

ISOLATION AND IDENTIFICATION OF POLYSACCHARIDES
DERIVED FROM BACTERIA ASSOCIATED WITH
SEA CUCUMBER, *Holothuria atra*.

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**ISOLATION AND IDENTIFICATION OF POLYSACCHARIDES DERIVED
FROM BACTERIA ASSOCIATED WITH SEA CUCUMBER, *Holothuria atra***

By

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**Research Report submitted in partial fulfillment of
the requirements for the degree of
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LIST OF ABBREVIATIONS

ADH	-	Arginine
α GLU	-	p-Nitrophenyl-N- α ,D-glucoside
BANA	-	N-Benzyl-arginine- β -naphtylamide
β GLU	-	p-Nitrophenyl-N- β ,D-glucoside
EST	-	Triglyceride
GLU	-	Glucose
GF/F	-	Whatman Glass Microfiber Filter
GGT	-	γ -Glutamyl- β -naphtylamide
HPLC	-	High Performance Liquid Chromatography
IND	-	Tryptophane
NO ₃	-	Sodium nitrate
NA	-	Nutrient agar
NAG	-	p-Nitrophenyl-N-acetyl- β ,D-glucosaminide
MR	-	Methyl-Red
ONPG	-	p-Nitrophenyl-N- β ,Dgalactoside
OXI	-	Oxidase
PC	-	Paper Chromatography
PHS	-	p-Nitrophenyl-phosphoester
PRO	-	Proline- β -naphtylamide
PYR	-	Pyrrolidonyl- β -naphtylamide

SIM	-	Sulfide Indole Motility
SSW	-	Sucrose Sea water
TRD	-	Aliphatic thiol
TRY	-	Tryptohane- β -naphtylamide
URE	-	Urea
Glu	-	Glucose
VP	-	Voges-Proskauer
μm	-	Micro meter

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ABSTRACT

Marine bacteria from sea cucumber, *Holothuria atra* was isolated from the species of sea cucumber, *Holothuria atra*. Six bacteria colony were cultured and named as H1, H2, H3, H4, H5 and H6. From all bacteria colony, only one bacterium was choose based on the pre-analysis to identify the possibility of the bacterium to produce high polysaccharide. The bacterium was identified as *Brevundimonas diminuta* based on morphology, biochemical tests and RapIDTM NF Plus system. The bacterium has characteristics of Gram-negative bacteria, straight curved rod (coccobacillus) and from the *Pseudomonas* species. The purification of the polysaccharide from the bacterium was done to analyzed the chemical composition of sugar contains by the polysaccharide. The average yield of crude polysaccharides produced by the bacterium was 244.4milligram (mg) per 1 liter (L) and 81.2mg per 1L for the acidic polysaccharides. Analyses of polysaccharide were done using the paper chromatograph (PC) and High Performance Liquid Chromatography (HPLC) techniques. The PC indicated the presence of glucose, lactose and raffinose. While HPLC showed the same results for glucose and lactose except the absence of raffinose. From the analyses, polysaccharide produced by *Brevundimonas diminuta* containing glucose, lactose and raffinose.

PENGHASILAN DAN PENULENAN POLISAKARIDA DARIPADA BAKTERIA YANG DIPENCILKAN DARIPADA TIMUN LAUT, *Holothuria atra*

ABSTRAK

Pemencilan bakteria telah dijalankan ke atas spesies timun laut, *Holothuria atra*. Enam koloni bakteria telah berjaya dituliskan dan dinamakan sebagai H1, H2, H3, H4, H5 dan H6. Kesemua bakteria ini telah dilakukan ujian awal untuk melihat penghasilan polisakarida. Bakteria yang telah dikenalpasti sebagai *Brevundimonas diminuta* didapati telah menghasilkan polisakarida yang banyak berbanding koloni bakteria yang lain. Pengenalpastian bakteria dilakukan melalui morfologi bakteria, ujian biokomia dan sistem RapID™ NF Plus. *Brevundimonas diminuta* adalah bakteria Gram-negatif, dan berbentuk coccobacillus. Penulenan ke atas polisakarida daripada bakteria ini telah dijalankan untuk mengenalpasti komposisi kimia polisakarida yang dihasilkan daripada bakteria tersebut. Jumlah purata pengeluaran polisakarida mentah yang dihasilkan daripada bakteria tersebut ialah 244.4miligram (mg) per 1 liter (L) dan purata hasil polisakarida asidik ialah 81.2mg per 1L. Analisa telah dijalankan ke atas polisakarida dengan menggunakan kaedah kertas kromatografi dan “High Performance Liquid Chromatography” (HPLC). Analisa terhadap polisakarida melalui kertas kromatografi menunjukkan kehadiran gula glukosa, laktosa dan raffinosa. Bagi HPLC, analisa menunjukkan kehadiran gula yang sama iaitu glukosa dan laktosa tetapi tidak menunjukkan kehadiran raffinosa. Polisakarida yang dihasilkan oleh *Brevundimonas diminuta* didapati mengandungi gula glukosa, laktosa dan raffinosa.