

CONTENTS

	PAGE NO
CHAPTER I: INTRODUCTION	
PREFACE	1-2
1.0 INTRODUCTION	2
1.1 Role of Metal ions in biological systems	2-3
1.1.1 Physiological role of Zinc	3
1.1.2 Physiological role of Chromium	4
1.1.3 Physiological role of Cadmium	4-5
1.2 Metals interaction with microorganisms	5-8
1.3 Metal homeostasis in <i>Pseudomonas</i> species	8-11
1.4 Pseudomonas Metal biology	12
1.4.1 Metal transporters (importers and exporters)	12-13
1.4.2 Metal Binding Proteins	13-14
1.5 Bioremediation	15
1.5.1 Genetic engineering of bacteria with membrane transporters to remove radionuclides and toxic metals from the environment	15-17
1.5.2 Genetic engineering of bacteria with Metal-binding proteins for bioremediation of heavy metals	17-18
SCOPE OF THE PRESENT INVESTIGATION	19-21

CHAPTER II: MATERIAL AND METHODS

2.1 *IN-SILICO* TOOLS: *In-silico* analysis of MreA for metal binding properties:

2.1.1 Steps in identification of Metal Binding Protein	22
2.1.2 Identification of Putative Transmembrane Domains and Hydropathy Plots	22-23
2.1.3 Phylogenetic Analysis	23-24
2.1.4 Predicting stability change on single amino acid polymorphism based on support vector machine (I-Mutant 2.0)	24
2.1.5 Analysis of secondary structure using Protein Structure Prediction Server (PSIPRED v3.0)	24
2.1.6 Homology modeling and metal binding sites identification with MODELLER	25
2.2 CHEMICALS AND REAGENTS	25-26
2.3 BACTERIAL STRAINS AND GROWTH CONDITIONS	26
Table 2.1: Details of the plasmids and bacterial strains	27
2.4 COMMONLY USED MEDIA AND BUFFERS	28
2.5 CLONING AND OVER-EXPRESSION OF MreA FROM <i>P. PUTIDA</i> KT2440	
2.5.1 DNA extraction procedures: Plasmid DNA extraction	29
2.5.3 PCR amplification	30
Figure 2.1: DNA and protein sequence of PP_2969 (MreA) From <i>P. putida</i> KT2440	
Table 2.2: Details of the primers used in this study for PCR, QRT-PCR analysis and SDM mutant's generation	31
2.5.4 Gel extraction	32

2.5.5 Preparation of <i>E.coli</i> Competent Cells by CaCl₂ Procedure and Chemical Transformation	32
2.5.6 Cloning of PP-2969 into pET-32a or pET-28a expression vectors	32-33
Figure 2.2.A: Vector map of pET-28a (+)	33
Figure 2.2.B: Vector map of pET-32a (+)	34
Figure 2.3: Cloning Strategy of PP_2969 (MreA) gene of <i>P. putida</i> KT2440 into <i>E. coli</i>	35
2.5.7 Generation of Site Directed Mutants	36
Figure 2.4: Overview of the Quick-Change II XL site-directed mutagenesis method	37
2.5.8 Over-expression of <i>E.coli</i>-BL21 (DE3)-MreA (pET32a-MreA or pET28a-MreA) and mutant proteins (pET28a-MreA-SDM-5, 6 and 7)	38
2.5.9 Polyclonal antibodies raising and purification	38-41
2.5.9.1 Raising polyclonal Antibodies against bacterial MreA protein and Immunization	38
2.5.9.2 Primary dose	39
2.5.9.3 Booster doses	39
2.5.9.4 Test bleeding and separating the serum	39
2.5.9.5 Antisera collection	40
2.5.9.6 Ammonium Sulphate Precipitation	40
2.5.9.7 DE-52 chromatography	40
2.5.9.8 Dot blot analysis	41

2.6 CHARACTERIZATION OF METAL BINDING PROTEIN	41-45
2.6.1 Metal-binding efficiencies evaluated by Atomic Absorption Spectroscopy (AAS)	41-42
2.6.2 Real-time PCR analysis and Western blot analysis of MreA expression in <i>P. putida</i> KT2440 (Wt), recombinant <i>E.coli</i>-BL21 (DE3) and SDM's (5,6 &7) during metal stress	
2.6.2.1 RNA isolation	42
2.6.2.2 Preparation of cDNA	43
2.6.2.3 Quantitative real time-PCR analysis	
2.6.3 Western blot analysis of MreA	44
2.7 MreA Mass prediction by Mass spectrophotometer (Peptide mass fingerprinting)	45
2.8 Statistical Analysis	45

CHAPTER III:

***IN-SILICO* ANALYSIS FOR METAL BINDING POTENTIAL OF MreA FROM *PSEUDOMONAS PUTIDA* KT2440**

3.1 Introduction	46
3.2 Results	47-61
3.3 Discussion	62-63

CHAPTER IV:

**CHARACTERIZATION OF METAL BINDING PROTEIN-MreA
FROM *PSEUDOMONAS PUTIDA* KT2440**

4.1 Introduction	64-66
4.2 Results	66-80
4.3 Discussion	81
SUMMARY	82-83
BIBLIOGRAPHY	84-97
PUBLICATIONS	98