

LP  
5  
FMSM  
2  
2007

1100054333

Perpustakaan Sultanah Nur Zahirah (UMT)  
Universiti Malaysia Terengganu



LP 5 FMSM 2 2007



1100054333

The production of exopolysaccharide from marine bacteria associated with marine sponge (Aaptos spp.) using sponge medium / Gunaseelan Munusamy.

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

1100054333		

Lihat sebelah



**THE PRODUCTION OF EXOPOLYSACCHARIDE FROM MARINE  
BACTERIA ASSOCIATED WITH MARINE SPONGE (*AAPTOS* SPP.) USING  
SPONGE MEDIUM**

**By**

**Gunaseelan S/O Munusamy**

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

**Department of Marine Sciences  
Faculty of Maritime Studies and Marine Science  
University Malaysia Terengganu**

**1100054333**

This report should be sited as;

Gunaseelan, M. 2007. The Production of Exopolysaccharide from Marine Bacteria Associated with Marine Sponge (*Aaptos* spp.) using Sponge Medium. Undergraduate thesis Bachelor of Science in Marine Science, Faculty of Maritime Studies and Marine Science, University Malaysia Terengganu, Terengganu.pp46.

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 MARIN  
FAKULTI PENGAJIAN MARITIM DAN SAINS  
UNIVERSITI MALAYSIA TERENGGANU (UMT)**

**PENGAKUAN DAN PENGESAHAN LAPORAN PROJEK PROJEK  
PENYELIDIKAN I DAN II**

Adalah diakui dan disahkan bahawa laporan penyelidikan bertajuk:

The Production of Exopolysaccharide from Marine Bacteria Associated with Marine Sponge, *Aptos.* spp using Sponge Medium oleh Gunaseelan A/L Munusamy, No. Matrik UK 9535 telah diperiksa dan semua pembetulan yang disarankan telah dilakukan. Laporan ini dikemukakan kepada Jabatan sains Marin sebagai memenuhi sebahagian daripada keperluan memperoleh Ijazah Sarjana Muda Sains (Sains Samudera) Fakulti Pengajian maritim dan Sains Marin.

Disahkan oleh:

Penyelia Utama

Nama: **DR. AHMAD SHAMSUDDIN BIN AHMAD**  
Ketua  
Cop Rasmi: Pusat Pembangunan dan Kebajikan Pelajar  
Bahagian Hal Ehwal Pelajar dan Alumni  
Universiti Malaysia Terengganu  
21030 Kuala Terengganu.

Tarikh: 6/5/07

Ketua Jabatan Sains Marin

Nama: **DR. RAZAK ZAKARIYA**  
Ketua Jabatan Sains Marin  
Cop Rasmi: Fakulti Pengajian Maritim dan Sains Marin  
Universiti Malaysia Terengganu  
(UMT)

Tarikh: 9/1/08

## **ACKNOWLEDGEMENT**

First of all, I like to thank the Almighty for His wonderful blessings on me to complete this thesis. I also want to express my deepest appreciation and grateful to my supervisor Dr. Ahmad Shamsuddin bin Ahmad for his guideline and supervision for making this thesis possible.

My appreciation also goes to En. Lukman, for his precious time and guidance for making my final year project running smoothly. I would like to thank my friends for their advice and criticism towards making my project a success. I really appreciate it.

I would like to dedicate this project to my family who had been supporting me throughout the years in UMT. Their love and encouragement make me stronger to carry on towards my goals.

## TABLE OF CONTENTS

<b>ACKNOWLEDGEMENTS</b>	ii
<b>LIST OF TABLES</b>	vi
<b>LIST OF FIGURES</b>	vii
<b>ABBREVIATIONS</b>	viii
<b>LIST OF APPENDICES</b>	ix
<b>ABSTRACT</b>	x
<b>ABSTRAK</b>	xi
<b>CHAPTER 1</b>	
<b>1.0 INTRODUCTION</b>	1
<b>OBJECTIVE</b>	1
<b>CHAPTER 2</b>	
<b>2.0 LITERATURE VIEW</b>	
2.1    Sponge and bacteria	4
2.2    Sponge, bioactive compounds and polysaccharide	6
2.3    Polysaccharide producing bacterium	9
<b>CHAPTER 3</b>	
<b>3.0 METHODOLOGY</b>	
3.1    Isolation and identification of bacteria	
3.1.1    Sampling	12

3.1.2	Bacterial Sampling and Isolation	12
3.1.3	Sponge Extract Preparation	13
3.1.4	Morphology of bacteria by Gram Staining Method	13
3.1.5	Biochemical Test	14
3.2	Isolation and purification of polysaccharides	16
3.2.1	Paper Chromatography	17
3.2.2	High-performance Liquid Chromatography (HPLC)	17

## **CHAPTER 4**

### **4.0 RESULT**

4.1	Bacterial Characteristics (Bacteria AO-1)	
4.1.1	Morphology of bacteria (Bacteria AO-1)	18
4.1.2	Gram staining	18
4.1.3	Biochemical characteristic	19
4.1.4	Bacterial identification kit	20
4.1.5	Isolation and purification of polysaccharide	21
4.1.6	Analysis of the polysaccharide	21
4.1.7	Analysis of the Polysaccharide (High Performance Liquid Chromatography) (Bacteria AO-1)	23
4.2	Bacterial Characteristics (Bacteria AI-1)	
4.2.1	Morphology of bacteria (Bacteria AI-1)	25
4.2.2	Gram staining	25
4.2.3	Biochemical characteristic	26
4.2.4	Bacterial identification kit	27



4.2.5	Isolation and purification of polysaccharide	28
4.2.6	Analysis of the polysaccharide	28
4.2.7	Analysis of the Polysaccharide (High Performance Liquid Chromatography) (Bacteria AO-1)	30

## **CHAPTER 5**

### **5.0 DISSCUSSION**

5.1	Isolation and identification of bacteria (Bacteria AO-1)	
5.1.1	Isolation and identification of bacteria (Bacteria AO-1)	32
5.1.2	Analysis of Polysaccharide (Bacteria AO-1)	34
5.2	Isolation and identification of bacteria (Bacteria AI-1)	
5.2.1	Isolation and identification of bacteria (Bacteria AI-1)	35
5.2.2	Analysis of Polysaccharide (Bacteria AI-1)	35

## **CHAPTER 6**

<b>6.0 CONCLUSION</b>	39
<b>REFERENCES</b>	40
<b>APPENDICES</b>	42

## LIST OF TABLES

<b>Table</b>		<b>Page</b>
4.1.3(a)	Biochemical characteristic of isolates bacteria of AO-1	17
4.1.3(b)	The growth of bacteria on sponge agar	17
4.1.4	Result of isolate bacteria AO-1 using RapID NF Plus System	18
4.2.3(a)	Biochemical characteristic of isolates bacteria of AI-1	22
4.2.3(b)	The growth of bacteria on sponge agar	22
4.2.4	Result of isolate bacteria AI-1 using RapID NF Plus System	23

## LIST OF FIGURES

Figure		Page
4.1.2	The gram staining of isolated bacteria	16
4.1.6(a)	The Paper Chromatography result for <i>Pseudomonas stutzeri</i> in SSW medium.	19
4.1.6(b)	The Paper Chromatography result for <i>Pseudomonas stutzeri</i> in sponge medium	20
4.1.7(a)	HPLC chromatogram sugar crude polysaccharide hydrolyzed by 4M HCL (Sample Bacteria AO-1, SSW Medium)	23
4.1.7(b)	HPLC chromatogram sugar crude polysaccharide hydrolyzed by 4M HCL (Sample Bacteria AO-1, Sponge Medium)	24
4.2.2	The gram staining of isolated bacteria	21
4.2.6(a)	The Paper Chromatography result for <i>Alcaligenes faecalis</i> in SSW medium	24
4.2.6(b)	The Paper Chromatography result for <i>Alcaligenes faecalis</i> in sponge medium	25
4.2.7(a)	HPLC chromatogram sugar crude polysaccharide hydrolyzed by 4M HCL (Sample Bacteria AI-1, SSW Medium)	30
4.2.7(b)	HPLC chromatogram sugar crude polysaccharide hydrolyzed by 4M HCL (Sample Bacteria AI-1, Sponge Medium)	31

## LIST OF ABBREVIATIONS AND SYMBOLS

$\alpha$	alpha
$\beta$	beta
$\gamma$	gamma
l	liter
g	gram
ml	milliliters
rpm	rote per minutes
NaCl	sodium chloride
HCL	hydrochloride acid
Glc	glucose
Gal	galactose

## LIST OF APPENDICES

<b>Appendix</b>		<b>Page</b>
<b>1</b>	Crude Polysaccharide-producing Bacterium (Bacteria AO-1)	33
<b>2</b>	Crude Polysaccharide-producing Bacterium (Bacteria AI-1)	33
<b>3</b>	Electrical light scattering detector for HPLC	34
<b>4</b>	Chrome Chamber	34
<b>5</b>	Freeze Dryer	35
<b>6</b>	Evaporator	35
<b>7</b>	Flow Chart of Isolation of Bacteria from Sponges, <i>Aaptos</i> spp	36

## ABSTRACT

The production of exopolysaccharide from the marine sponge using the sponge medium is one of the new research which is carried out. The objective of this study is to isolate and purified polysaccharide from gram negative bacteria associated with marine sponge *Aaptos spp* using both SSW medium and *Aaptos spp.* sponge medium. The study was also to identify isolated bacterium and the chemical analysis of the purified polysaccharide. The sponges were taken from Bidong Island and the samples were isolated from the outer layer of sponge. There are two bacteria which were isolated from this sponge which were labeled as bacteria AO-1 and bacteria AI-1. These isolated bacteria have been put for further analyses such as morphological characteristics, biochemical test and RapID™ NF Plus and ONE System. Bacteria AO-1 and bacteria AI-1 is a gram negative, straight rod and identified as *Pseudomonas stutzeri* and *Alcaligenes faecalis*. The production of crude polysaccharide from bacteria *Pseudomonas stutzeri* was 156 mg/l. The production of crude polysaccharide from this bacteria *Alcaligenes faecalis* was 240 mg/l. The analysis of sugars in this both bacteria was done by using Paper Chromatography (PC) and High Performance Liquid Chromatography (HPLC). From PC results, Bacteria AO-1 and Bacteria AI-1 showed the presence of maltose and glucose in SSW medium while trehalose and glucose in sponge medium.

**PENGHASILAN EKSPOLISAKARIDA DARIPADA BAKTERIA MARIN  
YANG ADA HUBUNGAN DENGAN SPAN MARIN, (AAPTOS SPP).  
DENGAN MENGGUNAKAN MEDIUM SPAN.**

**ABSTRAK**

Penghasilan eksopolisakarida daripada bakteria yang ada hubungan dengan span marin, *Aaptos spp.* dengan menggunakan medium span merupakan satu kaedah penyelidikan yang baru. Biasanya, kaedah penyelidikan dilakukan dengan menggunakan medium SSW (Sucrose Sea Water Medium). Objektif kajian ini adalah untuk memperoleh sampel polisakarida yang bersih untuk tujuan analisis seterusnya. Sampel span yang diperoleh dari Pulau Bidong, diekstrak untuk mendapatkan bahagian luar span. Terdapat dua bakteria yang diekstrak iaitu bakteria AO-1 dan bakteria AI-1 yang masing-masing dikenali sebagai *Pseudomonas stutzeri* dan *Alcaligenes faecalis*. Bakteria tersebut dikaji dan diselidik untuk analisis sifat, bentuk, ujian biokimia dan nama bakteria seperti di atas. Nama bakteria diperoleh dengan menggunakan Sistem RapID™ NF Plus and ONE. Bakteria AO-1 dan bakteria AI-1 merupakan bakteria gram-negatif dan berbentuk rod panjang. Sampel polisakarida yang diperolehi daripada bakteria AO-1 dan bakteria AI-1 masing-masing mempunyai berat 156 mg/L dan 240 mg/L. Analisa seterusnya dengan kertas kromatografi dan (High Performance Liquid Chromatography, HPLC) menunjukkan kehadiran gula maltosa dan glukosa dalam bakteria AO-1 dan bakteria AI-1 daripada medium SSW manakala kehadiran gula trehalosa dan glukosa dalam bakteria AO-1 dan bakteria AI-1 daripada medium span.