

TABLE OF CONTENTS:

CONTENTS	PAGE NO.
DECLARATION OF RESEARCHER	
CERTIFICATION OF SUPERVISOR	
ANTI-PLAGIARISM REPORT	
DRC CERTIFICATE	
ABSTRACT	I
PREFACE	IV
LIST OF FIGURES	XI
LIST OF TABLES	XII
1. Chapter 1. Introduction	1
2. Chapter 2. Literature review	6
2.1. Historical significance	6
2.2. Forms of mercury	8
2.2.1. Elemental mercury	8
2.2.2. Inorganic mercury	10
2.2.3. Organic mercury	10
2.3. Mercury and public health	11
2.3.1. Nervous system	12
2.3.2. Cardiovascular system	12
2.3.3. Gastrointestinal system	12
2.3.4. Pulmonary system	12
2.3.5. Excretory system	12
2.3.6. Endocrine system	12
2.3.7. Reproductive system	13
2.3.8. Immune system	13
2.4. Mercury and ecological health	14
2.5. Molecular mechanisms and toxicokinetics	16
2.5.1. Molecular interactions	17
2.5.1.1. Sulfhydryl groups	17
2.5.1.2. Cysteine	17
2.5.1.3. Glutathione	18
2.5.1.4. Proteins	18

CONTENTS	PAGE NO.
2.5.2. Biological effects of interaction	19
2.5.2.1. Mercury transport	19
2.5.2.2. Enzyme inactivation	20
2.5.2.3. Oxidative stress	20
2.5.2.4. Apoptosis	21
2.6. Biogeochemical cycle of mercury	21
2.6.1. Sources	23
2.6.1.1. Natural	23
2.6.1.2. Anthropogenic	23
2.6.2. Sinks	24
2.6.3. Phases of the biogeochemical cycle	24
2.6.3.1. Atmospheric	24
2.6.3.2. Aquatic	25
2.6.3.3. Freshwater	25
2.6.3.4. Marine	26
2.6.3.5. Terrestrial	27
2.6.4. Grasshopper effect	28
2.6.5. Global distribution	28
2.6.6. Regional distribution	30
2.7. Conventional strategies of remediation	31
2.7.1. Physical techniques of mercury remediation	32
2.7.1.1. Soil washing	32
2.7.1.2. Thermal treatment	33
2.7.1.3. Stabilization	33
2.7.1.4. Electro-remediation	34
2.7.1.5. Adsorption	34
2.7.2. Biological techniques of mercury remediation	34
2.7.2.1. Phytoremediation	34
2.7.2.2. Remediation through algae and aquatic plants	35
2.7.2.3. Remediation through yeast	36
2.7.2.4. Nanotechnology	36
2.8. Bacterial resistance metabolism	37
2.8.1. Reduced uptake	37

CONTENTS	PAGE NO.
2.8.2. Sequestration	37
2.8.3. Bioaccumulation	38
2.8.4. <i>mer</i> operon	38
3. Chapter 3, Materials and methods	45
3.1. Collection and soil samples from various regions in and around Darjeeling	45
3.2. Preliminary screening for tolerance against mercury	45
3.3. Isolation of pure culture of mercury-tolerant microbes	46
3.4. Determination of minimum inhibitory concentration of mercury against the isolates	47
3.5. Biochemical study of the isolated pure cultures	47
3.5.1. Gram staining	47
3.5.2. Methyl Red test (MR)	48
3.5.3. Voges-Proskauer test (VP)	49
3.5.4. Citrate utilization test	49
3.5.5. Triple sugar iron test (TSI)	50
3.5.6. Starch hydrolysis test	50
3.5.7. Gelatin hydrolysis	51
3.5.8. Motility test (SIM)	52
3.5.9. Nitrate reduction test (NR)	52
3.5.10. Urease test	53
3.5.11. Catalase test	54
3.5.12. Coagulase test	55
3.6. Carbohydrate utilization study	55
3.7. <i>In vitro</i> plant growth promoting (PGP) properties	56
3.7.1. Indole-3-acetic acid production	56
3.7.2. Hydrogen cyanide production	57
3.7.3. Ammonia production	58
3.7.4. Siderophore production	58
3.7.5. Phosphate solubilization	59
3.8. Cross tolerance of the isolates towards other heavy metals	60
3.9. Growth study of the isolates under mercury-free and mercury stress conditions	60
3.10. Molecular identification of pure culture isolates	60
3.10.1. Isolation of bacterial DNA	61

CONTENTS	PAGE NO.
3.10.2. Purity assessment of extracted DNA	62
3.10.3. PCR amplification of 16S amplicon	62
3.10.4. Quality check of 16S amplicons	63
3.10.5. Purification of PCR purified product	63
3.10.6. Sequencing of 16S rRNA region of DNA	64
3.10.7. Identification of pure culture isolates	64
3.11. Antibiotic susceptibility study on the isolates	64
3.12. Metabolomic profiling of pure culture isolates	66
3.12.1. Culture preparation of the isolates for GC-MS analysis	66
3.12.2. Preparation of extract for GC-MS analysis	66
3.12.3. Gas chromatography technique	66
3.12.4. Mass spectrometry	67
3.12.5. Metabolomics	67
3.13. Whole genome analysis	68
4. Chapter 4. Results and discussion	69
4.1. Collection of soil samples from various regions in and around Darjeeling	69
4.2. Preliminary screening for tolerance against mercury	71
4.3. Isolation of pure cultures of mercury-tolerant microbes	72
4.4. Determination of minimum inhibitory concentration of mercury against the isolates	74
4.5. Biochemical study of the isolated pure culture	76
4.5.1. Gram staining	76
4.5.2. Methyl Red test	79
4.5.3. Voges-Proskauer test	81
4.5.4. Citrate utilization test	84
4.5.5. Triple sugar iron test	86
4.5.6. Starch hydrolysis test	88
4.5.7. Gelatin hydrolysis	89
4.5.8. Motility test	92
4.5.9. Nitrate reduction test	94
4.5.10. Urease test	95
4.5.11. Catalase test	97
4.5.12. Coagulase test	98

CONTENTS	PAGE NO.
4.6. Carbohydrate utilization study	103
4.7. <i>In vitro</i> plant growth promoting properties	108
4.7.1. Indole-3-acetic acid production	109
4.7.2. Hydrogen cyanide production	109
4.7.3. Phosphate solubilization	112
4.7.4. Ammonia production	113
4.7.5. Siderophore production	114
4.8. Cross tolerance of the isolates towards other heavy metals	117
4.9. Growth study of the isolates under mercury-free and mercury stress conditions	132
4.10. Molecular identification of pure culture isolates	136
4.11. Antibiotic susceptibility study on the isolates	144
4.12. Metabolomic profiling of pure culture isolates	179
4.13. Whole genome analysis	206
CONCLUSION	220
APPENDIX	
A. LIST OF PUBLICATIONS	223
B. PARTIAL GENOMES	224
C. CULTURE MEDIA	225
D. SOFTWARE & DATABASES	226
E. ABBREVIATIONS	227
INDEX	228
BIBLIOGRAPHY	229