

## TABLE OF CONTENTS

<b>TITLE PAGE</b>	i
<b>APPROVAL</b>	ii
<b>CERTIFICATE OF AUTHENTICATION</b>	iii
<b>PREFACE</b>	iv
<b>TABLE OF CONTENTS</b>	v
<b>TABLE OF TABLES</b>	vii
<b>TABLE OF FIGURES</b>	viii
<b>TABLE OF APPENDIXES</b>	ix
<b>ABSTRACT</b>	x
<b>INTISARI</b>	xi
<b>CHAPTER I INTRODUCTION</b>	1
I.1 Background	1
I.2 Research Objectives	3
I.3 Research Benefits	4
<b>CHAPTER II LITERATURE REVIEW AND HYPOTHESIS FORMULATION</b>	5
II.1 Literature Review	5
II.1.1 Lipase enzyme	5
Lipase catalyzed chemical reaction	6
Genes encoding lipase	8
II.1.2 Recombinant microbial lipases and their applications	11
II.1.3 Bacterial isolate JG3	13
II.1.4 16 S rRNA genetic test	13
II.1.5 Amplification of lipase gene by PCR (polymerase chain reaction)	14
DNA isolation techniques	15
Primer design	16
II.1.6 Lipase gene sequence analysis	17
DNA sequencing	17
Nucleotide sequence analysis	18
Amino acid sequence analysis	18
II.1.7 Homology analysis	19
II.2 Hypothesis Formulation and Research Design	20
II.2.1 Hypotheses	20
II.2.2 Research design	21
<b>CHAPTER III RESEARCH METHODE</b>	23
III.1 Research Location	23
III.2 Instruments and Materials	23
III.2.1 Instruments	23
III.2.2 Materials	23
III.3 Research Methods	24
III.3.1 Preparation of bacterial sample	24
III.3.2 Bacterial identification	25

Amplification of 16S rRNA gene	25
Phylogenetic analysis	25
III.3.3 DNA Isolation	26
III.3.4 Qualitative analysis of isolated DNA	26
III.3.5 Quantitive analysis of isolated DNA	27
III.3.6 PCR primer design for lipase gene amplification	27
III.3.7 PCR amplification	27
III.3.8 Purification of amplified DNA	28
III.3.9 DNA sequencing	28
III.3.10 Homology analysis and protein structure prediction	29
<b>CHAPTER IV RESULTS AND DISCUSSION</b>	31
IV.1 Genotypic Characteristic of Bacterial Sample Strain JG3	31
IV.2 Lipase Gene Amplification	35
IV.2.1 DNA isolation	35
IV.2.2 Primer design	36
IV.2.3 Lipase DNA amplification by PCR	38
IV.2.4 Nucleotide sequence characterization of amplified DNA	40
IV.3 Deduced Amino Acid Sequence Analysis of Amplified DNA	42
IV.3.1 Translated amino acid sequence and BLASTp analysis	42
IV.3.2 Multiple sequence alignment of deduced amino acid sequence	46
IV.4 Homology Analysis	48
IV.4.1 Prediction of LipJG3 3-D structure	48
Secondary structure	48
Tertiary (3-D) Structure	49
IV.4.2 Protein Function	51
Characterization of Catalytic Site Residues	54
<b>CHAPTER V CONCLUSION AND SUGGESTION</b>	51
V.1 Conclusions	51
V.2 Suggestions	51
<b>REFERENCES</b>	57
<b>APPENDIXES</b>	64