STUDIES ON THE PURIFICATION OF POLYSACCHARIDES PRODUCED
[illegible] FROM MARINE SPONGES

[illegible]

[illegible]

FACULTY OF SCIENCE AND TECHNOLOGY
[illegible]

2008

STUDIES ON THE PRODUCTION OF POLYSACCHARIDES PRODUCED BY BACTERIUM ISOLATED FROM MARINE SPONGE, *Aaptos* sp.

By

Norhayati binti Zainon

Research Report submitted in partial fulfillment of
The requirements for the degree of
Bachelor of Science (Marine Biology)

Department of Marine Science
Faculty of Science and Technology
Kolej Universiti Sains dan Teknologi Malaysia
2006

This Project Report should be sites as:

Norhayati, Z. 2006. Studies On The Production of Polysaccharides Produced By Bacterium Isolated From Marine Sponge, *Aaptos* sp. Undergraduate thesis. Bachelor of Science in Marine Biology, Faculty of Science and Technology, Kolej Universiti Sains dan Teknologi Malaysia, Terengganu.

JABATAN SAINS SAMUDERA
FAKULTI SAINS DAN TEKNOLOGI
KOLEJ UNIVERSITI SAINS DAN TEKNOLOGI MALAYSIA

PENGAKUAN DAN PENGESAHAN LAPORAN PROJEK PENYELIDIKAN I DAN II

Adalah ini diakui dan disahkan bahawa laporan penyelidikan bertajuk :

STUDIES ON THE PRODUCTION OF POLYSACCHARIDES PRODUCED BY BACTERIUM ISOLATED FROM MARINE SPONGE, *Aaptos* sp.

oleh **NORHAYATI BINTI ZAINON,** No. Matrik **UK 8063**

telah diperiksa dan semua pembetulan yang disarankan telah dilakukan. Laporan ini dikemukakan kepada Jabatan Sains Samudera sebagai memenuhi sebahagian daripada keperluan memperolehi Ijazah **SARJANA MUDA SAINS (BIOLOGI MARIN)** Fakulti Sains dan Teknologi, Kolej Universiti Sains dan Teknologi Malaysia.

Disahkan oleh :

.....

Penyelia Utama

Nama : DR. AHMAD SHAMSUDDIN BIN AHMAD
Ketua
Pusat Pembangunan dan Kebajikan Pelajar
Bahagian Hal Ehwal Pelajar
Cop Rasmi : Kolej Universiti Sains Dan Teknologi Malaysia
Mengabang Telipot, 21030 K. Terengganu.

Tarikh : 16/4/2006

.....

Penyelia Kedua (jika ada)

Nama : ZAINUDIN BIN BACHOK
Pensyarah
Jabatan Sains Samudera
Fakulti Sains & Teknologi
Cop Rasmi : Kolej Universiti Sains dan Teknologi Malaysia
21030 Kuala Terengganu.

Tarikh : 17/4/2006

.....

Ketua Jabatan Sains Samudera

Nama :

Cop Rasmi : Tarikh :

Tesis ini didedikasikan khas buat bonda dan ayahanda,

Selma binti Ali dan Kapten (bersara) Zainon @ Zainol bin Abdullah
Kekanda kesayanganku, Noriman (Peter), serta adinda-adinda yang amat kurindui, Norhafidzah dan Norarifah.

Kalian adalah sumber inspirasiku, selamanya...

Buat teman-teman yang sentiasa bersama kala suka dan lara, kulayarkan kerinduan di lautan memori, mengimbau kenangan indah yang menyentuh emosi, masih segar diingatan saat mesra berputik, sesungguhnya kehadiran kalian membawa sinaran cahaya inspirasi.....Aida dan friends, Kak Nani and friends, sue, sal, Herni dan semua yang membantu di lab.

ACKNOWLEDGEMENT

Bismillahirrahmanirrahim,

First of all, I would like to express my deeply grateful to my first supervisor Dr. Ahmad Shamsuddin Ahmad for accepting me as his final year student. Thanks a lot for his permission to use his laboratory until completing this study and I appreciate his encouragement and support to finish this project.

I also dedicate a sincerely thank to my second supervisor, Mr. Zainudin Bachok for his attention and advice to me to completing this study. I also would like to express my deep sense to research assistant, Mr. Lukman Hakim and Mr. Zaidad Maraicar who have been most helpful in providing information, assistance and guidance to identify bacteria and expert instructions in polysaccharides analysis.

I also wish to express my cordial gratitude to all my friends who made cooperation and give a big support to me to finish this thesis. Thanks a lot and all of you will always be in my heart.

Finally, I express my love and appreciation to my parents Zainol@Zainon bin Abdullah and Selma binti Ali for their love, care and bless, my brother and sisters thanks because always be with me even sad or happy. Mom always said "when Allah drops needless and pains along your path of life, don't step away. Instead pick it up and collect them, they were designed to teach you to be strong and wise". May Allah bless all of you, Amin.

TABLE OF CONTENT

ACKNOWLEDGMENTS ii

TABLE OF CONTENTS iii

LIST OF TABLES v

LIST OF FIGURES vi

LIST OF ABBREVIATIONS AND SYMBOLS vii

ABSTRACT viii

ABSTRAK ix

CHAPTER 1

1.0 INTRODUCTION AND OBJECTIVES 1

CHAPTER 2

2.0 LITERATURE REVIEW

2.1 Marine Sponge 5
2.2 Marine Sponge and Bacteria 6
2.3 Polysaccharides and Bacteria 9

CHAPTER 3

3.0 MATERIALS AND METHOD

3.1 Sampling 12
3.2 Isolation and Identification of Bacteria
3.2.1 Gram Staining 12
3.2.2 Biochemical Test 13
3.3.3 Selective Media 13
3.3.4 REMEL Identification Kit 13
3.3 Isolation and Purification of Polysaccharide 14
3.4 Analytical Analysis of the Polysaccharide 15

CHAPTER 4

4.0 RESULTS

4.1 Morphological characteristic 17
4.2 Biochemical Test 19
4.3 Selective Media 21
4.4 REMEL Identification Kit 22
4.5 Isolation and Purification of Polysaccharides 23
4.6 Results of Paper Chromatography (PC)
4.6.1 Hydrolyzed of the crude polysaccharides with 2 mol TFA 24
4.6.2 Hydrolyzed of the acidic polysaccharides with 2 mol TFA 25
4.7 Results of High-performance liquid chromatography
4.7.1 Chromatograph of crude polysaccharides 26
4.7.2 Chromatograph of acidic polysaccharides 27

CHAPTER 5

5.0 DISCUSSION

5.1 Isolation and Identification of Bacteria 28
5.2 Isolation and Purification of Polysaccharides 30
5.3 Analytical Analysis of the Polysaccharide 31

CHAPTER 6

6.0 CONCLUSION 33

LIST OF TABLES

Table		Page
4.1	Morphological characteristic of bacteria from Gram staining results.	17
4.2.1	Biochemical test results of bacteria isolated with sponge, *Aaptos* sp.	19
4.2.2	Biochemical test results of bacteria isolated with sponge, *Aaptos* sp.	20
4.2.3	The growth of bacteria on sponge agar	20
4.3	The colonies of bacteria on selective agar and Tryptic Soy Agar.	21
4.4	Identification of *Acinetobacter calcoaceticus* using RaplD ™ ONE Plus System.	22
4.5	Average yields of crude polysaccharides and acidic Polysaccharides producing bacterium, *Acinetobacter calcoaceticus* from *Aaptos* sp.	23

LIST OF FIGURES

Figure		Page
4.1	Morphology of bacteria, *Acinetobacter calcoaceticus*	18
4.2	Chromatogram of paper chromatography of the crude Polysaccharides hydrolyzed with 2 mol TFA at 100°C for 12 hrs.	24
4.3	Chromatogram of Paper chromatography of the acidic polysaccharides hydrolyzed with 2 mol TFA at 100°C for 12 hrs.	25
4.4	Chromatograph of hyrolized crude polysaccharides with 2 mol TFA.	26
4.5	Chromatography of hydrolyzed acidic polysaccharides with 2 mol TFA.	27

LIST OF ABBREVIATIONS AND SYMBOLS

α	alpha
β	beta
γ	gamma
L	liter
g	gram
ml	milliliter
mg	milligram
rpm	rote per minutes
EPS	exopolysaccharides
NaCl	natrium chloride
HCL	hydrochloride acid
TFA	trifluoroacetic acid
Glc	glucose

ABSTRACT

Acinetobacter calcoaceticus was the marine bacterium associated with marine sponge, *Aaptos* sp. which was identified by using RapID^{TM} ONE Plus System identification kit in combination with their morphology and basic biochemical test. This bacterium was gram negative staphylococcus, 0.9-1.6 x 1.5-2.5 microns in size. *A.calcoaceticus* were produced an average 384.7 mg/L of crude polysaccharides with light cream in color and 465.6 mg/L of acidic polysaccharides with light pink in color. Paper chromatography (PC) and high-performance liquid chromatography (HPLC) were used to identify the sugar component of the polysaccharides. The purified polysaccharide consist raffinose, D-glucosamine, glucose and mannose with G_{glc} value 0.352, 0.730 and 1.190, respectively. HPLC was performed to reconfirm the results of PC. Raffinose, glucose and mannose are the neutral sugars in polysaccharides that were detected using HPLC and amino sugars like D-glucosamine can only be detected if HPLC is using amino acid analysis. The studies could generate more studies in this area to gain more participants to explore their novel compound.

KAJIAN KE ATAS BAKTERIA PENGHASIL POLISAKARIDA DARIPADA SPAN MARIN, *Aaptos* sp.

ABSTRAK

Acinetobacter calcoaceticus adalah bakteria marin yang hidup bersama span marin, *Aaptos* sp. yang telah dikenalpasti dengan menggunakan "RapID^{TM} ONE Plus System identification kit" berserta dengan morfologi dan ujian biokimia asas. Bakteria ini merupakan gram negatif staphylococcus, bersaiz 0.9-1.6 x 1.5-2.5 microns. *A. calcoaceticus* telah menghasilkan purata 384.7 mg/L polisakarida mentah yang berwarna krim cerah dan 465.6 mg/L asidik polisakarida yang memberikan warna merah jambu. Kertas kromatografi (PC) dan high-performance liquid kromatografi (HPLC) telah digunakan untuk mengenalpasti komponen gula yang terdapat pada polisakarida. Komponen gula yang didapati ialah raffinosa, D-glukosamin, glukosa dan mannosa dengan nilai G_{glc} masing-masing 0.352, 0.730 dan 1.190. Kaedah HPLC digunakan adalah untuk memastikan semula keputusan yang diperoleh daripada PC. Raffinosa, glukosa dan mannosa merupakan gula-gula yang neutral yang terdapat di dalam polisakarida. Walau bagaimanapun, D-glukosamin merupakan gula amino dan ia hanya boleh dikesan oleh HPLC sekiranya HPLC menggunakan colum analisis amino asid. Dengan adanya kajian ini diharapkan akan dapat meningkatkan lagi minat banyak pihak untuk lebih meneroka komponen-komponen yang terdapat pada span marin.