

PRODUCTION AND STUDY OF CHEMICAL COMPOSITION OF
POLYSACCHARIDE PRODUCED FROM MARINE BACTERIA
COLLECTED FROM BIDONG ISLAND

NUR HANINNADIA BINTI KHALIL

FACULTY OF MARITIME STUDIES AND MARINE SCIENCE
UNIVERSITI MALAYSIA TERENGGANU

2011

LP
33
FMSM
3
2011

CP: 8647

1100088842



LP 33 FMSM 3 2011



1100088842
Production and study of chemical composition of polysaccharide
produced from marine bacteria collected from Bidong Island /
Nur Haninnadia Khalil.

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

1100088842

1100088842		

Lihat sebelah

HAK MILIK
PERPUSTAKAAN SULTANAH NUR ZAHIRAH UMT

**PRODUCTION AND STUDY OF CHEMICAL COMPOSITION OF
POLYSACCHARIDE PRODUCED FROM MARINE BACTERIA COLLECTED
FROM BIDONG ISLAND**

By

Nur Haninnadia binti Khalil

**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**

2011

This project report should be cited as:

Nur Haninnadia, K., 2011. Production and Study of Chemical Composition of Polysaccharide Produced from Marine Bacteria Collected from Bidong Island. Undergraduate thesis, Bachelor of Science in Marine Biology, Faculty of Maritime Studies and Marine Science, Universiti Malaysia Terengganu; 53p.

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.



DEPARTMENT OF MARINE SCIENCE
FACULTY OF MARITIME STUDIES AND MARINE SCIENCE
UNIVERSITI MALAYSIA TERENGGANU

DECLARATION AND VERIFICATION REPORT
FINAL YEAR RESEARCH PROJECT

It is hereby declared and verified that this research report entitled:

Production and Study of Chemical Composition of Polysaccharide Produced from Marine Bacteria Collected from Bidong Island by Nur Haninnadia Binti Khalil Matric No. UK17720 have been examined and all errors identified have been corrected. This report submitted to the Department of Marine Science and as a partial fulfillment toward obtaining the Degree of Marine Biology, Faculty of Maritime Study and Marine Science, Universiti Malaysia Terengganu, Terengganu, Malaysia.

Verified by:

Supervisor

Name: **Dr. Ahmad Shamsuddin bin Ahmad**

Official stamp: **Deputy Director**
Institut Bioteknologi Marin
Universiti Malaysia Terengganu
21030 Kuala Terengganu

Date: 27/4/2024

Head of Department of Marine Science

Name: **Dr. Razak bin Zakariya**

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

Date: 29/4/11

ACKNOWLEDGEMENTS

Bismillahirrahmanirrahim,

All praises to Allah whose countless blessings enable me to finish the project report. I am fortunate in having the invaluable advices, comments and guidance of Dr. Ahmad Shamsuddin bin Ahmad as my project's supervisor. I am indebted to his kind assistance and supervision. Without them, this project would not have been carrying out.

No undertaking of this magnitude can be accomplished alone; it is grateful appreciation that I acknowledge the assistance of all laboratory staff especially Mr. Azahari bin Muda for their advices, cooperation and assistance during this project. I also wish to express my cordial gratitude to all my friends who made cooperation and helps. Special thanks dedicated to my friends Norsaidah binti Jailani and Norfazariah binti Ishak for always give help and a big support to finish this thesis.

My deepest gratitude also goes to my beloved parent, Khalil bin Jalil and Hazizah binti Ismail, brothers and sister and also my friend Mohd Sharulnaim bin Othman for their endless love and moral support during my day in university.

Finally, I would like to thank my colleagues for all their cooperation and loyalty that made me stronger in the challenge. May Allah bless all of you, Amin.

Thank you.

TABLE OF CONTENTS

	Page
ACKNOWLEDGEMENTS	ii
LIST OF TABLES	vii
LIST OF FIGURES	viii
LIST OF ABBREVIATIONS	ix
LIST OF APPENDICES	xi
ABSTRACT	xii
ABSTRAK	xiii
CHAPTER 1: INTRODUCTION	1
1.1 Introduction	1
1.2 Importance of Study	3
1.3 Objectives	4
CHAPTER 2: LITERATURE REVIEW	5
2.1 Bacteria	5
2.2 Marine bacteria	6
2.3 Polysaccharides	7
2.4 Microbial polysaccharides	8
2.5 Microbial polysaccharides classification	9
2.5.1 Viscosity-increasing polysaccharides	9
2.5.2 Gel-forming polysaccharides	9
2.5.3 Polysaccharides developed for specific application	10

2.6	Identification of bacteria	11
2.7	Methods used in bacterial identification	12
2.7.1	Microscopic morphology	12
2.7.2	Macroscopic morphology	13
2.7.3	Physiological/biochemical characteristics	13
 CHAPTER 3: METHODOLOGY		 14
3.1	Identification of selected marine bacteria	14
3.1.1	Gram staining	14
3.1.2	Biochemical test	15
3.1.2.a	Catalase test	15
3.1.2.b	Oxidase test	15
3.1.2.c	Sulfide-Indole-Motility medium (SIM)	16
3.1.2.d	Methyl red test (MR) and Voges-Proskauer (VP) test	16
3.1.3	REMEL identification kit	17
3.1.4	Antibacterial assay	19
3.2	Production of polysaccharide from selected marine bacteria	20
3.3	Determination of chemical composition of polysaccharide produced	21
3.3.1	High Performance Liquid Chromatography (HPLC)	21

CHAPTER 4: RESULTS	22
4.1 Identification of selected marine bacteria	22
4.1.1 Gram staining	22
4.1.2 Biochemical test	23
4.1.3 REMEL identification kit	24
4.1.4 Antibacterial assay	26
4.2 Production of polysaccharide from selected marine bacteria	27
4.3 Determination of chemical composition of polysaccharide produced	28
4.3.1 High Performance Liquid Chromatography (HPLC)	28
CHAPTER 5: DISCUSSIONS	29
5.1 Identification of selected marine bacteria	29
5.1.1 Gram staining	29
5.1.2 Biochemical test	31
5.1.2.a Catalase test	31
5.1.2.b Oxidase test	33
5.1.2.c Sulfide-Indole-Motility medium (SIM)	34
5.1.2.d Methyl red test (MR) and Voges-Proskauer (VP) test	37
5.1.3 REMEL identification kit	38
5.1.4 Antibacterial assay	39

5.2	Production of polysaccharide from selected marine bacteria	40
5.3	Determination of chemical composition of polysaccharide produced	41
CHAPTER 6: CONCLUSION		42
REFERENCES		43
APPENDICES		47
CURRICULUM VITAE		53

LIST OF TABLES

Table		Page
4.1	Biochemical characteristics of selected marine bacteria	23
4.2	RapID™ NF plus system result	24
4.3	Identification probability in suspected species of bacteria	25
4.4	Different sensitivity to the different antibacterial agents	26

LIST OF FIGURES

Figure		Page
4.1	Gram-negative bacteria cell observed under 100x magnification of light microscope	22
4.2	Crude polysaccharides produced from <i>Alcaligenes</i> sp.	27
4.3	HPLC chromatogram of hydrolyzed crude polysaccharide	28
5.1	Enzymatic degradation of trptophan	34
5.2	Indole reactions with <i>Kovac's</i> reagent	35

LIST OF ABBREVIATIONS

α GLU	-	p-Nitrophenyl- α ,D-glucoside
β GLU	-	p-Nitrophenyl- β ,D-glucoside
μ L	-	microliter
ADH	-	Arginine
BANA	-	N-Benzyl-arginine- β -naphthylamide
CO ₂	-	Carbon dioxide
ELSD	-	Evaporated light scattering detector
ERIC®	-	Electronic RapID™ Compedium
EST	-	Triglyceride
FeSO ₄	-	Ferrous sulfate
GF/F	-	Glass microfiber filter
GGT	-	γ -Glutamyl β -naphthylamide
GLU	-	Glucose
H ₂ O	-	Water
H ₂ O ₂	-	Hydrogen peroxide
H ₂ S	-	Hydrogen sulfide
HPLC	-	High performance liquid chromatography
IND	-	Tryptophane
L	-	Liter
MR	-	Methyl red
mg	-	milligram
NAG	-	p-Nitrophenyl-N-acetyl- β ,D-glucosaminide
NH ₃	-	Ammonia

NO ₃	-	Sodium nitrate
O ₂	-	Oxygen
ONPG	-	p-Nitrophenyl-β,D-galactoside
OXI	-	Oxidase
PHS	-	p-Nitrophenyl-phosphoester
PRO	-	Proline-β-naphthylamide
PYR	-	Pyrrolidine-β-naphthylamide
SIM	-	Sulfide-Indole-Motility
S ₂ O ₃ ⁼	-	Thiosulfates
SO ₄ ⁼	-	Sulfates
SO ₃ ⁼	-	Sulfites
TFA	-	Trifluoro acetic acid
TRD	-	Aliphatic thiol
TRY	-	Tryptophane β-naphthylamide
URE	-	Urea
VP	-	Voges-Proskauer

LIST OF APPENDICES

Appendix	Page
1 Freeze dryer	47
2 Precipitation of polysaccharide	47
3 Dialysis with tap water	48
4 Image analyser for Gram stain	48
5 Polysaccharide produced	49
6 Oxidase test	49
7 Antibacterial assay	50
8 Rotary evaporator	50
9 Analytical balance	51
10 High Performance Liquid Chromatography	51
11 RapID TM NF plus system (REMEL, USA)	52
12 RapID TM NF plus color guide	52

ABSTRACT

The selected marine bacteria from Bidong Island water was used in the study of the production of polysaccharides. The selected marine bacteria used had been carried out in the investigation of identification of the species of the bacteria. This study also identified the chemical composition of the polysaccharides that produced by the selected marine bacteria. RapID™ NF Plus System (REMEL, USA) was used in the identification of the selected marine bacteria that then namely as *Alcaligenes* sp. after the test was done. The biochemical test also was done in order to identify the bacteria since the entire biochemical test characterized the characteristics of the bacteria. The average yield of the polysaccharide produced from *Alcaligenes* sp. was 176.0 mg per 1 L of the medium and the polysaccharides represented by white to cream in color. The determination of chemical composition of the polysaccharides was performed by using High Performance Liquid Chromatography (HPLC). From HPLC chromatogram, sugar composition that presences in crude polysaccharides of *Alcaligenes* sp. were rhamnose and glucose.

Penghasilan dan Kajian Kandungan Polisakarida Yang Dihasilkan Daripada Bakteria Marin Daripada Pulau Bidong

ABSTRAK

Bakteria marin daripada Pulau Bidong yang telah dipilih digunakan dalam kajian penghasilan polisakarida. Bakteria marin yang digunakan telah dijalankan kajian mengenai pengenalpastian spesies bakteria tersebut. Kajian ini juga mengenalpasti kandungan kimia polisakarida yang dihasilkan oleh bakteria marin tersebut. RapID™ NF Plus System (REMEL, USA) telah digunakan di dalam proses pengenalpastian spesies bakteria tersebut yang kemudiannya dikenalpasti sebagai *Alcaligenes* sp. setelah kajian dijalankan. Kajian biokimia juga telah dijalankan dalam usaha mengenalpasti bakteria tersebut kerana keseluruhan kajian biokimia menggambarkan ciri-ciri bakteria tersebut. Purata polisakarida yang dihasilkan daripada *Alcaligenes* sp. adalah 176.0 mg setiap 1 L media dan polisakarida yang dihasilkan berwarna putih hingga krim. Penentuan kandungan kimia polisakarida telah dijalankan dengan menggunakan High Performance Liquid Chromatography (HPLC). Daripada chromatogram HPLC, kandungan gula yang hadir dalam polisakarida yang disailkan oleh *Alcaligenes* sp. ialah rhamnosa dan glukosa.