

SCREENING FOR LIPASE ACTIVITY FROM MD 2292  
BACTERIA ISOLATED FROM GORAL MURUS

NORALITA BINTI CHE ISA

FAKULTI SAINS DAN TEKNOLOGI  
UNIVERSITI MALAYSIA TERENGGANU  
2007



SCREENING FOR LIPASE ACTIVITY FROM MD 029a BACTERIA ISOLATED  
FROM CORAL MUCUS

By

Noralita Binti Che Isa

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

1100051149

This project report should be cited as:

Noralita, C.I. 2007. Screening for Lipase Activity from MD 029a Bacteria Isolated from Coral Mucus. Undergraduate thesis, Bachelor of Science (Biological Sciences), Faculty of Science and Technology, Universiti Malaysia Terengganu, Terengganu.

No part of this project may be produced by any mechanical, photographic, or electronic process, or in the 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 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 MD 029a BACTERIA ISOLATED FROM CORAL MUCUS** oleh **NORALITA BINTI CHE ISA**, no. matrik: **UK 10835** telah diperiksa dan semua pembetulan yang disarankan telah dilakukan. Laporan ini dikemukakan kepada Jabatan Sains Biologi sebagai memenuhi sebahagian daripada keperluan memperoleh Ijazah **SARJANA MUDA SAINS (SAINS BIOLOGI)**, Fakulti Sains dan Teknologi, Universiti Malaysia Terengganu.

Disahkan oleh: / Verified by:

Penyelia Utama / Main Supervisor  
**DR. MARIAN TAIB**  
Nama: **Pensyarah**  
**Jabatan Sains Biologi**  
Cop Rasmi: **Fakulti Sains dan Teknologi**  
**Universiti Malaysia Terengganu**  
**21030 Kuala Terengganu.**

Tarikh: 9/5/07

Penyelia Kedua (jika ada) / Co-Supervisor

Nama: **DR. AZIZ AHMAD**  
Cop Rasmi: **Pensyarah**  
**Jabatan Sains Biologi**  
**Fakulti Sains dan Teknologi**  
**Universiti Malaysia Terengganu**  
**21030 Kuala Terengganu.**

Tarikh: 8/5/2007

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: 8/5/2007

## ACKNOWLEDGEMENTS

Bismillahirrahmanirrahim. At last I can finally 'breathe' after spending long hours doing this final year project.

First of all, the biggest thank is to Allah The Almighty for giving me patience and strength. I also would like to express my deepest gratitude to my supervisor, Dr. Mariam Taib for being a good mentor, kind supervision and most importantly her timeless patience in helping me completing this study, without her this project will never be as it is. My deep appreciation also goes to Dr. Aziz Ahmad as my co-supervisor and also to my FYP coordinators, Dr. Noraznawati Ismail and Pn. Wahizatul Afzan for their priceless advices, kind guidance throughout the time of this project.

Sincere gratitude also goes out to the microbiological and biochemical lab crews, Kak Ina, Kak Tie, Pn. Fatimah and Pn. Ku for their patient assistance. My deepest gratitude also goes to Miss Norazlina Abdul Aziz as my science officers for her advices and guidance. To Kak Nor, thanks for your teaching and advices especially when it comes to identifying this marine bacteria.

A big thank goes to my dearest caring pals; the lipase group; Ayus, Najwa, Aza, Ana, Yan and Ucop who were always willing to give their helping hands in the lab and share the joy together. Not forgetting all my course mates, who also helped me a lot with my thesis, thanks for being my unforgettable-friends.

For someone who will be my special one, thanks for choosing me as your beloved one. Last but not least, for my family especially Ma and Abah, Fatimah Omar and Che Isa Muhamad and my beloved brothers and sisters, thanks a lot for all the guidance, support and the entire love you give. If, by any chances, any error in any part of my thesis, I offer my sincere request for forgiveness in advance.

## TABLE OF CONTENTS

	<b>Page</b>
<b>ACKNOWLEDGEMENTS</b>	<b>ii</b>
<b>LIST OF TABLES</b>	<b>v</b>
<b>LIST OF FIGURES</b>	<b>vi</b>
<b>LIST OF ABBREVIATIONS</b>	<b>vii</b>
<b>LIST OF APPENDICES</b>	<b>viii</b>
<b>ABSTRACT</b>	<b>ix</b>
<b>ABSTRAK</b>	<b>x</b>
<b>CHAPTER 1 INTRODUCTION</b>	<b>1</b>
1.1 Introduction	1
1.2 Objectives	2
<b>CHAPTER 2 LITERATURE REVIEW</b>	<b>3</b>
2.1 Lipases	3
2.2 Sources of Lipases	6
2.3 Applications of Lipases	7
2.3.1 Lipases in the detergent industry	8
2.3.2 Lipases in food industry	9
2.3.3 Lipases in pulp and paper industry	10
2.3.4 Lipases in resolution of racemic acids and alcohols	10
2.3.5 Lipases in oleochemical industries	11
2.3.6 Lipases in cosmetics	11
2.3.7 Lipases in medical applications	12
2.3.8 Lipases in oil biodegradation	13
2.4 Bacterial Lipases	14
2.4.1 Marine bacteria as lipase producers	14
2.5 Factors Affecting the Production and Activity of Lipases	16

<b>CHAPTER 3 METHODOLOGY</b>	<b>19</b>
3.1 Preparation of the Fresh Colonies of Pure Isolate	19
3.2 Confirmation of Identification of MD 029a Bacteria	19
3.2.1 Morphological characteristics	20
3.2.2 Basic phenotypic characteristics	20
3.3 Preparation for Induction of Lipases	24
3.3.1 Determination of the best culture medium for induction of lipases	24
3.3.2 Preparation of pre-inoculum	25
3.4 Induction of Lipases by MD 029a Bacteria	25
3.4.1 Determination of amount of protein	25
3.4.2 Lipase assay of crude enzyme	25
3.4.3 Determination of optimum condition for lipase induction by MD029a bacteria	26
3.4.4 Calculation of fatty acids released by hydrolysis	27
3.4.5 Statistical analysis	27
<b>CHAPTER 4 RESULTS</b>	<b>28</b>
4.1 Confirmation of Identification of MD 029a Bacteria	28
4.1.1 Morphological and basic phenotypic characteristics	28
4.2 Preparation for Induction of Lipases	33
4.2.1 Determination of the best culture medium for induction of lipases	33
4.2.2 Preparation of pre-inoculum	33
4.3 Induction of Lipases by MD 029a Bacteria	35
4.3.1 Determination of amount of protein	35
4.3.2 Lipase assay: effect of amount of enzyme	35
4.3.3 Hydrolysis of olive oil by MD 029a bacterial lipase	37
<b>CHAPTER 5 DISCUSSION</b>	<b>41</b>
<b>CHAPTER 6 CONCLUSIONS AND RECOMMENDATION</b>	<b>45</b>
<b>REFERENCES</b>	<b>46</b>
<b>APPENDICES</b>	<b>54</b>
<b>CURRICULUM VITAE</b>	<b>58</b>



## LIST OF TABLES

<b>Table</b>		<b>Page</b>
3.1	Composition of ZoBell's Modified Medium; Sucrose Sea Water (SSW) agar.	19
3.2	Different conditions of temperature, incubation time and amount of substrate for lipase induction.	26
4.1	Morphological and basic phenotypic characteristic of the isolated bacteria obtained from coral mucus of <i>Aeropora cervicornis</i> .	29

## LIST OF FIGURES

<b>Figure</b>		<b>Page</b>
2.1	The catalytic action of lipases.	3
4.1	Morphological characteristics of the MD 029a bacteria	30
4.2	Results of basic phenotypic characteristics tests for identification of MD 029a bacteria.	31
4.3	Lipid hydrolysis test using tributyrin agar.	32
4.4	Average number of cells of MD 029a bacterial culture at OD <sub>600</sub> in each selected media.	34
4.5	Preparation of pre-inoculum. The growth rate of MD 029a bacteria in ZoBell's modified medium to reach OD <sub>600</sub> = 0.5A.	34
4.6	Effect of amount of enzyme in lipase assay of MD029a bacteria	36
4.7	Effect of incubation time on the activity of MD 029a crude lipase.	38
4.8	Effect of temperature on the activity of MD 029a crude lipase.	39
4.9	Effect of amount of substrate on the activity of MD 029a crude lipase.	40

## LIST OF ABBREVIATIONS

A	-	Absorbance
BSA	-	Bovine Serum Albumin
CaCl <sub>2</sub>	-	Calcium Chloride
FFA	-	Free fatty acids
H <sub>2</sub> S	-	Hydrogen Sulfide
M	-	Molar
NaOH	-	Sodium hydroxide
OD	-	Optical Density
OF	-	Oxidative/Fermentative
g	-	Gram
mg	-	Milligram
ml	-	Milliliter
nm	-	Nanometer
°C	-	Degree Celsius
%	-	Percentage

## LIST OF APPENDICES

<b>Appendix</b>		<b>Page</b>
1	Standard curve of protein assay.	54
2	Effect of amount of enzyme in lipase assay of MD 029a bacteria.	54
3.	Effect of incubation time on the activity of MD 029a crude lipase (18.75µg/ml).	54
4.	Effect of temperature on the activity of MD 029a crude lipase (18.75µg/ml).	55
5.	Effect of amount of substrate on the activity of MD 029a crude lipase (18.75µg/ml).	55
6.	Analysis of variance in determination of the best culture medium for induction of lipase.	55
7.	Analysis of variance in determination of the effect of amount of enzyme to the lipase induction.	56
8.	Analysis of variance in determination of effect of incubation time.	56
9.	Analysis of variance in determination of effect of temperature.	57
10.	Analysis of variance in determination of effect of amount of substrate.	57

## ABSTRACT

The information on the marine bacteria producing lipase enzyme is quite limited. Lipases are hydrolases acting on the carboxyl ester bonds present in acylglycerols to liberate fatty acids and glycerols. In this study, the ability of the isolated marine bacteria to produce this enzyme was investigated. MD 029a marine bacteria, which was isolated from coral mucus at Pulau Bidong, Terengganu was chosen to induce this enzyme. The identification of the bacteria was confirmed using several biochemical tests based on the morphological and phenotypic characteristics. The results suggested that the bacteria is *Serratia* sp. Prior to the induction of lipase by the bacteria, the best culture medium among three different media was determined and the preparation of pre-inoculum has been carried out. ZoBell's modified media was found to be the best culture medium. With olive oil as substrate, three reaction parameters- incubation time, temperature and amount of substrate- were studied to obtain the optimum conditions for induction of lipase. The assays were done using 56.25 µg of crude enzyme as it is the suitable amount of enzyme to optimize the amount of fatty acids released. The results obtained showed that there was no significant difference ( $P>0.05$ ) in the incubation time between 6 and 12 hours and also between 18 and 24 hours, but there was significant difference ( $P<0.05$ ) observed between range of 6–12 hours and 18 hours. There was no significant differences ( $P>0.05$ ) observed in the temperature of incubation between 27°C and 37°C but 27°C was significantly higher ( $P<0.05$ ) compared to 15°C. Furthermore, there was no significant difference ( $P>0.05$ ) observed in the amount of substrates between 2% and 3% but 1% was significantly different ( $P<0.05$ ) compared to 3%. Therefore, optimum free fatty acids were released after 18–24 hours of incubation time, at temperature between 27°C–37°C and with 2%–3% of olive oil as substrate. These results indicate that *Serratia* sp. is capable of producing lipases.

# SARINGAN AKTIVITI LIPASE DARIPADA BAKTERIA MD 029a YANG DIPENCILKAN DARIPADA MUKUS BATU KARANG

## ABSTRAK

Penerangan mengenai bakteria marin yang menghasilkan enzim lipase adalah sangat terhad. Lipase adalah satu hidrolase yang bertindak ke atas ikatan karboksil ester yang hadir dalam acilgliserol untuk membebaskan asid lemak dan gliserol. Dalam kajian ini, keupayaan bakteria marin untuk menghasilkan enzim ini diselidiki. MD 029a yang diambil daripada mucus batu karang di Pulau Bidong telah dipilih untuk menghasilkan enzim ini. Identifikasi bakteria tersebut telah dipastikan dengan menggunakan beberapa ujian biokimia berdasarkan karakter morfologi dan fenotip. Keputusan yang diperolehi mendapati bahawa bakteria tersebut adalah *Serratia* sp. Terdahulu sebelum menggalakkan pengeluaran enzim lipase, media kultur yang terbaik telah ditentukan dan persediaan untuk pra-inoculum telah dilaksanakan. Medium ZoBell merupakan medium kultur terbaik. Dengan minyak zaitun sebagai substrat, tiga parameter telah dikaji untuk mencapai keadaan yang maksimum bagi penghasilan lipase- masa pengeraman, suhu dan amaun substrat. Pengujian ini dibuat dengan menggunakan 56.25 µg enzim mentah kerana ia adalah amaun enzim yang sesuai untuk memaksimumkan amaun asid lemak yang dibebaskan. Keputusan menunjukkan tiada perbezaan bererti ( $P>0.05$ ) dalam masa pengeraman antara 6 dan 12 jam dan juga antara 18 dan 24 jam, tetapi terdapat perbezaan bererti ( $P<0.05$ ) diperhatikan antara julat 6 – 12 jam dan 18 jam. Didapati tiada perbezaan bererti ( $P>0.05$ ) dalam suhu pengaraman antara 27°C dan 37°C, sebaliknya 27°C adalah lebih bererti ( $P<0.05$ ) berbanding dengan 15°C. Tambahan lagi, tiada perbezaan bererti ( $P>0.05$ ) diperhatikan dalam amaun substrat antara 2% dan 3%, tetapi 1% adalah berbeza dengan bererti ( $P<0.05$ ) berbanding dengan 3%. Justeru, amaun asid lemak yang optimum boleh dihasilkan selepas 18–24 jam masa pengeraman pada suhu antara 27°C–37°C dan dengan 2%–3% minyak zaitun sebagai substrat. Keputusan-keputusan ini menunjukkan bahawa *Serratia* sp. dapat menghasilkan lipase.