



**UNIVERSITI PUTRA MALAYSIA**

**PRODUCTION, EXPRESSION AND CHARACTERIZATION OF A  
HEAT-STABLE ORGANIC SOLVENT TOLERANT LIPASE FROM  
BACILLUS SP, STRAIN 42**

**MOHAMRD ABDALLAH ELTAWHEEL.**

**FBSB 2005 11**

**PRODUCTION, EXPRESSION AND CHARACTERIZATION OF A HEAT-  
STABLE ORGANIC SOLVENT TOLERANT LIPASE FROM  
*BACILLUS* SP. STRAIN 42**

**By**

**MOHAMED ABDALLAH ELTAWHEEL**

**Thesis Submitted to the School of Graduate Studies, Universiti Putra  
Malaysia, in Fulfillment of the Requirements for the Degree of  
Doctor of Philosophy**

**October 2005**



## **DEDICATION**

I dedicate this humble effort, the fruit of my thoughts and study,  
to the great and helpful wife Hawa Safar,  
dear sons Abdallah, Abdalrahman, Abdalraouf  
and sweet daughter Halima  
who have inspired me to higher ideals of life.



Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfillment of the requirement for the degree of Doctor of Philosophy

**PRODUCTION, EXPRESSION AND CHARACTERIZATION OF A HEAT-STABLE ORGANIC SOLVENT TOLERANT LIPASE FROM *BACILLUS* SP. STRAIN 42**

By

**MOHAMED ABDALLAH ELTAWHEEL**

October 2005

**Chairman: Associate Professor Raja Noor Zaliha Raja Abd Rahman, PhD**

**Faculty: Biotechnology and Biomolecular Sciences**

Ninety two bacterial strains were isolated from oil palm effluent from Bangi, Selangor; Kluang, Johor; Alor Gajah hot spring (up to 54 °C) Melaka and Slim River hot spring (up to 91°C) Perak. An enrichment culture technique was used to isolate bacteria utilizing olive oil as a substrate. Cultures were incubated at 60°C to select for the thermophilic bacteria. Eight isolates showed lipolytic activity on tributyrin and triolein agar plates. In order to screen for highest lipase producer, six production media were used. Isolate 42 was observed to produce the highest level (0.059 U/ml) after 72h. Its crude lipase retained its full activity when preincubated at 70°C for 30 min. It also showed high stability in several organic solvents (25% v/v). Furthermore, its activity was enhanced in benzene, hexane and hexadecane while, completely inhibited by butanol. Isolate 42 was



identified as *Bacillus* sp. Strain 42 using 16S rDNA. The nucleotide sequence deposited at GenBank under accession number AY 763118.

Further optimization studies were done in order to determine the best lipase production condition. Inoculum size of 3% proved to be the best for lipase production, with an optimum temperature of 50°C when, grown under shaking condition of 150 rpm. A combination of tryptone and yeast extract was the best nitrogen source. Lipase production was stimulated by olive oil.

The lipase gene was amplified by polymerase chain reaction using consensus primers based on multiple aligned sequences of thermophilic genes from other thermophilic *Bacillus* species. Nucleotide sequence comparison shared high homology with the thermostable genes in *Geobacillus* sp., *Bacillus stearothermophilus* and *Bacillus thermoleovorans*. Nucleotide sequence deposited at GenBank under accession number AY 787835. The amplified gene was successfully cloned using a pQE-30 UA expression vector and induced by IPTG at the optimum concentration of 0.75 mM.

The recombinant lipase was facilitated by the fusion of 6-histidine and this allowed a one-step purification of the lipase enzyme using Ni-NTA affinity chromatography. The histidine-tagged lipase was purified 6-fold with a yield of 21.7%. Purified lipase migrated as a single band with a molecular mass of ~43 KDa on SDS-PAGE.



The purified lipase showed high activity at 70°C with its optimum at pH 8.0. The enzyme was stable over a broad range of pH from 6.0 to 10.0. It also showed high stability with half-lives of 315 min at 60°C, 120 min at 65°C, and 45 min at 70°C. Preincubation enzyme activity was stimulated with Na<sup>+</sup>, K<sup>+</sup> and Ca<sup>2+</sup>. While, Zn<sup>2+</sup>, Mn<sup>2+</sup> and Fe<sup>2+</sup> at high concentration (10 mM) were greatly inhibitory. Protease inhibitors Bestatin and pepstatin stimulated the lipase activity while, phenylmethylsulfonyl fluoride (PMSF) completely inhibited the lipase activity. Tween 80 (0.1%) enhanced the lipase activity while higher concentration (1%) dramatically decreased the lipase activity. The activity of preincubated enzyme in heptanol (log *P* 2.4) and octanol (log *P* 2.9) was slightly enhanced while, remains very stable with other organic solvents tested. Solvents such as ethylbenzene (log *P* 3.1) and dodecane (log *P* 6.6) reduced the lipase activity up to 35% and 38%, respectively. The highest specificity was observed towards tricaprylin (C<sub>8</sub>), followed by tricaprin (C<sub>10</sub>). Its hydrolyzed all the natural oils tested, with highest hydrolysis rate on olive oil.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia  
Sebagai memenuhi keperluan untuk Ijazah Doktor Falsafah

**PENGHASILAN, PENGEKSPRESIAN DAN PENCIRIAN LIPASE TAHAN  
PELARUT ORGANIK DAN TERMOSTABIL DARI *BACILLUS* SP. STRAIN 42**

Oleh

**MOHAMED ABDALLAH ELTAWHEEL**

Oktober 2005

**Pengerusi: Profesor Madya Raja Noor Zaliha Raja Abd Rahman, PhD**

**Fakulti: Bioteknologi Dan Seins Biomolekul**

Sebanyak 92 strain bakteria telah dipencilkan daripada sisa kumbahan sawit yang di perolehi dari Bangi, Selangor; dan Kluang Johor, serta kolam air panas di Alor Gajah, Melaka (suhu 54°C) dan di Slim River, Perak (91 °C). Minyak zaitun sebagai substrat telah digunakan untuk memencilkan bakteria melalui kaedah pengkayaan kultur. Pengeraman pada suhu 60°C digunakan untuk menggalakkan pertumbuhan bakteria yang rintang suhu atau termofilik. Sebanyak lapan isolat telah menunjukkan aktiviti lipolitik tertinggi di atas plat agar tributirin and triolein. Di dalam media cecair, penghasilan tertinggi didapati pada strain 42. Bagi meningkatkan penghasilan lipase, sebanyak enam jenis media digunakan. Media M3 didapati menghasilkan lipase tertinggi, iaitu 0.059 U/mL pada 72 jam pengeraman, dengan kadar goncangan 150 rpm, pada suhu 60°C.



Kajian dilanjutkan bagi menentukan penghasilan terbaik enzim lipase. Inokulum bersaiz 3% terbukti menghasilkan enzim lipase tertinggi dengan suhu optimum pada 50°C. Penghasilan lipase paling tinggi adalah pada kadar goncangan 150 rpm per min. Penghasilan lipase adalah dirangsang oleh penambahan minyak zaitun sebagai substrat. Lipase mentah menunjukkan kestabilan yang tinggi sehingga mencapai suhu 70°C.

Ekstrak enzim mentah diuji terhadap beberapa pelarut organik berkepekatan 25% v/v, selama 30 min untuk menentukan kestabilannya. Peningkatan aktiviti berlaku di dalam pelarut benzena, heksana dan heksadekana, tetapi ia juga stabil di dalam pelarut toluena, xylina, dekanol, isooktana dan tetradekana. Aktiviti enzim menurun sebanyak 34.5% di dalam pelarut propanol dan 63.6% di dalam pelarut propilasetat berbanding kawalan dan direncatkan sepenuhnya oleh butanol. Gen lipase dari *Bacillus* sp. strain 42 telah digandakan melalui tindakbalas rantaian polimerase (PCR) menggunakan primer konsensus berdasarkan padanan jujukan berganda gen termofilik daripada spesies *Bacillus*. Perbandingan jujukan nukleotida menunjukkan gen lipase strain 42 mempunyai homologi yang tinggi dengan gen termostabil dari *Geobacillus* sp, *Bacillus stearothermophilus* dan *Bacillus thermoleovorans*. Gen yang digandakan ini telah berjaya diklon ke dalam vector pQE-30 UA dan telah diekspreskan dengan kehadiran IPTG pada kepekatan optimum 0.75 mM.

Penulenan enzim lipase rekombinan dipermudahkan dengan kehadiran 6-histidina pada vektor, ini membolehkan penulenan satu langkah dengan





menggunakan Ni-NTA kromatografi afiniti. Lipase pembawa histidina telah dituliskan sebanyak 5.65 kali dengan hasilan 21.7%. Lipase rekombinan tulen bergerak sebagai satu jalur dengan jisim molekular ~43 KDa pada SDS-PAGE. Lipase tulen menunjukkan aktiviti tertinggi pada suhu 70°C dengan pH optimum 8.0. Enzim adalah stabil pada julat pH dari 6.0 ke 10.0. Ia juga menunjukkan kestabilan tertinggi dengan tempoh separuh hayat 315 min pada 60°C, 120 min pada 65°C dan 45 min pada 70°C.

Lipase tulen menghidrolisiskan kesemua minyak semulajadi yang diuji dengan kadar hidrolisis tertinggi terhadap minyak zaitun. Spesifisiti substrat tertinggi adalah terhadap trikaprilin (C<sub>8</sub>) diikuti oleh trikaprin (C<sub>10</sub>). Aktiviti lipase meningkat dengan penambahan ion logam seperti Ca<sup>+</sup> dan Na<sup>+</sup>. Tween 80 pada kepekatan 0.1% meningkatkan aktiviti enzim tetapi aktiviti menurun pada 1%. Bestatin dan pepstatin juga meningkatkan sedikit aktiviti enzim tetapi EDTA tidak meningkatkan sebarang kesan. Sebaliknya aktiviti enzim direncatkan oleh fenilmetilsulfonilfluorida iaitu perencat protease serine.



## ACKNOWLEDGEMENTS

All praise is to the Almighty Allah, the Merciful and the Beneficent. Had it not been due to his will and favour, the completion of this study would not have possible.

I avail myself of this opportunity to record my sincerest thanks and appreciation to Associate Professor Dr. Raja Noor Zaliha Raja Abd Rahman, chairman of my supervisory committee, for her dedicated efforts, support, invaluable advice, intellectual guidance and encouragement in the conduct of my research and in the preparation of this thesis.

Grateful thanks are also due to my supervisory committee members, Professor Dr. Abu Bakar Salleh and Professor Dr. Mahiran Basri, for their constructive comment, advice and help throughout my studies and in the preparation of this final manuscript.

I am exceedingly grateful to the Libyan Higher Learning Popular Committee for their financial support. Thanks are also extended to all staff member in the department of Biotechnology and Biomolecular Sciences, lab mates and friends who have helped me in one way or another.

Finally, I also take this opportunity to express my deep gratitude to my affectionate Mother, brothers, sisters, helpful wife Hawa Safar, dear sons



Abdallah, Abdalrahman, Abdalraouf and sweet daughter Halima. I thank them for all their love, patience, support and encouragement throughout my study in UPM and whole life. Without their understanding and sacrifices this project would have been nigh impossible.



I certify that an Examination Committee met on 11<sup>th</sup> October 2005 to conduct the final examination of Mohamed Abdallah Eltaweel on his Doctor of Philosophy thesis entitled "Production, Expression and Characterization of a Heat-Stable Organic Solvent Tolerant Lipase from *Bacillus* sp. Strain 42" in accordance with Universiti Pertanian Malaysia (Higher Degree) Act 1980 and Universiti Pertanian Malaysia (Higher Degree) Regulations 1981. The Committee recommends that the candidate be awarded the relevant degree. Members of the Examination Committee are as follows:

**Norhani Abdullah, PhD**

Professor

Faculty of Biotechnology and Biomolecular Sciences

Universiti Putra Malaysia

(Chairman)

**Raha Abdul Rahim, PhD**

Associate Professor

Faculty of Biotechnology and Biomolecular Sciences

Universiti Putra Malaysia

(Internal Examiner)

**Johari Ramli, PhD**

Associate Professor

Faculty of Biotechnology and Biomolecular Sciences

Universiti Putra Malaysia

(Internal Examiner)

**Zainul Fadziruddin Zainuddin, PhD**

Professor

School of Health Sciences

Universiti Sains Malaysia

(External Examiner)



---

**GULAM RUSUL RAHMAT ALI, PhD**

Professor/Deputy Dean

School of Graduate Studies

Universiti Putra Malaysia

Date: 22 NOV 2005

This thesis submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfilment of the requirements for the degree of Doctor of Philosophy. The members of the Supervisory Committee are as follow:

**Raja Noor Zaliha Raja Abd Rahman, PhD**


Associate Professor  
Faculty of Biotechnology and Biomolecular Sciences  
Universiti Putra Malaysia  
(Chairman)

**Abu Bakar Salleh, PhD**

Professor  
Faculty of Biotechnology and Biomolecular Sciences  
Universiti Putra Malaysia  
(Member)

**Mahiran Basri, PhD**

Professor  
Faculty of Science  
Universiti Putra Malaysia  
(Member)



---

**AINI IDERIS, PhD**  
Professor/Dean  
School of Graduate Studies  
Universiti Putra Malaysia

Date: 08 DEC 2005



## DECLARATION

I hereby declare that the thesis is based on my original work except for quotations and citations which have been duly acknowledged. I also declare that it has not been previously or concurrently submitted for any other degree at UPM or other institutions.



---

**MOHAMED ABDALLAH ELTAWHEEL**

Date: 13.11.2005

## TABLE OF CONTENTS

	<b>Page</b>
DEDICATION	II
ABSTRACT	III
ABSTRAK	VI
ACKNOWLEDGEMENTS	IX
APPROVAL	XII
DECLARATION	XIII
LIST OF TABBLES	XVI
LIST OF FIGURS	XVII
LIST OF ABBREVIATIONS	XXI
<b>CHAPTER</b>	
1 INTRODUCTION	1
Objective of this study	7
2 LITERATURE REVIEW	8
2.1 Lipases	8
2.2 Sources of Lipases	10
2.2.1 Plant Lipases	10
2.2.2 Mammalian Lipases	11
2.2.3 Microbial Lipases	11
2.3 Thermostable Lipase	13
2.4 Organic Solvent Tolerant Lipase	16
2.5 Enzyme Catalysis in Organic Solvents	19
2.6 Selection of Organic Solvents	21
2.7 Detection and Screening of Lipolytic Microorganisms	24
2.8 Production of Microbial Lipases	27
2.9 Effect of Nutritional Factors on Lipase Production	28
2.9.1 Carbon Sources	28
2.9.2 Nitrogen Sources	30
2.9.3 Substrates	32
2.9.4 Minerals	33
2.10 Effect of Physical Factors on Lipase Production	34
2.10.1 Temperature	34
2.10.2 pH	35
2.10.3 Cultivation Period	35



2.10.4	Shaking Rate	36
2.11	Purification of Lipase	37
2.12	Properties of Purified Microbial Lipases	39
2.13	Substrate Specificity	41
2.13.1	Positional Specificity	41
2.13.2	Fatty Acid Specificity	42
2.13.3	Partial Glycerides Specificity	44
2.14	Polymerase Chain Reaction	44
2.15	Primer Design	45
2.16	Optimization of PCR	48
2.17	Selection of Vector	49
2.18	Selection of Host	51
2.19	Cloning and Expression	53
2.20	Cloning by PCR	54
2.21	Direct Selection Strategy of Lipase Gene	57
2.22	Expression of Lipase Gene	58
2.23	Application of Lipases	61
<b>3</b>	<b>MATERIALS AND METHODS</b>	<b>64</b>
3.1	Materials	64
3.2	Methods	71
3.2.1	Preparation of Media and Solutions	71
3.2.2	Bacterial Sources	80
3.2.3	Enrichment Culture Technique	80
3.2.4	Isolation of Bacteria	80
3.2.5	Screening of Lipase Producing Microorganisms	81
3.2.6	Slant Agar Stock Culture	81
3.2.7	Glycerol Stock Culture	82
3.2.8	Preparation of Inoculum	82
3.2.9	Assay of Lipase Activity	82
3.2.10	Effect of Different Liquid Media on Lipase Production	83
3.2.11	Effect of Temperature on Crude Enzyme Stability	84
3.2.12	Effect of Organic Solvents on Crude Enzyme Stability	84
3.2.13	Bacterial Identification	86
3.2.14	Growth Curve and Lipase Production of <i>Bacillus</i> sp. Isolate 42	88
3.2.15	Growth Optimization Study for Maximum Enzyme Production	88
3.2.16	Physical Factors Affecting the Growth and Lipase Production by <i>Bacillus</i> sp. Isolate 42	89
3.2.17	Nutritional Factors Affecting the Growth and Lipase Production by <i>Bacillus</i> sp. Isolate 42.	91
3.2.18	Cloning and Sequencing of Thermostable Organic	





	Solvent Tolerant Lipase Gene	95
3.2.19	Amplification of Thermostable Organic Solvent Tolerant Lipase Gene by PCR	97
3.2.20	PCR Amplification of the Lipase Gene from Genomic DNA	97
3.2.21	Purification of the Amplified PCR Product	99
3.2.22	Preparation of Competent <i>E. coli</i>	100
3.2.23	Cloning PCR Product using pQE-30 UA Vector	100
3.2.24	Transformation	101
3.2.25	Plasmid Isolation	101
3.2.26	Analysis of Positive Colonies	102
3.2.27	Stock Culture	103
3.2.28	Expression of Recombinant Thermostable Organic Solvent Tolerant Lipase Gene	103
3.2.29	Preparation of Culture Supernatant and Cell Extract	104
3.2.30	SDS-PAGE Analysis of Bacteria Protein	105
3.2.31	Optimum IPTG Concentration for Expression	106
3.2.32	Assay of Recombinant Lipase Activity	107
3.2.33	Purification of Thermostable Organic Solvent Tolerant Recombinant Lipase	107
3.2.34	Protein Determination	109
3.2.35	Characterization of Purified Thermostable Organic Solvent Tolerant Recombinant Lipase	109
<b>4</b>	<b>RESULTS AND DISCUSSION</b>	<b>117</b>
4.1	Isolation and Screening of Thermophilic Lipolytic Bacteria	117
4.2	Effects of Different Liquid Media on Lipase Production	118
4.3	Effect of Temperature on Crude Enzyme Stability	125
4.4	Effect of Organic Solvents on the Crude Lipase Activity	126
4.5	Bacterial Identification	130
4.5.1	16S rDNA Identification and Phylogenetic Tree Analysis	130
4.6	Growth Curve and Lipase Production by <i>Bacillus</i> sp. Isolate 42	138
4.7	Effects of Physical Factors on Growth and Lipase Production	141
4.7.1	Temperature	141
4.7.2	Agitation	143
4.7.3	pH	146
4.7.4	Inoculum Size	148
4.7.5	Medium Volume	150
4.8	Effect of Nutritional Factors on growth and Lipase Production	151
4.8.1	Carbon Sources	151
4.8.2	Inorganic Nitrogen Sources	156
4.8.3	Organic Nitrogen Sources	156



4.8.4	Formulation of Nitrogen Source for Optimal Production	160
4.8.5	Metal Ions	164
4.8.6	Substrates	166
4.8.7	Tweens	169
4.9	Cloning, Sequencing and Expression of the Organic Solvent Tolerant Lipase gene	171
4.9.1	Genomic DNA Extraction	171
4.10	Amplification of Thermostable Organic Solvent Tolerant Lipase Gene by Polymerase Chain Reaction (PCR)	173
4.11	Gene Analysis	180
4.11.1	Nucleotide Sequence and Deduced Amino Acid Sequence of <i>Bacillus</i> sp. Isolate 42	180
4.12	Cloning of Organic Solvent Tolerant Lipase Gene using pQE-30 UA vector	186
4.13	Analysis of Positive Colonies	188
4.14	Expression of Thermophillic Organic Solvent Tolerant Lipase Gene	195
4.16	SDS-PAGE Analysis of Bacterial Proteins	197
4.15	Optimum IPTG Concentration	197
4.17	Purification of Thermostable Organic Solvent Tolerant Lipase	200
4.17.1	Affinity Chromatography	200
4.18	Characterization of Purified Organic Solvent Tolerant Lipase	203
4.18.1	Determination of Molecular Weight	203
4.18.2	Effect of pH on Lipase Activity	205
4.18.3	Effect of pH on Lipase Stability	208
4.18.4	Effect of Temperature on Lipase Activity	210
4.18.5	Effect of Temperature on Lipase Stability	212
4.18.6	Effect of Metal Ions on Lipase Activity	214
4.18.7	Effect of Surfactants	217
4.18.8	Effect of Inhibitors	219
4.18.9	Substrate Specificity	222
4.18.10	Specificity of Purified Lipase Towards Natural Oils Activity	224
4.18.11	Effect of Organic Solvents on Lipase Activity	226
4.18.12	Positional Specificity	230
5	CONCLUSION	233
	REFERENCES	236
	APPENDICES	258
	BIODATA OF THE AUTHOR	266



## LIST OF TABLES

Table	Page
1 The log <i>P</i> Value of Common Solvents	23
2 Properties of Purified Lipases from some Bacterial Sources	40
3 Preparation of Free Fatty Acid Solutions	75
4 Bovine Serum Albumin Solutions	78
5 Thermophilic Lipase Producer from <i>Bacillus</i> sp Precursors Nucleotide Sequences Extracted from the NCBI Database.	98
6 Oligonucleotide Used as Primers for Specific Amplification of Genes Encoding for Organic Tolerant Lipase Gene Fragments	98
7 Screening the Activity of Lipolytic Thermophilic Bacteria Isolated from Different Local Regions in Malaysia	119
8 Effect of Different Liquid Media on the Isolates Lipase Production	120
9 Effect of Different Organic Solvents on Strain 42 Crude Lipase Activity	129
10 Summary of the Purification Recombinant 6 x His-tagged Lipase Produced with <i>E. coli</i> M15	202
11 Effect of Organic Solvents on Lipase Stability	227



## LIST OF FIGURES

Figure		Page
1	Hydrolytic and Synthetic Action of Lipase	9
2	Schematic Diagram of the PCR Process	46
3	Standard Curve for the Determination of Free Fatty Acid	76
4	Standard Curve for Protein Determination	79
5	Flow Chart for Lipase Assay Producer	85
6	<i>Bacillus</i> sp. Isolate 42 Streaked on Tributryin Plate with Control	122
7	<i>Bacillus</i> sp. Isolate 42 Streaked on Triolein Plate with Control	123
8	<i>Bacillus</i> sp. Isolate 42 Streaked on Rhodamine Plate with Control	124
9	Relative Stability of the Crude Lipase from Isolate 42	125
10	PCR Product of 16S rDNA Gene (1500 bp) from Isolate 42 Amplified using Universal Primers	131
11	16S rDNA Sequence of <i>Bacillus</i> sp. Strain 42	132
12	Comparison of 16S rDNA from <i>Bacillus</i> sp. BGSC W9A6 (AY 608903), <i>Bacillus</i> sp. BGSC W9A22 (AY 608987) and <i>Bacillus</i> sp Strain 42 (AY 763118).	136
13	Rooted Phylogenetic Tree Showing the Relationships of <i>Bacillus</i> sp. Strain 42 to other <i>Bacillus</i> spp.	137
14	Time Profile of Lipase Production by <i>Bacillus</i> sp. Strain 42	140
15	Effect of Temperature on Growth and Lipase	142
16	Effect of Agitation on Bacterial population and Lipase production	145
17	Effect of pH on Bacterial Growth and Lipase Production	147
18	Effect of Inoculum Size on Bacterial Growth and Lipase	



	Production	149
19	Effect of Medium Volume on Bacterial Growth and Lipase Production	152
20	Effects of Carbon Sources on the Growth and Lipase Production by <i>Bacillus</i> sp. Strain 42	153
21	Effect of Inorganic Nitrogen Sources on Growth and Lipase Production by <i>Bacillus</i> sp. Isolate 42.	157
22	Effect of Organic Nitrogen Sources on Growth and Lipase Production by <i>Bacillus</i> sp. Strain 42.	159
23	Effects of Tryptone with some Other Nitrogen Sources on Bacterial Growth and Lipase Production by <i>Bacillus</i> sp. Strain	162
24	Effects of Different Concentrations of Tryptone and Yeast extract on Growth and Lipase Production by <i>Bacillus</i> sp. Isolate 42.	163
25	Effect of Metal Ions ( $\text{Ca}^{2+}$ , $\text{Mg}^{2+}$ and $\text{Fe}^{3+}$ , Individually and in Combination, on the Growth and Lipase Production by <i>Bacillus</i> sp. Strain 42.	165
26	Effect of Heavy Metal Ions on the Growth and Lipase Production by <i>Bacillus</i> sp. Isolate 42.	167
27	Effects of Substrates on the Growth and Lipase Production by <i>Bacillus</i> sp. Strain 42.	168
28	Effect of Tween on the Growth and Lipase Production by <i>Bacillus</i> sp. Isolate 42	170
29	Genomic DNA Extraction from Strain 42	173
30	PCR Product of 308 bp Amplified by Using Hlip F1 and Hlip R1 Primers	175
31	PCR Product of 812 bp Amplified by Using Hlip F2 and Hlip R2 Primers	176
32	PCR Product of 771 bp Amplified by Using lip F12 and Hlip R 2 Primers	177
33	PCR Product of 790 bp Amplified by Using Hlip F2 and lip R8 Primers	178

34	PCR Product of 1251 bp Amplified by Using lip F12 and lip R12 Primers	179
35	Nucleotide and Deduced Amino Acid Sequences of Thermostable	182
36	The Putative Signal Peptide Cleavage Site	183
37	Comparison of the Amino Acids Sequence of Lipase Gene from <i>Bacillus</i> sp. Strain 42 with other Thermophilic Lipases Sequence Obtained from GenBank.	185
38	pQE-30 UA Vector	187
39	Clearing Zone of <i>E. coli</i> M 15 Harboring Thermostable Organic Solvent Tolerant Lipase Gene on Tributyrin Plate	189
40	Experimental Procedure of Cloning the Thermostable Lipase Gene of <i>Bacillus</i> sp. Strain 42	190
41	Cloning and Sequence Strategy of the Cloned Fragments by pQE-30 UA vector	191
42	Intensive Zone of <i>E. coli</i> M 15 Harboring Thermostable Organic Solvent Tolerant Lipase Gene on Triolein Plate	192
43	Orange Fluorescence Halo of <i>E. coli</i> M 15 Harboring Thermostable Organic Solvent Tolerant Lipase Gene on Rhodamine Plate	193
44	Double Digestion of Recombinant pQE-30 UA Vector	194
45	Expression of Thermostable Organic Solvent Tolerant Lipase by pQE-30 UA Vector	196
46	SDS-PAGE of Bacterial Proteins Analysis	198
47	Effect of different IPTG Concentration at 3h Induction in <i>E. coli</i> Harboring <i>Bacillus</i> sp. Strain 42 Lipase Gene	199
48	Immobilization Metal Affinity Chromatography of Recombinant 6 x His-tagged Lipase	201
49	SDS-PAGE of the Purified sp. 42 Lipase	204
50	Estimation of the Molecular Weight of the Purified Lipase by Gel	



	Filtration Chromatography.	206
51	Effect of pH on Purified Lipase Activity	207
52	Effect of pH on Purified 42 Lipase Stability	209
53	Effect of Temperature on Purified Lipase Activity	211
54	Effect of Temperature on Purified Lipase Stability	213
55	Effect of Metal Ions on Purified Lipase Activity	215
56	Effect of Surfactants on Purified Lipase Activity	218
57	Effect of Inhibitors on Purified Lipase Activity	220
58	Chain Length Specificity of Purified Lipase	223
59	Specificity of the Purified Lipase Towards Natural Oils.	225
60	Thin-layer Chromatography of the Hydrolysis Products Obtained with Ttriolein as Substrate	231



## LIST OF ABBREVIATIONS

POME	Palm Oil Mill Effluent
cm	Centimeter
dH <sub>2</sub> O	Distilled Water
DNA	Deoxyribonucleic Acid
dNTPs	Deoxynucleotide Triphosphates
EDTA	Ethylenediaminetetraacetic Acid
mM	Millimole
g/L	Gram per Liter
g	Gram
ng	Nanogram
Mg	Milligram
µg	Microgram
pmol	Picomole
L	Liter
M	Molar
mL	Milliliter
µl	Microliter
%	Percentage
µmole	Micromole
rpm	Rotation per Minute
x g	Gravity
UV	Ultraviolet
IPTG	Isopropyl-β-D- Thiogalactopyranoside
X-gal	5-bromo-4chloro-3-indolyl-β-D galactopyranoside
h	Hour
Min	Minute
PCR	Polymerase Chain Reaction
ORF	Open Reading Frame
GTE	Glucose Tris-HCl-EDTA
PCI	Phenol Chloroform Isoamylalcohol
SDS	Sodium Dodecyl Sulphate
SDS-PAGE	Sodium Dodecyl Sulphate Polyacrylamide Gel Electrophoresis
APS	Amonium Persulphate
TCA	Trichloroacetic Acid
TSB	Tryptone Soy Broth
LB	Luria-Bertani
BSA	Bovine Serum Albumin
U/mL	Unit per Milliliter
cfu	Colony Form Units
v/v	Volume per Volume
w/v	Weight per Volume





psi	Pound Persquare Inch
bp	Base Pair
Ni-NTA	Nickle-nitrilotriacetate acid
Kbp	Kilo Base Pair
Da	Dalton
KDa	Kilo Dalton
mA	Milliampere
sp	Specie
spp	Species
NCBI	National Center for Biotechnology Information

