BIOSYNTHESIS OF BIOSURFACTANT BY MARINE *PSEUDOMONAS AERUGINOSA*

By

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

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SCHOOL OF MARINE SCIENCE AND ENVIRONMENT

UNIVERSITI MALAYSIA TERENGGANU

DECLARATION AND VERIFICATION REPORT

FINAL YEAR RESEARCH PROJECT

It is hereby declared and verified that this research report entitled Biosynthesis of Biosurfactant By Marine *Psedomonas aeruginosa* by Mohd Saifuddin Bin Halim, Matric No. UK 26000 have been examined and all errors identified have been corrected. This report is submitted to the School of Marine Science and Environment as partial fulfillment towards obtaining the Degree Bachelor of Science (Marine Biology), School of Marine Science and Environment, Universiti Malaysia Terengganu.

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Praise the Lord of Heaven and Hell, for creating life on earth. Truly creative in His own way. My humble existence is none but to serve. A human is none but the sum of their memories.

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SAIFUDDIN

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LIST OF SYMBOLS AND ABBREVIATIONS

SYMBOLS AND ABBREVIATIONS	FULL NAME
%	Percentage
ß	Beta
°C	Degrees Centigrade
µg	Microgram
µL	Microliters
3HB	3-hydrohybutyrate
3HHx	3-hydroxyhexanoate
3HV	3-hydroxyvalerate
4HB	4-hydroxybutyrate
ACP	ß-ketoacyl-acyl carrier proteins
AlgC	Phosphomannomutase
C	Carbon
CDW	Cell Dry Weight
CME	Caprylic Methyl Ester
CO_2	Carbon dioxide
CoA	Coenzyme A
$CoCl_2.6H_2O$	Cobalt (II) chloride hexahydrate
CPKO	Crude palm kernel oil
$CuSO_4.5H_2O$	Copper sulphate pentahydrate
DAD	Diode array and multiple wavelength detector
DNA	Deoxyribonucleic acid

E_{24}	Emulsification index
EDTA	Ethylenediaminetetraacetic acid
ELSD	Evaporative light scattering detector
EtBr	Ethidium bromide
FabI	NADH-dependent enoyl-ACP reductase
FabG	NADPH-dependent ß-ketoacyl-ACP reductase
FAS II	Fatty acid synthesis II
$FeCl_3$	Iron (III) chloride
g	Gram
g/L	Gram per litre
GC	Gas chromatography
h	Hour
H_2O	Water
HAA	Hydroxyalkanoic acid
HCl	Hydrochloric acid
HPLC	High performance liquid chromatography
J	Joule
kb	Kilo base pairs
L	Liter
LB	Luria Bertani
LPS	Lipopolysaccharide
mcl-	Medium chain length
min	Minutes
mg	Milligrams
mL	Millilitres

mm	Millimetres
MSM	Mineral salts medium
N	Nitrogen
NaCl	Sodium chloride
NADPH	Reduced nicotinamide adenine dinucleotide phosphate
$NaNO_3$	Sodium nitrate
ng	Nanogram
NR	Nutrient rich
O	Oxygen
OD	Optical density
PHA	Polyhydroxyalkanoate
PhaC; *phaC*	PHA synthase; gene encoding PHA synthase
PhaG	(R)-3-hydroxydecanoyl-ACP:CoA transacylase
PHA_{MLC}	Medium chain length 3-hydroxy fatty acids
ppt	Parts per thousand
PTFE	Polytetrafluoroethylene
psi	Pounds per square
P(3HB)	Poly(3-hydroxybutyrate)
P(3HB-*co*-3HV)	Poly(3-hydroxybutyrate-*co*-3-hydroxyvalerate)
P(3HB-*co*-3HHx)	Poly(3-hydroxybutyrate-*co*-3-hydroxyhexanoate)
P(3HB-*co*-4HB)	Poly(3-hydroxybutyrate-*co*-4-hydroxybutyrate)
RhlA	(3-hydroxyalkanoyloxy)alkanoate synthase
RhlB	Rhamnosyltransferase 1
RhlC	Rhamnosyltransferase 2

RmlA	Glucose-1-phosphate thymidylyltransferase
RmlB	dTDP-D-glucose 4,6-dehydratase
RmlC	dTDP-4-dehydrorhamnose 3,5-epimerase
RmlD	dTDP-4-dehydrorhamnose reductase
RMM	Relative molecular mass
rpm	Rotations per minute
scl-	Short chain length
TAE	Tris-acetate-EDTA
TDP	Thymidine-diphosphate
TE	Trace elements
UV	Ultra violet
V	Volt
v/v	Volume per volume
wt%	Dry weight percent
w/v	Weight per volume
w/w	Weight per weight

ABSTRACT

This study emphasizes on the evaluation of the ability of the marine strain *Pseudomonas aeruginosa* to biosynthesize biosurfactant from the various carbon sources and to characterize the rhamnolipid produced. The need of viable alternatives from toxic synthetic surfactants are inevitable in the current status quo where environmental protection is being implemented. Biosurfactant provides an insight to this issue where its application does not produce adverse effects to the environment. The production of biosurfactant also opens up doors to utilize organic wastes as carbon source fuel for the biosynthesis process thus giving an additional value to these wastes. The study sees that the production of rhamnolipid is significant in all carbon sources with different capacity and concentrations. The diversity of the *P.aeruginosa* strain to survive in various terrain and condition may be a reason of the positive outcome from the tests. Collectively, the results obtained from this study may be able to provide additional information to the betterment of biosurfactant study. In the future, the need to obtain a sustainable process of mass production of biosurfactant may become reality.

BIOSINTESIS BIOSURFAKTAN OLEH PSEUDOMONAS AERUGINOSA MARIN

ABSTRAK

Kajian ini memberi penekanan kepada penilaian tentang kebolehupayaan spesis marin *Pseudomonas aeruginosa* untuk sintesis biosurfaktan secara biologinya daripada pelbagai sumber karbon dan untuk mencirikan ramnolipid yang terhasil. Keperluan untuk alternatif yang berdaya maju berbanding surfaktan sintetik yang bertoksin adalah tidak dapat dielakkan dalam keadaan semasa dimana pelindungan alam sekitar adalah diterapkan. Biosurfaktan memberi jalan keluar kepada isu ini dimana penggunaannya tidak memberi kesan buruk kepada alam sekitar. Penghasilan biosurfaktan juga membuka pintu untuk menggunakan sisa organik sebagai sumber bahan mentah karbon untuk proses biosintesis lantas memberikan nilai tambah kepada sisa tersebut. Kajian ini melihat bahawa penghasilan rhamnolipid adalah signifikan di dalam semua jenis sumber karbon dengan kapasiti dan kepekatan yang berbeza. Kepelbagaian *P.aeruginosa* untuk hidup dalam keadaan dan tempat yang berbagai-bagai mungkin merupakan satu sebab keputusan positif daripada ujian-ujian yang dijalankan. Secara keseluruhannya, keputusan yang didapati daripada kajian ini mungkin mampu untuk menyediakan maklumat tambahan untuk pembaikan kajian biosurfaktan. Di masa hadapan, keperluan untuk mendapatkan satu proses penghasilan biosurfaktan secara besar-besaran yang berdaya maju akan menjadi kenyataan.