

THE PRODUCTION OF POLISACCHARIDE AND DETERMINATION
OF ITS CHEMICAL COMPOSITION FROM MARINE BACTERIA,
Acinetobacter sp. SAMPLED AT BIDONG ISLAND

NORFAZARIHAH BT ISHAK

FACULTY OF MARITIME STUDIES AND MARINE SCIENCE
UNIVERSITI MALAYSIA TERENGGANU

2011

LP
28
FMSM
3
2011

**THE PRODUCTION OF POLISACCHARIDE AND DETERMINATION OF ITS
CHEMICAL COMPOSITION FROM MARINE BACTERIA, *Acinetobacter* sp.
SAMPLED AT BIDONG ISLAND**

**By
NORFAZARIHAH BT ISHAK**

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

**Department of Marine Science
Faculty of Maritime Studies and Marine Science
UNIVERSITI MALAYSIA TERENGGANU
2011**



DEPARTMENT OF MARINE SCIENCE
FACULTY OF MARITIME STUDIES AND MARINE SCIENCE

DECLARATION AND VERIFICATION REPORT
FINAL YEAR RESEARCH PROJECT

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

The production of polysaccharide and determination of its chemical composition from marine bacteria, *Acinetobacter* sp. sampled at Bidong Island by Norfazariah bt Ishak, Matric No. UK 16805 have been examined and all errors identified have been corrected. This report is submitted to the Department of Marine Science as partial fulfillment towards obtaining the Degree of marine biology, Faculty of Maritime Studies and Marine Science, Universiti Malaysia Terengganu.

Verified by:

Principal Supervisor

Name: Dr. Ahmad Shamsuddin Ahmad

DR. AHMAD SHAMSUDDIN BIN AHMAD
Deputy Director
Institut Bioteknologi Marin
Universiti Malaysia Terengganu
21030 Kuala Terengganu

Official stamp:

Date: 24/4/2011

Second Supervisor (where applicable)

Name: Dr. Habsah bt. Mohamad

Official stamp:

Date: 29/4/11

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

.....

Head of Department of Marine Science

Name: Dr. Razak bin Zakariya

Official stamp:

Date:

ACKNOWLEDGEMENTS

Assalamualaikun, first of all I would like to thankful to Allah because of His blessing on me to complete this thesis or project. I also would like to express my very sincere thank to my supervisor, Dr. Ahmad Shamsudin bin Ahmad for accepting me as his final year student. Thanks a lot for always giving me some advices, guideline, and supervision me to complete this project.

Besides that, I also would like to dedicate my appreciation to science officers and all laboratory staff for their cooperation. I would like to thank En. Azahari for his advice, cooperation and guidance while doing my laboratory work at Instrumentation Laboratory. Unforgettable, thank also to my senior for their help and encouragement in doing this project.

Last but not least, special thanks to my family for their support and pray toward my success throughout this study. I am also grateful for all my friends' supports and helps during the completion of this project. Thank you.

TABLE OF CONTENTS

List	Page
ACKNOWLEDGEMENTS	ii
TABLE OF CONTENTS	iii
LIST OF TABLES	vi
LIST OF FIGURES	vii
LIST OF ABBREVIATIONS	viii
LIST OF APPENDICES	ix
ABSTRACT	x
ABSTRAK	xi
CHAPTER 1: INTRODUCTION	1
1.1 Important of study	2
1.2 Objectives	3
CHAPTER 2: LITERATURE REVIEW	4
2.1 Bacteria	4
2.2 Marine bacteria	5
2.3 Polysaccharide	6
2.4 Bacterial polysaccharide	8
2.5 Extracellular polysaccharide	9
CHAPTER 3: METHODOLOGY	10
3.1 Culture of selected bacteria	10
3.2 Gram staining	10

3.3	REMEL identification kits	11
3.4	Sensitivity of bacteria to antibacterial agent	11
3.5	Biochemical characteristics	12
3.5.1	Oxidase test	12
3.5.2	Methyl red test (MR)	12
3.5.3	Sulphate- Indole Motility Medium (SIM)	13
3.5.4	Catalase test	13
3.6	Physical characteristics	14
3.7	Isolation and purification of polysaccharide	14
3.7.1	Paper chromatography (PC)	15
3.7.2	High performance liquid chromatography (HPLC)	16
CHAPTER 4: RESULTS		17
4.1	Identification of bacteria	17
4.1.1	Gram staining	17
4.1.2	REMEL identification kits	18
4.1.3	Sensitivity of bacteria towards antibacterial agents	19
4.1.4	Biochemical test	20
4.1.5	Physical test of isolated bacteria	21
4.1.5a	Effect of different salinity on growth of bacteria	21
4.1.5b	Effect of different temperature on the growth of bacteria	22
4.2	Production of polysaccharide	23
4.3	Analysis of polysaccharide	23
4.3.1	Paper chromatography (PC)	24

4.3.2 High performance liquid chromatography (HPLC)	26
CHAPTER 5: DISCUSSION	29
5.1 Identification of selected bacteria	29
5.2 Physical characteristic of selected bacteria	31
5.3 Production of polysaccharide	32
5.4 Analysis of polysaccharide	32
CHAPTER 6: CONCLUSION	35
REFERENCES	36
APPENDICES	42
CURICULUM VITAE	47

LIST OF TABLES

Tables	Page
4.1.2a Result of test RapID™ NF Plus for selected bacteria	21
4.1.2b The identification probability in suspected species of bacteria	21
4.1.3 Different sensitivity of selected bacteria to the antibacterial agent	22
4.1.4 Biochemical characteristics of isolated bacteria	23
4.1.5a Bacteria growth in different percentage of sea water	24
4.1.5b Effect of temperature on growth of bacteria	25
4.2 Yield of polysaccharide produced by isolated bacteria	26

LIST OF FIGURES

Figure	Page
4.3.1 Results of Paper Chromatography (PC) for crude polysaccharide of selected bacteria.	27
4.3.2a Results of high performance liquid chromatography (HPLC) chromatogram of sample (crude polysaccharide) with retention time 3.947 minutes and 4.009 minutes.	28
4.3.2b Result of high performance liquid chromatography (HPLC) chromatogram of standard mannose with retention time is 3.949 minutes.	29
4.3.2c Result of high performance liquid chromatography (HPLC) chromatogram of standard glucose with retention time, 4.065 minutes.	29
4.3.2d Result of high performance liquid chromatography (HPLC) chromatogram of standard mannose and glucose with retention time, 3.940 minutes and 4.009 minutes.	30

LIST OF ABBREVIATIONS

PAH	- Polycyclic aromatic hydrocarbons
EPS	- Extracellular polysaccharide
TSA	- Tryptic soy agar
MR	- Methyl red
SIM	- Sulphate- Indole Motility
HPLC	- High performance liquid chromatography
PC	- Paper chromatography
°C	- degree Celsius
%	- percentage
mL	- Milliliter
Rpm	- Revolution per minute
Cm	- Centimeter
G	- Gram
Mm	- Millimeter
NaOH	- Sodium Hydroxide
H ₂ O	- Water

LIST OF APPENDICES

Appendix	Page
Appendix 1.0 Table of standard zone diameter by Kirby-Bauer method	44
Appendix 2.0 Chromatogram of standard glucose and mannose from HPLC	44
Appendix 3.0 Antibacterial disc	45
Appendix 4.0 REMEL identification kit (RapID NF Plus system)	45
Appendix 5.0 Culture of bacteria in different salinity	46
Appendix 6.0 Crude polysaccharide	46
Appendix 7.0 Gram staining method	47
Appendix 8.0 Freeze dry	47
Appendix 9.0 Dialysis process of polysaccharide	48
Appendix 10.0 High Performance Liquid Chromatography (HPLC)	48

ABSTRACT

Isolation of bacteria that collected from the marine environment at Bidong Island was carried out. The pure cultured bacteria used for this study were provided by Institute Marine Biotechnology from University Malaysia Terengganu. The purpose of this study is to identify the bacterium culture on crude oil and analysis the chemical composition from polysaccharide that contain in the bacterium. From the study that has been conducted, the isolated bacteria were identified by using different type of methods. The method that had been used is gram staining, biochemical test, and REMEL identification kits. The bacterium was identified as *Acinetobacter* through RapID™ NF Plus (REMEL identification kits). The bacterium, *Acinetobacter* yield 345.16 mg/L of crude polysaccharide from the three batch of culture medium. The analysis of chemical polysaccharide was conducted by using High Performance Liquid Chromatography (HPLC) and Paper Chromatography (PC) and its show the sugar composition obtained were glucose and mannose.

**PENGHASILAN POLISAKARIDA DAN PENGENALPASTIAN KOMPOSISI
KIMIA DARIPADA BAKTERIA MARIN, *Acinetobacter* sp. DARI PULAU
BIDONG**

ABSTRAK

Pemencilan bakteria yang di pungut dari persekitaran marin di Pulau Bidong telah dijalankan. Kultur tulen bakteria yang digunakan dalam kajian ini disediakan oleh Institut Marin Bioteknologi, Universiti Malaysia Terengganu. Tujuan kajian ini adalah untuk mengenal pasti jenis bakteria dan menganalisis komposisi kimia dari polisakarida yang terdapat dari bakteria tersebut. Daripada kajian yang dijalankan, bakteria tersebut telah dikenal pasti dengan menggunakan pelbagai kaedah. Bacteria tersebut telah dikenal pasti sebagai *Acinetobacter* hasil daripada ujian dengan menggunakan REMEL Identification Kits, RapID NF Plus. Pengenalpastian ini dicapai hasil daripada gabungan keputusan sifat biokimia bakteria tersebut. Jumlah pengeluaran polisakarida yang terhasil ialah sebanyak 345.16 mg/L dari bakteria *Acinetobacter*. Analisis mengenai komposisi kimia dalam polisakarida telah dikenal pasti dengan menggunakan kertas kromatografi dan High Performance Liquid Chromatography (HPLC) dan kandunagn gula yang hadir dalam kedua-dua kaedah tersebut adalah glukosa dan mannose.