soil properties | 130-130 |
| 4.9. Effect of application of rhizobacteria on growth of jute plants | 130-154 |
| 4.10. Biochemical changes in jute leaves induced by application of rhizobacteria | 155-170 |
| 4.11. Effect of rhizobacteria on disease development of jute | 171-175 |
| 4.12. Biochemical changes induced in jute leaves by application of rhizobacteria | 175-189 |
| 4.12.1. Biochemical components | |
| 4.12.2. Defense enzymes | |
| 5. Discussion | 190-212 |
| 6. Conclusion | 213-216 |
| 7. Bibliography | 217-265 |
| Appendices | A ₁ -A ₅ |