

PRODUCTION AND DETERMINATION OF CHEMICAL COMPOSITION
OF POLYSACCHARIDE FROM MARINE BACTERIA
(*Ps. orizyhabitans*) SAMPLED IN BIDONG WATER

NORSAIDAH BT. JAILANI

FACULTY OF MARITIME STUDIES AND MARINE SCIENCE
UNIVERSITI MALAYSIA TERENGGANU

2011

LP
30
FMSM
3
2011

**PRODUCTION AND DETERMINATION OF CHEMICAL COMPOSITION OF
POLYSACCHARIDE FROM MARINE BACTERIA (*Ps. orizyhabitans*) SAMPLED IN
BIDONG WATER**

By

Norsaidah bt. Jailani

**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



**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:
Production and determination of chemical composition of polysaccharide from marine bacteria (*Ps. orizyhabitans*) sampled in Bidong water by Norsaidah bt. Jailani, Matric No. UK 16769 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

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

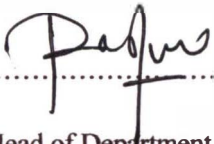
Date: 24/4/2021

Second Supervisor (where applicable)

Name: Dr. Habsah bt. Mohamad

Official stamp:

Date:


.....

Head of Department of Marine Science

Name: Dr. Razak bin Zakariya

Official stamp:

Date: 29/4/11.....

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

ACKNOWLEDGEMENT

Alhamdulillah, I thank to Allah SWT for the strength and good health that He has gave to me while struggling to finish this project.

I hereby sincerely would like to thank my supervisor, Dr. Ahmad Shamsuddin b. Ahmad whose been encouraged me to finish my Final Year Project. Thank you for the continuous guidance and support that have been given to me all along my journey of finishing my project. Your guidance, support and advices are most appreciated. Thanks to Dr. Habsah bt. Mohamad too for being the second supervisor for the project.

Not forgotten, my beloved family whose been put their trust on me and support me on my up and down through all the years in UMT. For the unfailing trust given, for the advices and encouragement; thank you so much.

Apart from that, I would like to dedicate my thank to En. Azahari, Cik Mardiah Hayati, En. Mohd Zan, En. Manaf and also staffs in Laboratory of Institute Marine Biotechnology (IMB) for all the cooperation and advices they gave. Without their help, the task of finishing this project would be more difficult for me.

Last but not least, to all my friends who been constantly support and cheer me up during the years of togetherness, thank you.

TABLE OF CONTENT

Contents	Page
ACKNOWLEDGEMENT	ii
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 Introduction	1
1.2 Significance of study	3
1.3 Objectives	5
CHAPTER 2: LITERATURE REVIEW	
2.1 Marine bacteria	6
2.2 Polysaccharide	7
2.3 Source of polysaccharide	8
2.4 Bacterial polysaccharide	9
2.5 Extracellular polysaccharides	10
2.6 Applications of bacterial polysaccharides	11

CHAPTER 3: METHODOLOGY

3.1 Identification of selected marine bacteria	
3.1.1 Biochemical properties	13
3.1.2 Gram staining	15
3.1.3 Physical properties	16
3.1.4 REMEL identification kit	17
3.1.5 Sensitivity towards antimicrobial agents	17
3.2 Production of crude polysaccharide	18
3.3 Determination of chemical composition of polysaccharide produced	
3.3.1 High Performance Liquid Chromatography (HPLC)	19
3.3.2 Paper Chromatography (PC)	20

CHAPTER 4: RESULT

4.1 Identification of selected marine bacteria	
4.1.1 Biochemical properties	21
4.1.2 Gram staining	22
4.1.3 Physical properties	23
4.1.4 REMEL identification kit	25
4.1.5 Sensitivity towards antimicrobial agents	26
4.2 Production of crude polysaccharide	27
4.3 Determination of chemical composition of polysaccharide produced	
4.3.1 Paper chromatography (PC)	28
4.3.2 High Performance Liquid Chromatography (HPLC)	31

CHAPTER 5: DISCUSSION	
5.1 Identification of selected marine bacteria	33
5.2 Production of crude polysaccharide	35
5.3 Chemical analysis of polysaccharide	36
CHAPTER 6: CONCLUSION	37
REFERENCES	39
APPENDICES	44
CURRICULUM VITAE	46

LIST OF TABLES

Table		Page
4.1.1	Biochemical properties of tested bacteria	22
4.1.3a	Effect of bacterial growth cultured in different percentage of sea water medium agar	24
4.2.3b	Effect of bacterial growth cultured in different temperatures	25
4.1.4	Reaction of inoculated bacteria tested with RapID™ NF PLUS	25
4.1.5	Sensitivity of bacteria towards different antimicrobial agents	26
4.2	Yield production of polysaccharide	28

LIST OF FIGURES

Figure		Page
4.1.2	Gram staining and simple staining of bacteria	23
4.3.1	Result of Paper Chromatography of polysaccharide sample	30
4.3.2a	Retention time of polysaccharide sample	31
4.3.2b	Retention time of sugar component mannose	32
4.3.2c	Retention time of sugar component glucose	32

LIST OF ABBREVIATIONS

MNA	-	Marine Nutrient Agar
mg	-	milligram
EPS	-	exopolysaccharide
SIM	-	Sulfide Indole Motility
MR	-	Methyl Red
TFA	-	Trifluoroacetic acid
HPLC	-	High Performance Liquid Chromatography
PC	-	Paper chromatography
ml	-	mililiter
ELSD	-	Evaporate Light Scattering Detector

LISTOF APPENDICES

Appendix		Page
Appendix 1.0	Antimicrobial sensitivity using different antimicrobial discs	44
Appendix 2.0	Crude polysaccharide produced from the bacteria	44
Appendix 3.0	Retention time for mixture of glucose and mannose	45

ABSTRACT

Marine bacteria used in this studied were sampled in Bidong Island's water of Terengganu. It was taken from stock cultured in Laboratory of Institute of Marine Biotechnology (IMB). The bacteria were identified and were used for the production of crude polysaccharide. The product (crude polysaccharide), used to determined the sugar components present in the samples by using High Performance Liquid Chromatography (HPLC) and Paper Chromatography (PC). The selected bacteria were identified as *Pseudomonas oryzihabitans* by using RapID™ NF PLUS species identification kit. The polysaccharides yielded from the bacteria were about 265mg per 1 L culture for every batch. The analysis of sugar component in the crude polysaccharides; glucose and mannose were observed as its monomer.

**PENGHASILAN DAN PENENTUAN KOMPOSISI KIMIA DALAM
POLISAKARIDA DARI BAKTERIA MARIN YANG DIAMBIL DARI AIR LAUT
BIDONG**

ABSTRAK

Bakteria marin yang digunakan di dalam kajian ini merupakan sampel dari air laut Pulau Bidong, Terengganu. Bakteria ini diambil dari kultur simpana Makmal Institut Bioteknologi Marin (IMB). Bakteria tersebut telah dikenalpasti spesiesnya serta digunakan dalam penghasilan “crude” polisakarida. Polisakarida yang dihasilkan kemudian telah digunakan untuk mengenalpasti kandungan gula “monomer” yang terkandung di dalam sebatian tersebut menggunakan High Performance Liquid Chromatography (HPLC) dan kertas kromatografi (PC). Bakteria tersebut telah dikenalpasti sebagai *Pseudomonas oryzae* menggunakan kit RapID™ NF PLUS. Dengan menggunakan HPLC dan PC untuk menentukan kandungan gula yang wujud dalam polisakarida berkenaan, keputusan analisis menunjukkan bahawa polisakarida berkenaan mengandungi glukosa dan mannososa sebagai monomernya.