

CONTENTS

SUMMARY	IV
ACKNOWLEDGEMENT	VI
CONTENTS	i
LIST OF TABLES	7
LIST OF FIGURES	8
1. INTRODUCTION	11
1.1. Classification of Bacteria	12
1.2. <i>Geobacillus</i> species.....	14
1.3. Enzymes.....	14
a. Oxidoreductases (EC 1).....	14
b. Transferases (EC 2)	15
c. Hydrolases (EC 3).....	15
d. Lyases (EC 4)	15
e. Isomerases (EC 5)	15
f. Ligases (EC 6)	16
1.3.1. Hydrolytic Enzymes	16
1.3.2. Lipases (EC 3.1.1.3).....	17
1.4.1. Classification of lipases.....	17
1.4.2. Structure of Lipases	18
1.4.3. Applications of lipases	19
1.4.3.1. Biodegradable materials.....	20
1.4.3.2. Food Industry.....	20
1.4.3.3. Detergent industry	21
1.4.3.4. Pharmaceutical Industry.....	21
1.5. Proteases	22
1.5.1. Classification of proteases.....	22
1.5.2. Carboxypeptidases	23
1.5.2.1. Classification of carboxypeptidase	23
1.5.2.2. Metallocoarboxypeptidases	23
1.5.2.3. Structure and catalytic mechanism of metallocoarboxypeptidases	24

1.5.3. Applications of carboxypeptidases.....	24
1.5.3.1. Protein C-terminal sequencing.....	24
1.5.3.2. Detergent industry	25
2. MATERIALS AND METHODS	26
2.1. Strain isolation.....	27
2.1.1. Sample collection and isolation.....	27
2.1.2. Growth conditions for the isolate	27
2.1.2.1. Growth media.....	27
2.1.2.2. Optimum growth temperature	28
2.1.2.3. Optimum pH for the growth.....	28
2.1.2.4. Effect of salt on growth	28
2.1.2.5. Antibiotic sensitivity	28
2.2. Characterization of the microorganism.....	29
2.2.1. Biochemical Characterization	29
2.2.2. Characterization on the basis of 16S rRNA.....	29
2.2.2.1. Genomic DNA isolation.....	29
2.2.2.2. Agarose gel electrophoresis	30
2.2.2.3. Quantification of DNA	30
2.2.2.4. Cloning of 16S rRNA gene.....	31
2.2.2.5. Sequence analyses of 16S rRNA	32
2.2.2.6. Nucleotide sequence accession number.....	32
2.2.3. Characterization on the basis of chapronin gene sequence	33
2.2.3.1. PCR amplification and sequence analyses of chapronin gene	33
2.2.3.2. Nucleotide sequence accession number	33
2.3. Cloning and characterization of Lipase	33
2.3.1. PCR amplification of the <i>lip_{SBS}</i> gene.....	33
2.3.2. Ligation of the <i>lip_{SBS}</i> gene in pTZ57R/T.....	34
2.3.3. Restriction analyses of pTZ-lip.....	34
2.3.4. Expression of the <i>lip_{SBS}</i> gene.....	34
2.3.5. Sodium dodecyl sulfate polyacrylamide gel electrophoresis	35

2.3.6. Estimation of protein concentration	35
2.3.7. Solubilization and refolding of LIP _{SBS}	36
2.3.8. Purification of the LIP _{SBS}	37
2.3.9. Lipase activity assay.....	37
2.3.10. Molecular mass determination	38
2.3.11. Characterization of LIP _{SBS}	38
2.3.11.1. Effect of temperature on the LIP _{SBS} activity	38
2.3.11.2. Effect of pH on the LIP _{SBS} activity.....	38
2.3.11.4. Effect of metal ions and detergents on the activity of the LIP _{SBS}	39
2.3.11.5. Degradation of oils by LIP _{SBS}	39
2.3.11.6. Kinetic studies of LIP _{SBS}	39
2.3.12. X-Ray crystallographic studies of the LIP _{SBS}	40
2.3.12.1. Crystallization and data collection	40
2.3.12.2. Structure determination and refinement	40
2.3.13. Molecular Dynamics (MD) simulation studies of the LIP _{SBS}	41
2.3.14. Nucleotide sequence accession number.....	42
2.4. Cloning and characterization of carboxypeptidase	43
2.4.1. PCR amplification of the <i>cbp</i> _{SBS} gene	43
2.4.2. Ligation of the <i>cbp</i> _{SBS} gene	43
2.4.3. Restriction analyses of pTZ-cbp.....	44
2.4.4. DNA sequencing and phylogenetic analyses of <i>cbp</i> _{SBS} gene.....	44
2.4.5. Expression of the <i>cbp</i> _{SBS} gene	44
2.4.6. Production of the CBP _{SBS} in the presence of a chapronin	45
2.4.7. Solubilization and refolding of CBP _{SBS}	45
2.4.8. Cloning of the <i>cbp</i> _{SBS} gene in pET-28a	45
2.4.9. Colony PCR of the transformants	46
2.4.10. Restriction analyses of pET28-cbp.....	47
2.4.11. Production of the CBP _{SBS} with 6x-His-Tag (His-CBP _{SBS})	47
2.4.12. Purification of His-CBP _{SBS}	47
2.4.13. Molecular mass determination	48

2.4.14. Enzyme activity assay	48
2.4.15. Characterization of His-CBP _{SBS}	48
2.4.15.1. Effect of temperature on His-CBP _{SBS} activity	48
2.4.15.2. Effect of pH, detergents and metal ions on the activity of His-CBP _{SBS}	49
2.4.15. Nucleotide sequence accession number	49
3. RESULTS	50
3.1. Strain isolation.....	51
3.2: Growth conditions for the isolate	51
3.2.1. Growth media.....	51
3.2.2. Optimum growth temperature	52
3.2.3. Optimum pH for the growth.....	52
3.2.4. Salt tolerance.....	53
3.2.5. Antibiotic sensitivity	54
3.3. Identification of SBS-4S	54
3.3.1. Biochemical characterization	54
3.3.2. Characterization on the basis of 16S rRNA sequence	56
3.3.2.1. Genomic DNA isolation.....	56
3.3.2.2. Cloning of 16S rRNA gene.....	56
3.3.2.3. Sequence analyses of 16S rRNA	58
3.3.3. Characterization on the basis of chapronin sequence	59
3.3.3.1. PCR amplification of chapronin gene	59
3.3.3.2. Sequence analyses.....	60
3.4. Gene cloning and characterization of LIP _{SBS}	61
3.4.1. PCR amplification of <i>lip</i> _{SBS} gene	61
3.4.2. Restriction analyses of pTZ-lip.....	61
3.4.3. Sequence analyses of <i>lip</i> _{SBS} gene	62
3.4.4. Expression of the <i>lip</i> _{SBS} gene.....	63
3.4.5. Refolding of LIP _{SBS}	65
3.4.6. Purification of the LIP _{SBS}	65
3.4.7. Molecular mass determination	67

3.4.8. Characterization of LIP _{SBS}	67
3.4.8.1. Effect of temperature on LIP _{SBS} activity.....	67
3.4.8.2. Effect of pH on LIP _{SBS} activity.....	69
3.4.8.3. pH stability of LIP _{SBS}	69
3.4.8.4. Effect of metal ions on activity of LIP _{SBS}	70
3.4.8.5. Effect of detergents on the activity of LIP _{SBS}	70
3.4.8.6. Degradation of oils by LIP _{SBS}	71
3.4.8.7. Kinetic studies of LIP _{SBS}	71
3.4.9. X-Ray crystallographic studies of LIP _{SBS}	73
3.4.9.1. Crystallization of LIP _{SBS}	73
3.4.9.2. Structure determination and refinement.....	73
3.4.10. Molecular dynamics simulation studies of LIP _{SBS}	76
3.4.11. Configurational comparison of helical lid of LIP _{SBS}	77
3.5. Gene cloning and characterization of CBP _{SBS}	79
3.5.1. PCR amplification of <i>cbp</i> _{SBS} gene.....	79
3.5.2. Ligation and restriction analyses of pTZ-cbp.....	79
3.5.3. Sequence analyses of <i>cbp</i> _{SBS} gene.....	80
3.5.4. Expression of <i>cbp</i> _{SBS} gene.....	82
3.5.5. Production of CBP _{SBS} in the presence of a chapronin.....	84
3.5.6. Solubilization and refolding of CBP _{SBS}	84
3.5.7. Cloning of <i>cbp</i> _{SBS} in pET-28a.....	85
3.5.8. Colony PCR of the transformants.....	85
3.5.9. Restriction analyses of pET28-cbp.....	86
3.5.10. Production of CBP _{SBS} with 6x-His-Tag.....	86
3.5.11. Purification of His-CBP _{SBS}	87
3.5.12. Molecular mass determination.....	89
3.5.13. Characterization of His-CBP _{SBS}	90
3.5.13.1. Effect of temperature on His-CBP _{SBS}	90
3.5.13.2. Thermostability analyses of His-CBP _{SBS}	91
3.5.13.3. Effect of pH on the activity of His-CBP _{SBS}	92

3.5.13.4. Effect of detergents on the activity of His-CBP _{SBS}	93
3.5.13.5. Effect of metal ions on the activity of His-CBP _{SBS}	94
3.5.13.6. Kinetic studies of His-CBP _{SBS}	95
4. DISCUSSION	97
5. REFERENCES	105
6. APPENDIX (PUBLISHED WORK)	114