

ISOLATION AND IDENTIFICATION OF POLYSACCHARIDE  
PRODUCING BACTERIA FROM MARINE SPONGE

*By Nurul Hafidza*

MEMORANDUM

FAKULTI SAINS DAN TEKNOLOGI  
KOLEJ UNIVERSITI SAINS DAN TEKNOLOGI MALAYSIA

2015

1100034651

LP 40 FST 2 2005



1100034651

Isolation and identification of polysaccharide producing bacterium from marine sponge (haliclona sp).



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

1100034651

1100034651		

Lihat sebelah

HAK MILIK  
PERPUSTAKAAN KUSTEM

**ISOLATION AND IDENTIFICATION OF POLYSACCHARIDE PRODUCING  
BACTERIUM FROM MARINE SPONGE, *Haliclona* sp.**

**By  
Wan Aaisyah binti Wan Mohamad**

**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 SAINS AND TECHNOLOGY MALAYSIA.  
2005**

**1100034651**

Wan Aaisyah W.M. 2005. Isolation and Identification of Polysaccharide Producing Bacterium From Marine Sponge *Haliclona* sp. Undergraduate thesis, Bachelor of Science in Marine Biology, University College of Science and Technology Malaysia, Terengganu. 52p.

No parts of this project may be reproduced 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 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 Identification of Polysaccharide Producing Bacterium from Marine Sponge  
*Haliclona* sp. oleh Wan Aaisyah binti Wan Mohamad, UK 6684 telah diperiksa dan  
semua pembetulan yang disarankan telah dilakukan. Laporan ini dikemukakan kepada  
Jabatan Sains Samudera sebagai memenuhi sebahagian daripada keperluan memperoleh  
Ijazah Sarjana Muda Sains (Biologi Marin), Fakulti Sains dan Teknologi, Kolej  
Universiti Sains dan Teknologi Malaysia.

Disahkan oleh:

Penyelia Utama **DR. AHMAD SHAMSUDDIN B. AHMAD**  
Ketua  
Nama: Jabatan Sains Samudera  
Fakulti Sains dan Teknologi  
Cop Rasmi: Kolej Universiti Sains dan Teknologi Malaysia  
21030 Kuala Terengganu

Tarikh: 24/3/05

Penyelia Kedua (jika ada)  
Nama: **NAJIAH BINTI MUSA (Ph. D)**  
*Pensyarah*  
Cop Rasmi: Jabatan Sains Perikanan dan Akuakultur  
Fakulti Agroteknologi dan Sains Makanan  
Kolej Universiti Sains dan Teknologi Malaysia  
21030 Kuala Terengganu

Tarikh: 24.3.05

Ketua Jabatan Sains Samudera  
Nama: **DR. AHMAD SHAMSUDDIN B. AHMAD**  
Ketua  
Cop Rasmi: Jabatan Sains Samudera  
Fakulti Sains dan Teknologi  
Kolej Universiti Sains dan Teknologi Malaysia  
21030 Kuala Terengganu

Tarikh: 24/3/05

## **ACKNOWLEDGEMENT**

Alhamdulillah, thank to God because of blessing and greatly this study was accomplishing in successfully. First of all, a grateful acknowledge to my first supervisor Dr. Ahmad Shamsuddin Ahmad because of accepting me as his student final year project. Thanks for his sincere care, supports and guidance to fulfill my study successfully. Also grateful acknowledge for Dr. Najiah Musa as my second supervisor and Dr. Siti Aishah as my mentor.

Never forget, thanks to research assistance in Instrumentation Laboratory, Mr. Lukman and Mr. Zaidad for their guidance and support in determination of bacteria and purification of polysaccharide successfully to complete in this study.

Also thanks to my lovely friends in same field of study Mas, Chillu, Z, Aini and Sya for their assistances and helps during my study.

Lastly, a special thank to my family especially to my beloved parents because of their blessing and support, I accomplished this study successfully.

## TABLE OF CONTENTS

<b>CONTENTS</b>	<b>PAGE</b>
<b>ACKNOWLEDGEMENTS</b>	ii
<b>TABLES OF CONTENTS</b>	iii - v
<b>LIST OF TABLE</b>	vi
<b>LIST OF FIGURES</b>	vii
<b>LIST OF ABBREVIATIONS</b>	viii
<b>LIST OF APPENDICES</b>	ix
<b>ABSTRACT</b>	x
<b>ABSTRAK</b>	xi
<b>CHAPTER I          INTRODUCTION</b>	1 - 3
<b>CHAPTER II          LITERATURE REVIEW</b>	
2.1      Sponge and Bacteria	4 - 5
2.2      Sponge, Bioactive Compounds and Polysaccharide	6
2.3      Bacteria Produce Polysaccharide	7

## **CHAPTER III      METHODOLOGY**

3.1	Isolation and identification of bacteria	
3.1.1	Sampling	8
3.1.2	Isolation	8
3.1.3	Gram Staining	9
3.1.4	Biochemical Test	9
3.1.5	Selective Media	10
3.1.6	Anaerobic Agar	10
3.1.7	REMEL Identification kit	10
3.2	Isolation and Purification of Polysaccharides	11
3.3	Analyses of Polysaccharide	12

## **CHAPTER IV      RESULT**

4.1	Isolation of Bacteria	13
4.2	Identification of Bacteria	
4.2.1	Staining and Morphology Characteristics	13
4.2.2	Cultural Characteristics	14
4.2.3	Physiological Characteristics	15
4.2.4	Biochemical Characteristics	16
4.3	REMEL Identification Kit	17 – 22
4.4	Isolation and Purification of Polysaccharide	23



4.5	Analyses of Polysaccharide	24
4.5.1	<i>Enterobacter sakazaki</i>	25
4.5.2	<i>Enterobacter intermedium</i>	26
4.5.3	<i>Citrobacter freundii</i>	27
4.5.4	<i>Citrobacter koseri</i>	28
4.5.5	<i>Oligella urethalis</i>	29
4.5.6	<i>Pantoea agglomerans</i>	30
<b>CHATER V                    DISSCUSSION</b>		
5.1	Isolation and Identification of Bacteria	
5.1.1	Isolation	31
5.1.2	Morphology Characteristics	32
5.1.3	Selective Media	33
5.1.4	Anaerobic Agar	34
5.1.5	Biochemical Test	34
5.1.6	REMEL Identification Kit	34
5.2	Isolation and Purification of Polysaccharide	35-36
5.3	Analyses of Polysaccharide	36
<b>CHAPTER VI                    CONCLUSSION</b>		37
<b>REFERENCES</b>		38 - 40
<b>APPENDICES</b>		41-52
<b>CURRICULUM VITAE</b>		

## LIST OF TABLES

Tables		Page
4.2.2	Cultural characteristics of the isolates in NA agar	14
4.2.3	Growth of the isolates in Selective Media and Anaerobic Agar	15
4.2.4	Biochemical test of the isolates	16
4.3.1	Results of biochemical test for H5 by RapID™ NF System (Remel, USA)	17
4.3.2	Results of biochemical test for H1 by RapID™ ONE System (Remel,USA)	18
4.3.3	Results of biochemical test for H2 by RapID™ ONE System (Remel,USA)	19
4.3.4	Results of biochemical test for H3 by RapID™ ONE System (Remel,USA)	20
4.3.5	Results of biochemical test for H4 by RapID™ ONE System (Remel,USA)	21
4.3.6	Results of biochemical test for H6 by RapID™ ONE System (Remel,USA)	22
4.4	Yields of polysaccharide produced by bacteria associated with <i>Haliclona</i> sp.	23
4.5	Sugar compositions in all isolates bacteria	24

## LIST OF FIGURES

Figure		Page
4.5.1	Paper chromatography of the hydrolyzate of the polysaccharide from <i>Enterobacter sakazaki</i> .	25
4.5.2	Paper chromatography of the hydrolyzate of the polysaccharide from <i>Enterobacter intermedium</i> .	26
4.5.3	Paper chromatography of the hydrolyzate of the polysaccharide from <i>Citrobacter freundii</i> .	27
4.5.4	Paper chromatography of the hydrolyzate of the polysaccharide from <i>Citrobacter koseri</i> .	28
4.5.5	Paper chromatography of the hydrolyzate of the polysaccharide from <i>Oligella urethalis</i> .	29
4.5.6	Paper chromatography of the hydrolyzate of the polysaccharide from <i>Pantoea agglomerans</i> .	30

## LIST OF ABBREVIATIONS

$\mu\text{m}$	micron meter
sp.	species
$\alpha$	alpha
$\beta$	beta
$\gamma$	gamma
PC	paper chromatography
glc	glucose
$\text{H}_2\text{O}_2$	hydrogen peroxide
$\text{AgNO}_3$	argentum nitrate

## LIST OF APPENDICES

Appendix		Page
1	Sampling	41
2	Isolation	41
3	Gram staining	42
4	Selective Media	43
5	Anaerobic jar	43
6	Isolation and purification of polysaccharide	44-46
7	Fig. 3.2.1. Isolation and purification of polysaccharide	47
8	Analyses of polysaccharide	48-49
9	REMEL Identification Kit	50
10	Figure of Sampling Area (Bidong's Island)	51
11	Figure of Growth Curve of Bacteria	51

## ABSTRACT

This investigation was performed to isolate and to identify the sugar compositions of polysaccharides from marine bacteria isolate from marine sponge, *Haliclona* sp. which was collected from Bidong's Island. Six marine bacteria were isolated *Oligella urethalis*, *Pantoea agglomerans*, and two bacteria from genus *Enterobacter*; *Enterobacter sakazaki* and *Enterobacter intermedium* and two bacteria from genus *Citrobacter*; *Citrobacter freundii* and *Citrobacter koseri*. All bacteria isolated were bacteria gram negative and successfully produce polysaccharides. Sugar compositions in polysaccharides were identified using paper chromatography method. The sugar compositions determined in the polysaccharides were mannose, glucose, raffinose, xylose, arabinose and rhamnose.

PEMENCILAN BACTERIA DAN PENENTUAN POLISAKARIDA DARIPADA  
SPAN *Haliclona* sp.

**ABSTRAK**

Kajian ini dijalankan untuk memencilkan dan mengenalpasti sebatian komposisi gula yang terkandung di dalam polisakarida dari bakteria marin yang dipencilkan dari span *Haliclona* sp. yang diambil dari Pulau Bidong. Enam bakteria berjaya dipencilkan, *Oligella urethalis*, *Pantoea agglomerans*, dua bakteria daripada genus *Enterobacter*; *Enterobacter sakazaki* dan *Enterobacter intermedium* dan dua bakteria daripada genus *Citrobacter*; *Citrobacter freundii* dan *Citrobacter koseri*. Kesemua bakteria ini adalah bakteria gram negatif dan berupaya menghasilkan polisakarida. Komposisi gula yang terkandung di dalam polisakarida dikenal pasti menggunakan kaedah kertas kromatografi. Komposisi gula yang ditemui di dalam polisakarida daripada bakteria ini adalah terdiri daripada mannososa, glukosa, raffinosa, xylosa, arrabinosa dan rhamnosa.