CONTENTS

Acknowledgements	
Abbreviations	i - iii
Preface	1 - 5
Chapter I: Review of literature	6 - 41
1.1 M. tuberculosis	6
1.2 Pathogenesis	6
1.3 Immune response to M. tuberculosis	. 8
1.4 Comparative genomics	11
1.5 Function of RD1 region	13
1.6 Antisense technology	14
1.7 Technique to identify bacterial genes expressed specifically during infection	15
1.8 Reporter systems for indirect monitoring bacterial mRNA levels in vivo	18
1.9 Tuberculosis vaccines	21
1.10 Mycobacterial antigens	24
1.11 Diagnosis of tuberculosis	28
1.12 Drug targets of M. tuberculosis	29
1.13 Asymmetric protein localization in bacterial cell.	31
1.14 <i>M. smegmatis</i> mc ² 155: A surrogate host for analysis of <i>M. tuberculosis</i>	33
1.15 Glycopeptidolipids (GPLs)	34
1.16 Protein protein interaction	35
1.17 Animal models	38
1.18 Physiological stress and mycobacteria.	40
Chapter II: materials and methods	42 - 62
2.1 Bacterial strains and plasmids	42
2.2 Standard Markers	44
2.3 Media and buffers	44
2.4 Chemicals and biochemicals	44
2.5 Primers	45
2.6 General methods	45
2.6.1 Staining of acid fast bacilli	45
2.6.2 Isolation of genomic DNA from mycobacteria	45

2.6.3 Agarose gel electrophoresis	46
2.6.4 Dephosphorylation of plasmid DNA	46
2.6.5 Transformation of E. coli	47
2.6.6 Electroporation of plasmids into mycobacteria	47 ·
2.6.7 Restriction endonucleases digestion	47
2.6.8 Ligation	48
2.6.9 Nucleotide sequencing of cloned inserts	48
2.6.10 Protein estimation	49
2.6.11 Methods in protein expression and purification	50
2.6.12 Denatured SDS- polyacrylamide gel electrophoresis	51
2.6.13 Optimizing expression and solubilization of protein	51
2.6.14 Large scale purification of recombinant protein using His Bind resin	52
2.6.15 Western blot analysis	52
2.6.16 Generation of antibody against recombinant protein	53
2.6.17 ELISA	53
2.6.18 Southern hybridization	53
2.6.19 Immuno- fluorescence microscopy	55
2.6.20 Localization of Rv3878	56
2.6.21 Preparation of M. tuberculosis subfractions	57
2.6.22 Generation of M. tuberculosis genomic library	57
2.6.23 BATCH complementation assay	57
2.6.24 Assessment of cell surface hydrophobicity by Congo red binding assay	58
2.6.25 Assessment of cell surface hydrophobicity by adherence of bacteria with xylene	58
2.6.26 Autoaggregation of mycobacteria by sedimentation assay	59
2.6.27 Isolation of total lipids and glycopeptidolipids (GPLs)	59

2.6.28 Thin layer chromatography	60
2.6.29 GFP transcriptional fusion assay during growth cycle	60
2.6.30 GFP translational fusion assay in different stress conditions	60
2.6.31 GFP transcriptional fusion assay in acidic condition	60
2.6.32 GFP transcriptional fusion assay in nutrient starvation model	61
2.6.33 Expression analysis in ex-vivo condition	61,
2.6.34 Circular Dichroism measurements	61.
2.6.35 The thermal denaturation of protein Rv 3878	62
2.6.36 Chemically induced unfolding of protein Rv3878	62
2.6.37 Chemical cross linking of Rv3878 protein	62
2.6.38 Tyrosine fluorescence measurement	62
Chapter III: Results	63 - 104
3.1 PCR amplification of Rv3878	63
3.2 Cloning of PCR amplified product in TOPO-TA cloning vector	63
3.3 Nucleotide sequencing of cloned Rv 3878 in TOPO- TA vector	64
3.4 Subcloning of Rv3878 in pET-14b vector and expression of protein	64
3.5 Protein purification using His bind resin	65
4. Biophysical characterization	68
4.1 CD spectrum in presence of TFE.	68
4.2 CD spectrum in presence of SDS	68
4.3 CD spectra at different pH	70
4.4 CD spectra at different pH in presence of 4 mM SDS	70
4.5 Thermal and chemical denaturation with GuHCl in presence of SDS	70
4.6 Tyrosine fluorescence measurement	71
4.7 Chemical cross linking of Rv3878 protein with glutaraldehyde	72
5 Localization of Rv3878 in different mycobacteria	74
5.1 Southern hybridization	74

6.4.1 GFP transcriptional fusion assay in acidic condition.	98	
6.4.2 Expression of Rv3878 in nutrient starvation conditions	99	
6.4.3 Expression analysis in ex-vivo condition	100	
7. Bioinformatic analysis	101	
Chapter IV: Discussion	105 - 116	
Chapter V: Bibliography	117 - 138	
appendix	A1 – A12	