

SCREENING FOR LIPASE ACTIVITY FROM MOORSE
BACTERIA ISOLATED FROM CORAL MUCUS

NATWA MUSTAFAR

FAKULTASAINS DAN TEKNOLOGI
UNIVERSITIMALAMALAKA TERENGGANU
2007

**SCREENING FOR LIPASE ACTIVITY FROM MD030 BACTERIA ISLATED
FROM CORAL MUCUS**

By

Najwa Mustafar

Research Report submitted in partial fulfillment of
the requirements for the degree of
Bachelor of Science (Biological Sciences)

Department of Biological Sciences
Faculty of Science and Technology
UNIVERSITI MALAYSIA TERENGGANU
2007

1100051146

This project should be cited as:

Najwa, M. 2007. Screening for Lipase Activity from MD030 Bacteria Isolated from Coral Mucus. Undergraduate thesis, Bachelor of Science (Biological Sciences), Faculty of Science and Technology, Universiti Malaysia Terengganu. 51p.

No part of this project report may be produced by any mechanical, photographic, or electronic process, or in form of phonographic recording, nor may it be stored in a retrieval system, transmitted, or otherwise copied for public or private use, without written permission from the author and the supervisor (s) of the project.



**JABATAN SAINS BIOLOGI
FAKULTI SAINS DAN TEKNOLOGI
UNIVERSITI MALAYSIA TERENGGANU**

**PENGAKUAN DAN PENGESAHAN LAPORAN
PROJEK PENYELIDIKAN I DAN II
RESEARCH REPORT VERIFICATION**

Adalah ini diakui dan disahkan bahawa laporan penyelidikan bertajuk: Screening for Lipase Activity from MD030 Bacteria Isolated from Coral Mucus oleh Najwa Bt Mustafar no. matrik: UK 10502 telah diperiksa dan semua pembetulan yang disarankan telah dilakukan. Laporan ini dikemukakan kepada Jabatan Sains Biologi sebagai memenuhi sebahagian daripada keperluan memperolehi Ijazah Sarjana Muda Sains (Sains Biologi), Fakulti Sains dan Teknologi, Universiti Malaysia Terengganu.

Disahkan oleh: / Verified by:

Penyelia Utama / Main Supervisor

Nama: **DR. MARIAM TAIB**

Cop Rasmi: Pensyarah
Jabatan Sains Biologi
Fakulti Sains dan Teknologi
Universiti Malaysia Terengganu
21030 Kuala Terengganu.

Tarikh: 9/5/07

Penyelia Kedua (jika ada) / Co-Supervisor (if applicable)

Nama:

Cop Rasmi

DR. AZIZ AHMAD
Pensyarah
Jabatan Sains Biologi
Fakulti Sains dan Teknologi
Universiti Malaysia Terengganu
21030 Kuala Terengganu.

Tarikh:

Ketua Jabatan Sains Biologi / Head, Department of Biological Sciences

Nama:

Cop Rasmi

DR. AZIZ BIN AHMAD
Ketua
Jabatan Sains Biologi
Fakulti Sains dan Teknologi
Universiti Malaysia Terengganu
21030 Kuala Terengganu

Tarikh: 10/5/2007

ACKNOWLEDGEMENTS

I am grateful to Allah s.w.t, whom with His blessing, I had the strength to complete all my work on time. I would like to express my sincere appreciation to my supervisor, Dr Mariam Taib for her guidance, advice, encouragement and understanding. Without her cooperation, patience and full support, this thesis will not survive. A special thank for my co-supervisor, Dr Aziz Ahmad for his advice and opinions in helping me to finish my research.

I also would like to take this opportunity to thank the science officers, Miss Norazlina Abdul Aziz and Puan Ku Naiza Ku Nordin for their constant help in using the labs especially Microbiology Lab and Biochemistry Lab. I also would like to thank the lab assistances, Kak Ina and Kak Ti for their kindness, guidance and their valuable time in helping me to complete my laboratory works.

Special appreciations go to my friends, especially Ana, Azza, Yan, Ita, Ayus and Ucop, who were always willing to give their full support during the process of completing this study. Thanks for your encouragement, caring, understanding, teamwork and patience. And also thanks to all Biological Sciences final year students for everything.

Last but not least, my warmest gratitude also goes to my family. Without their encouragement, criticism, understanding and support, I would not finish this thesis on time.

TABLE OF CONTENTS

	Page
ACKNOWLEDGEMENTS	ii
LIST OF TABLES	vi
LIST OF FIGURES	vii
LIST OF APPENDICES	viii
LIST OF ABBREVIATIONS	ix
ABSTRACT	x
ABSTRAK	xi
CHAPTER 1 INTRODUCTION	1
1.1 Introduction	1
1.2 Objectives	3
CHAPTER 2 LITERATURE REVIEW	4
2.1 Microbial Enzymes	4
2.2 Lipases	6
2.3 Sources of Lipases	8
2.4 Applications of Lipases	10
2.4.1 Uses in detergent industries	11
2.4.2 Uses in food industries	11
2.4.3 Uses in pharmaceuticals industries	11
2.5 Marine Organisms	12
2.5.1 Marine sponges	12
2.5.2 Corals	13
2.5.3 Marine microbes as lipase producers	14

CHAPTER 3	METHODOLOGY	15
3.1	Preparation of Fresh Stock Culture of Pure Isolate	15
3.2	Confirmation of the MD030 Bacteria Identification	15
3.2.1	Morphological test	15
3.2.2	Lipid hydrolysis	16
3.2.3	Oxidase test	16
3.2.4	Catalase test	16
3.2.5	Triple Sugar Ion test (TSI)	16
3.2.6	Oxidation- Fermentation test	17
3.2.7	Citrate utilization test	17
3.3	Induction of Lipase by MD030 Bacteria	17
3.3.1	Determination of best culture medium	17
3.3.2	Preparation of preinoculum	17
3.3.3	Protein assay	18
3.3.4	Lipase assay of crude enzyme	18
3.3.5	Induction of lipase in media containing olive oil as substrate	18
3.3.6	Calculation of free fatty acids	19
3.3.7	Statistical Analysis	19
CHAPTER 4	RESULTS	20
4.1	Confirmation of MD030 Bacterial Identification	20
4.2	Determination of the Best Medium for Bacterial Growth	20
4.3	Preparation of Preinoculum	22
4.4	Protein Assay	22
4.5	Lipase Assay: Effect of Amount of Enzyme	22
4.6	Induction of Lipase Activity by MD030	25
4.6.1	Effect of incubation time	25
4.6.2	Effect of temperature	25
4.6.3	Effect of substrate	25
CHAPTER 5	DISCUSSION	29
CHAPTER 6	CONCLUSION AND RECOMMENDATIONS	33

REFERENCES	34
APPENDICES	39
CURRICULUM VITAE	45

LIST OF TABLES

Table	Page
Table 3.1 ZoBell's modified medium; Sucrose Sea Water (SSW)	15
Table 3.2 Different parameters investigated for induction	19
Table 4.1 Morphological and biochemical characterizations of MD030 bacteria	20

LIST OF FIGURES

Figure		Page
Figure 2.1	Lipase-catalysed reactions	7
Figure 4.1	Determination of the best medium for growth of MD030 bacteria.	21
Figure 4.2	Preparation of pre-inoculum of MD030 bacteria in Nutrient Broth in Sea Water.	23
Figure 4.3	Effect of amount of enzyme in lipase assay by MD030	24
Figure 4.4	Effect of incubation time on the activity of MD030 crude lipase (150µg/ml).	26
Figure 4.5	Effect of incubation temperature on the activity of MD030 of crude lipase (150µg/ml).	27
Figure 4.6	Effect of amount of substrates on the activity of MD030 crude lipase (150µg/ml)	28

LIST OF APPENDICES

Appendix		Page
Appendix 1	Determination of the Best Medium for Bacterial Growth	40
Appendix 2	Lipase Assay: Effect of Amount of Enzymes	40
Appendix 3	Effect of incubation times	40
Appendix 4	Effect of incubation temperatures	40
Appendix 5	Effect of substrates	40
Appendix 6	Standard curve of Bovine Serum Albumin (BSA) for Bradford method.	41
Appendix 7	ANOVA (Determination of best media for bacterial growth)	41
Appendix 8	ANOVA (Effect of amount of enzymes)	41
Appendix 9	ANOVA (Effect of incubation times)	42
Appendix 10	ANOVA (Effect of incubation temperature)	43
Appendix 11	ANOVA (Effect of substrates)	43

LIST OF ABBREVIATIONS

°C - celcius

g - gram

μg - microgram

ml - milliliter

M - molar

rpm - rotation per minute

NaOH - natrium hydroxide

sp - species

ABSTRACT

There are very few environmental studies to date, focusing on lipase activities in marine microbes such as bacteria. In this research, the ability to produce lipase by marine bacteria MD030, isolated from coral mucus was investigated. The identification of the MD030 bacteria was confirmed by several biochemical tests based on their morphological and phenotypic characteristics. The results suggested that the bacteria are *Moraxella* sp. Prior to induction of lipase by the bacteria, three different media have been tested to determine the best culture medium: Nutrient Broth in Distilled Water, Nutrient Broth in Sea Water and ZoBell's modified medium. In this study, Nutrient Broth in Sea Water medium was chosen as the medium for lipase induction. The protein content of the crude enzyme was 150 μ g/ml. The induction of lipase with olive oil as substrate was initiated with the preparation of pre-inoculum. Several reaction parameters influencing lipase activity such as incubation time, temperature and amount of substrate have been determined. There was no significant difference ($p>0.05$) between all incubation times tested. Free fatty acids released (ml) at 27°C was significantly higher ($p<0.05$) compared to 15 and 37°C. No significant difference ($p>0.05$) was observed for 2 and 3 %, but there was significant difference ($p<0.05$) observed between 1 and 3 %. Therefore, the optimum conditions chosen for lipase activity were 18 hours of incubation time, at 27°C with 2-3% of olive oil as substrate, in a lipase assay using 300 μ g crude enzyme. The results indicate that MD030 bacteria is capable in producing lipase.

PENYARINGAN AKTIVITI LIPASE DARIPADA BAKTERIA MD030 YANG DIPENCILKAN DARI MUKUS BATU KARANG

ABSTRAK

Setakat ini hanya terdapat beberapa kajian persekitaran mengenai aktiviti lipase daripada mikrob marin seperti bakteria. Dalam kajian ini, keupayaan bakteria marin MD030 untuk menghasilkan lipase yang dipencilkan dari batu karang telah dikaji. Dalam melakukan pengesahan identiti bakteria MD030, beberapa ujian biokimia telah dilakukan berdasarkan morfologi dan ciri- ciri fenotopik bakteria tersebut. Keputusan yang diperolehi mencadangkan bakteria tersebut adalah *Moraxella* sp. Sebelum mengaruh penghasilan lipase daripada bakteria, tiga media yang berbeza telah diuji untuk menentukan medium kultur yang paling baik; 'Nutrient Broth' dalam air suling, 'Nutrient Broth' dalam air laut dan medium ZoBell yang diubahsuai. Dalam kajian ini, medium 'Nutrient Broth' dalam air laut telah dipilih sebagai media untuk penghasilan lipase. Kandungan protein oleh enzim yang ditentukan ialah 150 μ g/ml. Lipase diaruh menggunakan minyak zaitun sebagai substrat dan dimulakan dengan penyediaan pra-inokulum. Beberapa parameter tindakbalas terhadap aktiviti lipase seperti masa pengeraman, suhu dan jumlah substrat telah dilakukan. Tiada perbezaan signifikan ($p>0.05$) antara kesemua masa pengeraman yang diuji. Asid lemak bebas yang dibebaskan (ml) pada 27°C adalah signifikan yang tinggi ($p<0.05$) dibandingkan dengan 15 dan 37°C. Tiada perbezaan signifikan ($p>0.05$) telah diperhatikan untuk 2 dan 3%, tetapi terdapat perbezaan signifikan ($p<0.05$) diperhatikan antara 1 dan 3%. Oleh itu, keadaan optimum yang dipilih untuk aktiviti lipase adalah pada 18 jam masa pengeraman pada suhu 27°C menggunakan 2-3 % minyak zaitun sebagai substrat, di dalam asai lipase yang menggunakan 300 μ g enzim kasar. Keputusan menunjukkan bakteria MD030 berkebolehan menghasilkan lipase.