

---

# Contents

	Page no.
A. Chapter 1: Introduction	1-3
B. Aim and objective	4
C. Chapter 2: Review of literature	5-15
2.1 Proteases	
2.2 Classification of proteases	
2.3 Microbial proteases	
2.4 Bacterial alkaline proteases	
2.5 Application of alkaline proteases	
2.5.1 Food and feed industry	
2.5.2 Leather industry	
2.5.3 Management of industrial and household waste	
2.5.4 Photographic industry	
2.5.5 Medical usage	
2.5.6 Skin degumming	
2.5.7 Detergent industry	
2.6 Cloning and overexpression of bacterial protease gene	
2.6.1 Bacterial expression systems	
2.6.1.1 pET expression system	
2.6.1.2 Expression host strain	
D. Chapter 3: Materials and methods	16-22
3.1 Materials	
3.1.1 Bacterial strains	
3.1.2. Chemicals and reagents:	
3.2 Methods	
3.2.1 Culture of alkaline protease producing bacteria	
3.2.2 Fibrinolytic activity assay	
3.2.3 Isolation of protease gene	
3.2.4 Isolation of the PCR product	
3.2.5 Restriction digestion	
3.2.6 Cloning of the protease gene	
3.2.7 Preparation of competent cells	
3.2.8 Transformation by heat-shock method	
3.2.9 Isolation of plasmid	
3.2.10 Restriction digestion of recombinant plasmid	
3.2.11 Expression of recombinant protein	
3.2.12 Sodium Dodecyl Sulfate-Polyacrylamide Gel Electrophoresis (SDS-PAGE)	
3.2.13 Effect of IPTG on expression of recombinant protein	
3.2.14 Purification of recombinant protein under denaturing condition	
3.2.14.1 Preparation of cell lysate using 8 M Urea	
3.2.14.2 Purification of recombinant protein using His-tag column	
3.2.15 Biochemical characterization of recombinant protein	
E. Chapter 4: Results	23-30

---

4.1 Fibrinolytic activity of <i>P. tezipurensis</i>	
4.2 Amplification of Protease gene	
4.3 Restriction digestion of insert and vector plasmid	
4.4 Plasmid isolation	
4.5 Restriction digestion of recombinant plasmid	
4.6 Expression of recombinant protein	
4.7 Effect of IPTG on expression of recombinant protein	
4.8 Purification of recombinant protein under denaturing condition	
4.9 Biochemical characterization of the recombinant protein	
F. Chapter 5: Discussions	31-33
G. Chapter 6: Conclusions and future directions	34
H. References	35-39

---

## List of abbreviations used

Amp	:	Ampicillin
APS	:	Ammonium persulfate
CaCl <sub>2</sub>	:	Calcium chloride
DNA	:	Deoxyribonucleic acid
FC	:	Folin-Ciocalteu
IPTG	:	Isopropyl $\beta$ -D-1-thiogalactopyranoside
LB	:	Luria-Bertani
M	:	Mole
min	:	Minute
ml	:	Milliliter
mM	:	Millimole
NB	:	Nutrient broth
OD	:	Optical density
PCR	:	Polymerase chain reaction
rpm	:	Rotation per minute
SDS	:	Sodium dodecyl sulfate
SDS-PAGE	:	Sodium Dodecyl Sulfate-Polyacrylamide Gel Electrophoresis
sec	:	Second
TCA	:	Tricarboxylic acid
TEMED	:	Tetramethylethylenediamine
$\mu$ l	:	Microliter

---