SCREENING FOR LIPASE ACTIVITY BY NITROGEN
BACTERIA ISOLATED FROM SOIL SAMPLES

NUR FARIZA BINTI MOHD TAJUDIN

FAKULTI SAINS DAN TEKNOLOGI
UNIVERSITI KEBANGSAAN MALAYSIA BANGI
2007

SCREENING FOR LIPASE ACTIVITY BY MD031 BACTERIA ISOLATED FROM CORAL MUCUS

By

Nur Fairuz Puteri Binti Mohd Taman

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
UNIVERSITY MALAYSIA TERENGGANU
2007

UNIVERSITI MALAYSIA TERENGGANU

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 BY *BRUCELLA* SP. ISOLATED FROM CORAL MUCUS** oleh **NUR FAIRUZ PUTERI BINTI MOHD TAMAN** no. matrik: **UK 10623** telah diperiksa dan semua pembetulan yang disarankan telah dilakukan. Laporan ini dikemukakan kepada Jabatan Sains Biologi sebagai memenuhi sebahagian daripada keperluan memperolehi ljazah **BACHELOR OF SCIENCE (BIOLOGICAL SCIENCES)**, 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: 19/5/17

.....

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

Nama: DR. AZIZ AHMAD

Cop Rasmi: 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: 19/5/2017

TABLE OF CONTENTS

ACKNOWLEDGEMENTS

I would like to give my very special thanks my supervisor, Dr Mariam bt Taib and co-supervisor, Dr Aziz bin Ahmad for their efforts in guiding me through out this study. Also special thanks to laboratory assistance, Kak Ina and Kak Tie for giving major helps and co operation while doing my lab work. Not forgetting other lab assistance of biochemistry, biotechnology and microbiology laboratories.

Not to forget supporting friends, Ita, Najwa, Azah, Yan, Ana and Ucop. Special thanks also to Suvik, Aizat, Kak Suzi, Rohana and all friends of Biological Sciences third year students for their cares and shares during my research. Not forgetting my best friends, Azlan at UTM Skudai and Fadhil, second year junior of Biology course who have always been there for me. To Ina and Yun, my housemates, and neighbours, Ain and Baiyah thanks a lot for all supports and concern. Even simple kindness and thoughts have brought strength for me to succeed in finishing this thesis.

Special thanks are dedicated to my family especially my beloved father, Mohd Taman bin Mansor and mother, Junaidah bt Md Som. Without them I would not be able to gain knowledge and success now. Thousands of thanks, mum and dad. Thanks also to my brothers and sisters, Kak Ajim, Angah, Abang, Adik, Wawa and Uki for their non stop moral supports and loves.

For all that have helped me during my study and all co-operations, may Allah bless every one of us.

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

A	-	Absorbance
BSA	-	Bovine Serum Albumin
$CaCl_2$	-	Calcium Chloride
FFA	-	Free Fatty Acid
g	-	Gram
H_2S	-	Hydrogen Sulfide
M	-	Molar
mg	-	milligram
ml	-	milliliter
NaOH	-	Sodium hydroxide
nm	-	nanometer
OD	-	Optical Density
rpm	-	rotation per minute

ABSTRACT

This study was carried out to investigate the production of lipase by MD031 bacteria isolated from coral mucus at Bidong Island, Terengganu. The identification of MD031 bacteria was confirmed by several biochemical tests based on the morphological and phenotypic characteristics. The results suggested that MD031 bacteria is *Brucella* sp. The best medium for lipase induction has been determined between three different media: Zobell modified medium, nutrient broth in sea water and nutrient broth in distilled water. Zobell modified medium has been chosen as the best medium for culture growth. For the pre-inoculum preparation, it was determined that MD031 bacteria took five hours to achieve OD reading of 0.5 at 600nm, which indicates the mid-log phase of bacteria. Different parameters were determined to find the optimum reaction conditions for lipase production: incubation time, temperature, and amount of substrate. The assays were done using 48.05µg 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 were no significant differences ($P>0.05$) 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. No significant difference ($P>0.05$) was observed 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 1% and 2% but 2% was significantly different ($P<0.05$) compared to 3%. The optimum conditions chosen for MD031 bacteria to produce lipase was in the range of 18-24 hour, at 27°C-37°C and 3% of substrate, using 43.05µg of enzyme in the lipase assay. The results indicate that the MD031 bacteria is capable of producing lipase.

SARINGAN AKTIVITI LIPASE DARIPADA BAKTERIA MD031 YANG DIASINGKAN DARIPADA MUKUS BATU KARANG.

ABSTRAK

Kajian ini dilakukan untuk menentukan penghasilan enzim lipase oleh bakteria MD031 yang dipencilkan daripada mukus batu karang dari Pulau Bidong, Terengganu. Identiti bakteria MD031 telah disahkan dengan beberapa ujian biokimia berdasarkan ciri-ciri morfologi dan fenotipik. Keputusan yang diperolehi mencadangkan bahawa bakteria MD031 adalah *Brucella* sp. Medium kultur terbaik untuk digunakan bagi proses induksi lipase telah diuji pada tiga jenis medium berbeza: Zobell, broth nutrien dalam air laut and broth nutrien dalam air suling; medium Zobell telah didapati sebagai medium terbaik bagi proses pertumbuhan kultur. Bagi persediaan pre-inokulum, bakteria MD031 didapati memerlukan lima jam bagi mencapai bacaan OD=0.5 pada 600nm, iaitu fasa eksponen bagi bakteria. Parameter yang berbeza diuji bagi menentukan takat optimum kadar penghasilan lipase: masa pengeraman, suhu dan jumlah substrat. Pengujian ini dibuat dengan menggunakan 48.05µg 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 1% dan 2%, tetapi 2% adalah berbeza dengan bererti ($P<0.05$) berbanding dengan 3%. Keadaan optimum yang dipilih bagi penghasilan lipase oleh bakteria MD031 adalah di antara 18-24 jam, pada suhu 27°C-37°C dan 3% substrat, menggunakan 43.05µg enzim dalam asai lipase. Keputusan kajian menunjukkan bahawa bakteria MD031 mampu untuk menghasilkan lipase.