LIST OF ABBREVIATIONS

	Amp	:	Ampicillin
	APS	:	Ammonium persulfate
-	CaCl ₂	:	Calcium chloride
	DNA	:	Deoxyribonucleic acid
	IPTG	:	Isopropyl β-D-1-thiogalactopyranoside
	LB	:	Luria Bertani
• •	LBA	:	Luria Bertani Agar
•	MHA	:	Mueller Hinton Agar
	M	:	Mole
	Ml	:	Milliliter
	^{ie} mM	:	Millimole
· · · · ·	min	:	Minute
	sec	:	Second
	OD	:	Optical density
•	PCR	:	Polymerase Chain Reaction
2 · ·	Rpm	:	Rotation per minute
	SDS	:	Sodium Dodecyl Sulfate
	SDS-PAGE	:	Sodium Dodecyl Sulfate-Pplyacrylamide Gel Electrophoresis
	TCA	:	Tricarboxylic acid
	TEMED	:	Tetramethylethylenediamine
	μl .	:	Microliter

Contents

		Page no.			
A. Chapter 1 :	Introduction	1 - 7			
1.1 Pathogen	L Contraction of the second				
1.2 History					
1.3 Therapy					
1.3.1	Drugs and date of discovery				
1.3.2	New drug trials				
1.4 MDR & 2	1.4 MDR & XDR strains of Mtb				
1.4.1	MDR strains				
1.4.2	XDR strains	i			
1.5 Latency					
1.6 TB-AIDS	5				
B. Aim and obj	jectives	8			
C. Chapter 2 : Review of literature		9 - 17			
2.1 Why stu	dy about tuberculosis?				
2.2 Various	2.2 Various drug targets of Mycobacterium tuberculosis (Mtb)				
2.3 Some pr	2.3 Some prevalent drug candidates against Mtb				
2.4 Thymidy	2.4 Thymidylate synthase (TS) complementing protein gene as a potential drug target				
in Mtb					
2.5 Cloning	and overexpression of the Mtb TS complementing protein	n gene			
2.5.1	Host strains for cloning				
2.5.2	Bacterial expression systems				

- 2.5.2.1 pET expression system
- 2.5.2.2 Expression host strain

2.6 Study of the mycobacterial TS complementing protein gene expression at RNA level

2.6.1 Total RNA isolation after treatment with Ascorbic acid

D. Chapter 3 Materials and methods

.

,

.

• ;

18-27

3.1 Materials

3.1.1 Bacterial strains

3.1.2 Chemicals and reagents

3.2 Methods

3.2.1 Culture of bacterial strains

3.2.2 Staining of *M.smegmatis* (Acid-fast staining)

3.2.3 Isolation of M.smegmatis (MS) DNA

3.2.4 PCR of the TS complementing protein gene

3.2.5 Isolation of the PCR product

3.2.6. Restriction Digestion

3.2.7. Cloning of the TS complementing protein gene

3.2.8. Preparation of competent cells

3.2.9 Transformation by heat-shock method

3.2.10 Isolation of plasmid

3.2.11 Restriction digestion of recombinant plasmid

3.2.12 Expression of recombinant protein

3.2.13 Sodium Dodecyl Sulfate-Polyacrylamide Gel Electrophoresis (SDS-PAGE)

3.2.14 Antibacterial Assay

3.2.15 Determination of Minimum Inhibition Concentration (MIC) of ascorbic acid

3.2.16 Growth curve kinetics

3.2.17 Total RNA isolation from *M.smegmatis* on the basis of the time-kill assay of both ascorbic acid treated and untreated cells

3.2.18 Electrophoresis of RNA through Agarose Gel Containing Formaldehyde

3.2.19 First strand cDNA synthesis

3.2.20 PCR of the TS complementing protein gene from the first-strand cDNA

E. CHAPTER 4: RESULTS

2 - .

28 - 39

4.1 Amplification of TS complementing protein gene

4.2 Restriction digestion of insert and vector plasmid

4.3 Transformation

4.4 Plasmid isolation

4.5 Restriction digestion of recombinant plasmid

4.6 Effect of IPTG (Isopropyl β -D-1-thiogalactopyranoside) on expression of recombinant protein

4.7 Antibacterial Assay

4.8 Determination of Minimum Inhibition Concentration (MIC) of ascorbic acid 4.9 Growth curve kinetics

4.10 Total RNA isolation from *M.smegmatis* on the basis of the time-kill assay of bothb ascorbic acid treated and untreated cells

4.11 PCR of the TS complementing protein gene from the first strand cDNA

F. DISCUSSION	40 - 41
G. CONCLUSION	42
H. FUTURE DIRECTIONS	43 – 47
I. REFERENCES	48-50