

ANALYSIS OF HYDROLYZABLE POLYMER FROM MARINE  
BIOMASS ASSOCIATED WITH BIVALVE SPECIES, *Tridacna* sp.

SARINAH B. AB. RAHIM

FACULTY OF SCIENCE AND TECHNOLOGY  
KOLEJ UNIVERSITI SAINS DAN TEKNOLOGI MALAYSIA

2006

1100042421



LP 37 FST 2 2006



1100042421

Analysis of exopolysaccharide isolated from marine bacterium associated with marine sponges, theomella sp. / Rathi Sai d/o Muniandy.

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

1100042421

1100042421		

Lihat sebelah

**HAK MILIK**  
**PERPUSTAKAAN KUSTEM**

**ANALYSIS OF EXOPOLYSACCHARIDE ISOLATED FROM MARINE  
BACTERIUM ASSOCIATED WITH MARINE SPONGES, *Theonella* sp.**

By  
Salbiah bt Ab. Manaf

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

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

**1100042421**

This Project Report should be sites as:

Salbiah, A.B. 2006. Analysis of Exopolysaccharide Isolated from Marine Bacterium Associated with Marine Sponges, *Theonella* sp. Undergraduate thesis, Bachelor of Science in Marine Biology, Faculty of Science and Technology, Kolej Universiti Sains dan Teknologi Malaysia, Terengganu.pp31.

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

Analysis of Exopolysaccharide Isolated from Marine Bacterium Associated with Marine Sponges, *Theonella* sp. oleh Salbiah bt. Ab. Manaf No. Matrik UK 8359

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 Malaysia, Kolej Universiti Sains dan Teknologi Malaysia.

Disahkan oleh:

Penyelia Utama

Nama: Dr. Ahmad Shamsuddin Bin Ahmad

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

Tarikh: 16/4/2006

Penyelia Kedua

Nama: En. Zainudin Bin Bachok

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

Tarikh: 16/4/2006

## **ACKNOWLEDGMENT**

Assalamualaikum w.b.t. Firstly, I like to thank Allah s.w.t for His wonderful blessing on me to complete this thesis. I also want to wish million thanks to my supervisor, Dr. Ahmad Shamsudin bin Ahmad and my second supervisor, En. Zainudin bin Bachok for their guidances, supports and valuable suggestions.

Unforgettable, I would like to thank to research assistant, Mr. Zaidad and Mr. Luqman for their constant helping and encouragement since I start of my thesis. I also like to wish a great thank to my beloved parent, Ab.Manaf bin Tahir and Rakiah bt Othman for their moral supports and blessing on me towards my success.

Finally, a special thank to all my friends for their million ideas and cooperation in completing this thesis. Last but not least, to anybody that had raised a hand to help me I owe a big thankful to them and only God can repay for their kindness on helping me.

Thank You.

## CONTENTS

<b>ACKNOWLEDGMENT</b>	ii
<b>LIST OF TABLE</b>	v
<b>LIST OF FIGURES</b>	vii
<b>LIST OF ABBREVIATION AND SIMBOLS</b>	viii
<b>LIST OF APPENDICS</b>	ix
<b>ABSTRACT</b>	x
<b>ABSTRAK</b>	xi
<b>CHAPTER 1</b>	
<b>1.0 INTRODUCTION AND OBJECTIVES</b>	<b>1</b>
1.1 Introduction	1
<b>CHAPTER 2</b>	
<b>2.0 LITERATURE REVIEW</b>	
2.1 Sponges	4
2.2 Bacteria and sponges	5
2.3 Exopolysaccharide	6
2.4 Bacteria and polysaccharide	7
<b>CHAPTER 3</b>	
<b>3.0 METHODOLOGY</b>	
3.1 Sampling	10
3.2 Morphological Characteristic	10
3.3 Gram Stain	11

3.4 Biochemical Test Characteristic	11
3.4.1 Oxidase test	11
3.4.2 Catalase test	12
3.5 Bacterial Identification Kit	12
3.6 Isolation and purification of the polysaccharide	12
3.7 Analysis of the polysaccharide	
3.7.1 Hydrolysis	13
3.7.2 Paper chromatography	14
3.7.3 HPLC	14

## **CHAPTER 4**

<b>4.0 RESULT</b>	<b>16</b>
4.1 Morphological of isolated bacteria using Gram stain	16
4.2 Biochemical test	17
4.2.1 Result of biochemical test	17
4.3 Selective Agars	18
4.4 Remel Identification	19
4.5 Isolation and purification of the polysaccharides	20
4.6 Paper Chromatography	21
4.6.1 Hydrolyzed of the crude polysaccharides	21
4.6.2 Hydrolyzed of the acidic polysaccharides	22
4.7 HPLC	23



**CHAPTER 5**

<b>5.0 DISCUSSION</b>	<b>25</b>
5.1 Isolation and identification of bacteria	25
5.2 Isolation and purification of polysaccharides	26
5.3 Analysis of polysaccharides	27

**CHAPTER 6**

<b>6.0 CONCLUSION</b>	<b>28</b>
<b>REFERENCES</b>	<b>29</b>
<b>APPENDICES</b>	<b>31</b>

## LIST OF TABLES

<b>Tables</b>	<b>Page</b>
4.2.1 Results of biochemical test	17
4.3.1 The growth of bacteria on selective agars	18
4.4 Results of biochemical test by RapID ONE System (Remel, USA)	19
4.4.1 Yield of crude and acidic polysaccharide-producing bacterium	20

## LIST OF FIGURES

Figures		Page
4.1	Morphological of isolated bacteria Gram staining using the light microscope under 100 times	16
4.6.1	Paper chromatography of the hydrolyzed of the crude polysaccharides with 2 mol TFA at 100 <sup>0</sup> C for 12 hours.	21
4.6.2	Paper chromatography of the hydrolyzed of the acidic polysaccharides with 2 mol TFA at 100 <sup>0</sup> C for 12 hours.	22
4.7.1	Chromatogram for acidic polysaccharides hydrolyzed by 2 mol TFA at 100 <sup>0</sup> C for 12 hours.	23
4.7.2	Chromatogram for crude polysaccharides hydrolyzed by 2 mol TFA at 100 <sup>0</sup> C for 12 hours	24

## LIST OF ABBREVIATION AND SYMBOLS

ml	millimeter
g	gram
Glc	glucose
TFA	trifluoroacetic acid
NaCl	Natrium Chloride
S.t.d	Standard
S	Sample

## LIST OF APPENDICES

Appendices	Page
1 Crude polysaccharides from isolated bacterium	31
2 Examples of selective agars (XLD agar, Hektoen agar, Mac Conkey	32

**ANALYSIS OF EXOPOLYSACCHARIDE ISOLATED FROM MARINE  
BACTERIUM ASSOCIATED WITH MARINE SPONGES, *Theonella* sp.**

**Abstract**

A bacterium producing polysaccharides associated with marine sponges, *Theonella* sp. collected in Bidong's island, Terengganu has been isolated and identified. The identification of bacteria was done by using the biochemical test Remel Identification Kit. The bacteria were identified as *Alcaligene faecalis*. Crude and acidic polysaccharides were hydrolyzed by using 2 M Trifluoroacetic (TFA). Paper Chromatography method was performed to determine the sugars component in the polysaccharide-producing bacterium. Ten standards of sugar monomers have been used. The results showed that sample polysaccharides hydrolyzed by 2 M TFA contain 4 sugars which were raffinose, glucose, mannose, and unknown compound. The polysaccharides also were analyzed with the High Performance Liquid Chromatography (HPLC) to determine neutral sugars.

**ANALISIS EXOPOLISAKARIDA YANG DIPENCIL DARIPADA BAKTERIA  
MARIN YANG ADA HUBUNGAN DENGAN SPAN MARIN, *Theonella* sp.**

**ABSTRAK**

Bakteria dari span marin, *Theonella* sp. dari pulau bidong, Terengganu yang menghasilkan polisakarida telah dipencilkan dan dikenalpasti. Bakteria yang telah dianalisa melalui teknik Remel Identification Kit dikenalpasti sebagai *Alcaligene faecalis*. Polisakarida mentah dan polisakarida asidik dihidrolisis dengan 2 Molar Trifluoroacetic (TFA). Seterusnya, kaedah kertas kromatografi dilakukan untuk menentukan komponen gula bakteria penghasil polisakarida. Sepuluh standard gula monomer digunakan untuk TFA. Bagi HCL keputusan menunjukkan 4 gula dikesan iaitu raffinose, glucose, mannose, dan satu komponen gula tidak dapat dikenalpasti. Analisis polisakarida dilakukan dengan kaedah High Performance Liquid Chromatography (HPLC) untuk menentukan gula neutral.