

ISOLATION, IDENTIFICATION AND PURIFICATION OF
POLYSACCHARIDES DERIVED FROM BACTERIUM ASSOCIATED
WITH SEA CUCUMBER, *Holothuria edukis*

FARHANA BT. NORMAN

FAKULTI PENGAJIAN MARITIM DAN SAINS MARIN
UNIVERSITI MALAYSIA TERENGGANU

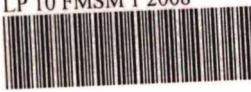
2008

C/N 6406

1100061831

Peroustakaan Sultanah Nur Zahirah (UMT)
Universiti Malaysia Terengganu

LP 10 FMSM 1 2008



1100061831
Isolation, identification and purification of polysaccharides derived from bacterium associated with sea cucumber, *Holothuria edulis* / Farhana Norman.

PERPUSTAKAAN SULTANAH NUR ZAHRAH
UNIVERSITI MALAYSIA TERENGGANU (UMT)
21030 KUALA TERENGGANU

1000618\$1

100061881

Lihat sebelah



ISOLATION, IDENTIFICATION AND PURIFICATION OF POLYSACCHARIDES
DERIVED FROM BACTERIUM ASSOCIATED WITH SEA CUCUMBER, *Holothuria*
edulis.

By

FARHANA BT. NORMAN

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

Department of Marine Science
Faculty of Maritime Studies and Marine Science
UNIVERSITI MALAYSIA TERENGGANU
2007

This project should be cited as:

Farhana, N. 2008. Isolation and Identification and Purification of polysaccharides derived from bacteria associated with sea cucumber, *Holothuria edulis*. Undergraduate thesis, Bachelor of Science in Marine Biology, Universiti Malaysia Terengganu. 56p.

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

1100061831

1
201



JABATAN SAINS MARIN
FAKULTI PENGAJIAN MARITIM DAN SAINS MARIN
UNIVERSITI MALAYSIA TERENGGANU

PENGAKUAN DAN PENGESAHAN LAPORAN
PROJEK PENYELIDIKAN I DAN II

Adalah ini diakui dan disahkan bahawa laporan penyelidikan bertajuk:

Isolation, Identification and Purification of Polysaccharides derived from marine bacterium associated with sea cucumber, *Holothuria edulis* oleh Farhana Bt. Norman, No.Matrik UK12839 telah diperiksa dan semua pembetulan yang disarankan telah dilakukan. Laporan ini dikemukakan kepada Jabatan Sains Marin sebagai memenuhi sebahagian daripada keperluan memperolehi Ijazah Sarjana Muda Sains (Biologi Marin), Fakulti Pengajian Maritim dan Sains Marin, Universiti Malaysia Terengganu.

Disahkan oleh:

Penyelia Utama **DR. AHMAD SHAMSUDDIN BIN AHMAD**
Pensyarah

Nama: Jabatan Sains Marin
Fakulti Pengajian Maritim dan Sains Marin
Universiti Malaysia Terengganu (UMT)
21030 Kuala Terengganu.
Cop Rasmi:

Tarikh: **30/4/2008**

Penyelia Kedua (jika ada)

Nama: **DR. ZAINUDIN BACHOK**
Lecturer
Department of Marine Science
Faculty of Maritime Studies and Marine Science
Universiti Malaysia Terengganu (UMT)
21030 Kuala Terengganu.
Cop Rasmi

Tarikh: **4.5.2008**

.....

Ketua Jabatan Sains Marin

Nama:

Cop Rasmi:

Tarikh:

ACKNOWLEDGEMENTS

Assalamualaikum, first of all I am thankful to Allah for his wonderful blessing on me to complete this thesis. I would like to express my deep gratitude and sincere thank to my first supervisor Dr. Ahmad Shamsudin bin Ahmad and my second supervisor Dr. Zainuddin bin Bachok for accepting me as their final year student. Thank a lot for their guideline and supervision for making this thesis possible.

I also dedicate my appreciation to the research assistant, Mr. Lukman for his valuable suggestion and patient in guiding me to complete this project. Unforgettable, a bunch of thanks to my friends Muhammad Asyraf and Shafarini for their help and support during this project was carried out.

Last but not least, I extend my gratitude and sincere thanks especially to my beloved mother, Che Elina Mat Adam and my family for their unfailing faith, pray towards my success and also for their support throughout this study. Thank you.

TABLE OF CONTENTS

	Page
ACKNOWLEDGEMENTS	ii
TABLE OF CONTENTS	iii
LIST OF TABLES	vii
LIST OF FIGURES	viii
LIST OF ABBREVIATIONS	ix
ABSTRACT	x
ABSTRAK	xi
CHAPTER 1: INTRODUCTION	1
1.1 Introduction	1
1.2 Importance of Study	4
1.3 Objectives	4
CHAPTER 2: LITERATURE REVIEW	5
2.1 Sea cucumber or Holothurians	5
2.2 Bacteria and Holothurians	6
2.3 Polysaccharides and Bacteria	8
CHAPTER 3: METHODOLOGY	10
3.1 Sampling	10
3.2 Isolation of Bacteria	10

3.3	Morphology Characteristic	11
3.4.1	Gram staining	11
3.4	Cultural and Physiological Characteristics	11
3.4.1	Growth in different media	11
3.4.2	Growth in seawater	12
3.4.3	Effect of temperature on the growth	12
3.5	Biochemical test	12
3.5.1	Oxidase test	12
3.5.2	Catalase test	13
3.5.3	Indole production	13
3.5.4	Methyl Red (MR) and Voges-Proskauer (VP)	14
3.5.5	Citrate utilization	14
3.5.6	Hydrolysis of gelatin	14
3.5.7	Starch hydrolysis	15
3.5.8	Sensitivity to antibacterial agent	15
3.6	Identification of bacteria	16
3.6.1	REME Identification Kits	16
3.7	Production and Purification of Polysaccharides	16
3.8	Chemical Analysis of Polysaccharides	17
3.8.1	Hydrolysis of polysaccharides	17
3.8.2	Paper Chromatography (PC)	18
3.8.3	High Performance Liquid Chromatography (HPLC)	18

CHAPTER 4: RESULTS	20
4.1 Isolation of Bacteria	20
4.2 Gram staining	21
4.3 Cultural and Physiological Characteristics	22
4.3.1 Growth in different media	22
4.3.2 Growth in seawater	23
4.3.3 Effect of temperature on the growth	24
4.4 Biochemical test	25
4.4.1 Sensitivity to antibacterial agent	26
4.5 REMEL Identification Kits	27
4.6 Polysaccharide production	28
4.7 Analysis of polysaccharide	29
4.7.1 Paper Chromatography (PC)	29
4.7.2 High Performance Liquid Chromatography (HPLC)	32
CHAPTER 5: DISCUSSION	36
5.1 Isolation of bacteria	36
5.2 Gram staining	36
5.3 Cultural and Physiological Characteristics	37
5.4 Biochemical test	38
5.4.1 Oxidase test	38
5.4.2 Catalase test	39
5.4.3 Indole production	39

5.4.4 Methyl Red (MR) and Voges-Proskauer (VP)	40
5.4.5 Citrate utilization	41
5.4.6 Hydrolysis of gelatin	41
5.4.7 Starch hydrolysis	42
5.4.8 Sensitivity to antibacterial agent	42
5.5 REMEL Identification Kits	43
5.6 Analysis of Polysaccharide	43
CHAPTER 6: CONCLUSION	46
REFERENCES	48
APPENDICES	51
CURICULUM VITAE	56

LIST OF TABLES

TABLE	PAGE
4.1 Morphological characteristics of bacteria on Sucrose Sea Water Agar	19
4.2 Growth of bacterium in two different medium	21
4.3 Bacterium growth in different % of seawaters	22
4.4 Effect of temperature on the growth of a bacterium	23
4.5 Biochemical characteristic of the bacteria	24
4.6 Sensitivity to anti bacterial agent	25
4.7 REMEL identification of bacteria	26
4.8 Yield of polysaccharide produced by <i>Acinetobacter calcoaceticus</i> associated with <i>Holothuria edulis</i>	27
4.9 Sugar composition contain in polysaccharide from Paper Chromatography (PC) analysis	28
4.10 Sugar composition contain in polysaccharide from HPLC analysis	31

LIST OF FIGURES

FIGURE		PAGE
4.1	Gram staining result for isolated bacteria of S (U)	20
4.2	Paper Chromatography for crude polysaccharide hydrolyze with 4M TFA	29
4.3	Paper Chromatography for acidic polysaccharide hydrolyze with 4M TFA	30
4.4	HPLC chromatogram for crude polysaccharide hydrolyze with 4M TFA	32
4.5	HPLC chromatogram for acidic polysaccharide hydrolyze with 4M TFA	33
4.6	HPLC chromatogram for twelve standard sugars	34

LIST OF ABBREVIATIONS

mg	-	miligram
g	-	gram
M	-	molarity
ml	-	mililiter
⁰ C	-	degree Calcious
cm	-	centimeter
mm	-	millimeter

ABSTRACT

Isolation of bacteria from different part of sea cucumber, *Holothuria edulis*, body that was collected from Bidong Island was carried out. From the study that has been made, there were 6 different colonies of bacteria was isolated from every part of the *H.edulis* body (skin, surface of the body, internal mucus and cloacae opening). From experiment in the production of polysaccharides, bacteria identified as *Acinetobacter calcoaceticus* was believe to produce high yield of polysaccharides and was chosen for further test. This bacterium is of the type gram-negative. This identification achieve with the combination result from biochemical test and also by using RapIDTM ONE System (RETEL, USA). Average of crude polysaccharide which has been produced by *Acinetobacter calcoaceticus* was 235.9mg / L while average of acidic polysaccharide produced was 165.1mg / L. From Paper Chromatography (PC) and High Performance Liquid Chromatography (HPLC) analysis shows that polysaccharides produced by *Acinetobacter calcoaceticus* contain basic sugar compositions which were galactose, glucose, lactose and raffinose.

KAJIAN PENGHASILAN POLISAKARIDA DARI BAKTERIA MARIN YANG BERGABUNG DENGAN GAMAT

ABSTRAK

Pemencilan bakteria daripada beberapa bahagian badan timun laut, *Holothuria edulis* yang diambil dari Pulau Bidong telah dijalankan. Bahagian-bahagian yang terlibat adalah kulit, permukaan badan, mukus dalam dan bukaan kloka. Sebanyak 6 koloni bakteria telah berjaya ditularkan. Melalui ujikaji penghasilan polisakarida, didapati bakteria yang dikenalpasti sebagai *Acinetobacter calcoaceticus* didapati menghasilkan polisakarida tertinggi dan seterusnya dipilih untuk ujikaji seterusnya. Bakteria ini adalah dari jenis gram negatif. Pengenalpastian ini dicapai dengan menggabungkan keputusan dari sifat biokimianya dan juga dengan menggunakan RapIDTM ONE System (RETEL, USA). Purata jumlah polisakarida mentah yang telah dihasilkan oleh bakteria *Acinetobacter calcoaceticus* adalah sebanyak 235.9mg/L manakala purata jumlah penghasilan polisakarida asidik tulen pula sebanyak 165.1mg/L. Analisa melalui PC dan juga HPLC menunjukkan bahawa polisakarida yang dihasilkan oleh *Acinetobacter calcoaceticus* mengandungi gula galaktosa, laktosa dan raffinosa.