

100% OF THE INDUSTRIAL OUTPUT IS PRODUCED BY
INDUSTRIES WHICH ARE OWNED OR CONTROLLED BY
THE STATE.

THE NEW INDIA

FROM SCIENCE AND TECHNOLOGY
TO INDUSTRIAL DEVELOPMENT

1100042395

Perpustakaan
Kolej Universiti Sains dan Teknologi Malaysia (KUSTEM)
95

LP 11 FST 2 2006



1100042395

Isolation and purification of polysaccharide produce by an isolated bacterium associated with marine sponge, *Aaptos* sp. / Herni Kadir.



PERPUSTAKAAN

**KOLEJ UNIVERSITI SAINS & TEKNOLOGI MALAYSIA
21030 KUALA TERENGGANU**

Lihat sebelah

HAK MILIK
PERPUSTAKAAN KUSTEM

**ISOLATION AND PURIFICATION OF POLYSACCHARIDE PRODUCED BY
AN ISOLATED BACTERIUM ASSOCIATED WITH MARINE SPONGE,
Aaptos sp.**

By
Herni binti Kadir

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

Department of Marine Sciences
Faculty of Science and Technology
UNIVERSITY OF COLLEGE SCIENCE AND TECHNOLOGY MALAYSIA
2006

This project should be cited as:

Herni, K. 2006. Isolation and purification of polysaccharide produced by an isolated bacterium associated with marine sponge, *Aaptos* sp. Project report of B. Sc. (Marine Biology). Faculty of Science and Technology. Kolej Universiti Sains dan Teknologi Malaysia. 47 p.

No part of this project report may be reproduced 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 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:

Isolation and purification of polysaccharide produced by an isolated bacterium associated with marine sponge, *Aaptos* sp. oleh Herni binti Kadir, No. Matrik UK7821 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
Cop Rasmii: Ketua
Pusat Pembangunan dan Kebajikan Pelajar
Bahagian Hal Ehwal Pelajar
Kolej Universiti Sains Dan Teknologi Malaysia
Miengabang Telipot, 21030 K. Terengganu.

Tarikh: 18/4/2006

Penyelia Kedua

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

Tarikh: 17/4/2006

Ketua Jabatan Sains Samudera

Nama:

Cop Rasmi:

Tarikh:

ACKNOWLEDGEMENT

First and foremost, I would like to start by thanking God for the gift of life and makes all things possible.

Then, I would like to thank my first supervisor Dr. Ahmad Shamsuddin bin Ahmad and also my second supervisor, Mr. Zainudin bin Bachok for their support, care and concern.

Next, my thanks go to Mr. Lukman Hakim bin Mohd. Din and Mr. Mohd. Zaidad Maraicar for anything and everything they had done in helping me with this study. Thanks a lot for always being there for me with consideration and guidance.

Thanks to my friends for their advices and accompanying. Last but not least, I would love to give my special thanks to my lovely parents for supporting and comforting me from behind. Thank you so much.

TABLE OF CONTENTS

CONTENTS	PAGE
ACKNOWLEDGEMENTS	ii
TABLES OF CONTENTS	iii
LIST OF TABLE	vi
LIST OF FIGURES	vii
LIST OF ABBREVIATIONS	viii
LIST OF APPENDICES	x
ABSTRACT	xi
ABSTRAK	xii
CHAPTER I INTRODUCTION AND OBJECTIVES	1
CHAPTER II LITERATURE REVIEW	
2.1 Sponges and the bioactive compound	4
2.2 Bacteria, bioactive compounds and polysaccharides	7
CHAPTER III METHODOLOGY	
3.1 Sampling	9
3.2 Bacteria isolation	9
3.3 Identification of bacteria	10

3.3.1	Gram Staining	10
3.3.2	Oxidase Test and Catalase Test	10
3.3.3	Sulfide, Indole, Motility Test	11
3.3.4	Simmon's Citrate Test	11
3.3.5	Methyl Red Test & Voges Proskauer Test	11
3.3.6	TSI & KIA Test	12
3.3.7	Selective Medium	12
3.3.8	Sponge Agar Test	13
3.3.9	REMEI Identification Kit	13
3.4	Isolation and Purification of Polysaccharides	13
3.4.1	Acidic	13
3.4.2	Crude	14
3.5	Analyses of Polysaccharide	15

CHAPTER IV RESULTS

4.1	Identification of Bacteria	17
4.1.1	Staining Result	17
4.1.2	Biochemical Test	18
4.1.3	Selective Medium	19
4.1.4	Sponge Agar Test	20
4.1.5	REMEI Identification Kit	20
4.2	Isolation and Purification of Polysaccharide	21

4.3 Analyses of Polysaccharide	22
--------------------------------	----

CHAPTER V DISCUSSION

5.1 Identification of Bacteria	28
5.1.1 Gram Staining	28
5.1.2 Oxidase and Catalase Test	28
5.1.3 Sulfide, Indole, Motility Test	29
5.1.4 Methyl Red and Voges Proskauer Test	30
5.1.5 Simmon's Citrate Agar	30
5.1.6 KIA and TSI Test	30
5.1.7 Selective Medium	31
5.1.8 Sponge Agar	31
5.1.9 Identification Kit	32
5.2 Analyses of Polysaccharide	32

CHAPTER VI CONCLUSION

34

REFERENCES

35

APPENDICES

40

CURRICULUM VITAE

LIST OF TABLES

Tables	Page
4.1.1 Staining result	17
4.1.2 Biochemical characteristics of the bacteria	18
4.1.3 Growth characteristics of the bacteria	19
4.1.4 The growth of bacteria on Sponge Agar	20
4.1.5 REMEL Identification Kit result	21
4.3.1 Sugar composition in polysaccharide using HPLC analyses	22
4.3.2 Sugar composition in acidic polysaccharide using PC analyses	23

LIST OF FIGURES

Figure		Page
1	Gram stain bacteria	17
2	Paper Chromatography analyses for crude polysaccharide hydrolyzed with TFA.	24
3	Paper Chromatography analyses for acidic polysaccharide hydrolyzed with TFA.	25
4	HPLC analyses for crude polysaccharide hydrolyzed with TFA	26
5	HPLC analyses for acidic polysaccharide hydrolyzed with TFA	27

LIST OF ABBREVIATIONS

URE	Urea
ADH	Arginine
ODC	Ornithine
LDC	Lysine
TET	Aliphatic thiol
LIP	Fatty acid ester
KSF	Sugar aldehyde
SBL	Sorbitol
GUR	-Nitrophenyl- β ,D-glucuronide
ONPG	o-Nitrophenyl- β ,D-galactoside
β GLU	p-Nitrophenyl- β ,D-glucoside
β XYL	p-Nitrophenyl- β ,D-xyloside
NAG	p-Nitrophenyl-n-acetyl- β ,D-alucosaminide
MAL	Malonate
PRO	Proline- β -naphthylamide
GGT	γ -Glutamyl- β -naphthylamide
PYR	Pyrrolidonyl- β -naphthylamide
ADON	Adonitol
IND	Tryptophane
CTAB	Cetyltrimethylammonium bromide

TFA	Trifluoroacetic acid
HCl	Hydrochloric acid
SSW	Sucrose Sea Water
nm	Nanometer
L	Liter
g	Gram
sp.	Species
glc	Glucose
μ L	Micro liter

LIST OF APPENDICES

Appendix		Page
1	Isolated bacteria	40
2	Polysaccharide	41
3	Identification Kit Result	42
4	Instrument used	42

ABSTRACT

The study was done in order to identify the selected polysaccharide-producing bacterium isolated from the outer part of marine sponges, *Aaptos* sp., to isolate and purify polysaccharide produced by the bacterium, and to investigate the chemical properties of the polysaccharide. The isolated bacterium associated with marine sponge, *Aaptos* sp. was identified as *Shigella* sp. after the biochemical tests were carried out, with the combination of RapID™ ONE Plus System (Remel, USA) identification kit. All the biochemical tests characterized the characteristics of the bacterium. This gram negative bacterium gave an average yield of 228mg/L crude and 121mg/L acidic polysaccharide. The polysaccharide contains glucose, galactose, rhamnose and another two unknown residues. R_{glc} value of galactose and rhamnose were 0.952 and 1.951 for crude polysaccharide while 0.945 and 1.655 for acidic polysaccharide. All the analyses were carried out and conducted by using Chromatography Paper (PC) and High Performance Liquid Chromatography (HPLC).

PEMENCILAN DAN PENULENAN POLISAKARIDA OLEH BAKTERIA YANG
BERGABUNG DENGAN SPAN, *Aaptos* sp.

ABSTRAK

Kajian yang telah dilakukan bertujuan untuk mengenalpasti bakteria yang bergabung dengan Span Marin, *Aaptos* sp., untuk menghasilkan polisakarida daripada bakteria yang telah dikenalpasti, dan untuk mengkaji komponen gula yang terkandung dalam polisakarida tersebut. Bakteria tersebut telah dikenalpasti sebagai *Shigella* sp. setelah menjalani ujian biokimia beserta dengan kit RapID™ ONE Plus System (Remel, USA). Ujian tersebut telah mencerminkan ciri-ciri bakteria ini. Bakteria gram negatif ini telah berjaya menghasilkan sebanyak 228mg/L polisakarida mentah dan 121mg/L polisakarida asidik yang mengandungi glukosa, galaktosa, rhamnosa dan dua lagi komponen gula yang tidak diketahui. Nilai R_{glc} bagi galaktosa dan rhamnosa untuk polisakarida mentah adalah 0.952 dan 1.951 sementara untuk polisakarida asidik pula adalah 0.945 and 1.655. Semua analisa yang telah dilakukan adalah dengan menggunakan teknik Kertas Kromatografi (PC) dan kaedah High Performance Liquid Chromatography (HPLC).