

CONTENTS

<i>Preface</i>	<i>xi</i>
<i>About the Authors</i>	<i>xv</i>
1. Introduction	1
1.1 Enzymes	4
1.2 Etymology and Historical Aspects of Industrial Enzymes	4
1.3 Industrial Enzymes: Development of Bioindustrial Sector	5
1.4 Classification of Enzymes	9
1.5 Lipase	12
1.6 Classification of Bacterial Lipolytic Enzymes	15
References	21
2. Application of Lipases	25
2.1 Detergent Industry	26
2.2 Food Processing, Flavour Development and Improving Quality	27
2.3 Bakery Industry	27
2.4 Fat and Oleochemical Industry	27
2.5 Textile Industry	28
2.6 Cosmetic Industry	28
2.7 Pulp and Paper Industry	29
2.8 Oil Biodegradation	29
2.9 Production of Biodegradable Polymers	29
2.10 Diagnostic Tool	30
2.11 Degreasing of Leather	30
2.12 Waste/Effluent/Sewage Treatment	31
2.13 Production of Biodiesel	31
2.14 Tea Processing	32

2.15 Biosensors.....	32
2.16 Environmental Management	32
2.17 Major Obstacles and Future Prospect of Microbial Lipases ..	33
References	33
3. Metagenomics and Unculturable Bacteria	35
3.1 Microbial Communities.....	35
3.2 Soil as a Microbial Habitat.....	36
3.3 Unculturable Microorganisms	36
3.4 Culturable Microorganisms	38
3.5 Microbial Species for Beneficial Products and Processes.....	38
3.6 Metagenomics.....	38
3.7 Soil Metagenomics for Desirable Genes	39
3.8 Soil Metagenomics: Tool for Novel Compounds	41
3.9 Sequence-Based Metagenomics	43
3.10 Function-Driven Metagenomics	44
3.11 Metagenomic Library Construction and Its Analysis.....	46
3.12 Approaches to Metagenomics	47
References	48
4. Accessing Metagenomics	53
4.1 Methods	53
4.2 Expression Vector/Host System	55
4.3 Functional Screening of Metagenomic Libraries	56
References	58
5. Metagenomics for Lipase.....	61
5.1 Collection of Environmental Samples.....	62
5.2 Extraction, Purification and Quantification of Soil Metagenomic DNA.....	65
5.3 Isolation and Purification of Mgdna from Gram ⁺ and Gram ⁻ Bacteria	72
5.4 Polymerase Chain Reaction.....	75

5.5	Restriction Digestion by <i>Eco</i> RI, <i>Hind</i> III and BamHI	75
5.6	Metagenomic DNA Library Construction	76
5.7	Sequence-Based Metagenomic Approach	76
5.8	Construction of 16S rDNA Gene Libraries	79
5.9	Preparation of <i>E. coli</i> Competent Cells and Transformation ...	81
5.10	Analysis of Transformation Efficiency Using Non-Recombinant Plasmid.....	82
5.11	Screening of Recombinants by α Complementation	83
5.12	Purification of PCR Products	84
5.13	Transformant Confirmation by Colony PCR Using Universal Primers	85
5.14	Isolation and Restriction of Recombinant/Nonre- combinant Plasmid DNAs	88
5.15	Analysis of 16S rDNA Gene	90
5.16	Phylogenetic Analysis	91
5.17	Genbank Submission and Accession Numbers	91
5.18	Phylogeny	91
	References	94
6.	Functional Approach for Metagenomic Library Construction...	97
6.1	Restriction Digestion of mgDNA.....	98
6.2	Transformation of <i>E. coli</i> BL21 Competent Cells.....	98
6.3	Functional Screening of Metagenomic Libraries for the Lipase Gene	103
6.4	Isolation and Restriction Digestion of Recombinant Plasmid.....	104
6.5	Sequencing of Cloned DNA	106
6.6	Sequence Analysis and Phylogenetic Tree Construction.....	107
6.7	Analysis of the Cloned Lipase Gene	107
6.8	Phylogeny of Recombinant Protein.....	108
6.9	Amino Acid Sequence of Recombinant Protein.....	108
6.10	Multiple Sequence Alignment	108

6.11	Storage of Recombinant Plasmid	109
	References	110
7.	Overexpression of Recombinant Protein	111
7.1	Purification of Expressed Protein	111
7.2	Determination of Active Lipase Using Zymographic Study ..	114
7.3	Determination of Specific Activity, % Yield and Protein Fold Purification	116
7.4	Homology Model and Validation for Protein Structure Prediction	117
	References	124
8.	Biochemical Characterization of Purified Lipase.....	127
8.1	Dose-Dependent Enzyme Activity	127
8.2	Substrate Specificity and Effect of Substrate Concentration	128
8.3	Enzyme Kinetics.....	129
8.4	Effect of Temperature and pH	131
8.5	Effect of Surfactants	133
8.6	Effect of Other Factors	134
	References	139
9.	Genomic Study of Culture Dependent Bacteria.....	141
9.1	Isolation and screening for lipase producing culturable bacteria	141
9.2	Pure culture of lipase-producing bacterial isolates.....	142
9.3	Inoculum preparation.....	143
9.4	Zone of hydrolysis.....	143
9.5	Growth kinetics and lipase production	144
9.6	Thermal Stability	145
9.7	Evaluation of Crude Enzyme in Detergent Formulation.....	147
9.8	Stability of Culture Supernatant Under Storage.....	148
9.9	Taxonomic Identification of Lipase-Producing Bacteria.....	148

9.10 Extracellular Enzyme Activity	156
9.11 Spectrophotometric Growth Determination	159
References	162
10. Genomic Study of Culturable Bacteria.....	163
10.1 Genomic DNA Extraction of Lipase-Producing Culturable Bacteria.....	163
10.2 Agarose Gel Electrophoresis	164
10.3 Quantification and Purity Determination of the Isolated DNA.....	165
10.4 Molecular Genetic Assessment of Lipase- Producing Bacteria	167
10.5 Phylogenetic Analysis	169
10.6 Optimization of Culture Condition for Lipase Production..	176
10.7 Effect of Carbon and Nitrogen Sources for the Growth of Culturable Bacteria	177
10.8 Time Course with Lipase Production	182
10.9 Effect of Other Factors	183
References	192
11. Microbial Assay of Culture Supernatant Containing Crude Lipase	193
11.1 Determination of Antibacterial Activity	193
11.2 Determination of Antifungal Activity.....	196
References	198
12. Critical Observations.....	201
Index.....	207