UNIVERSITI PUTRA MALAYSIA

PRODUCTION AND CHARACTERIZATION OF BIOSURFACTANT BY ISOLATED Pseudomonas aeruginosa RS6 STRAIN

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PRODUCTION AND CHARACTERIZATION OF BIOSURFACTANT BY ISOLATED *Pseudomonas aeruginosa* RS6 STRAIN

By

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Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfillment of the Requirements for the Degree of Master of Science

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Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the degree of Master of Science

PRODUCTION AND CHARACTERIZATION OF BIOSURFACTANT BY ISOLATED *Pseudomonas aeruginosa* RS6 STRAIN

By

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February 2016

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Biosurfactants are becoming important alternative to chemical surfactants in almost every sector in the modern industry because of their improved properties compared to their chemical counterparts. Rhamnolipid is among the best known glycolipid type biosurfactants with effective surface properties and high productivity. This study aims to isolate and characterize isolated bacterial strain from different environment conditions with the capacity to produce rhamnolipid-biosurfactants when grown on blue agar plate (BAP) selective medium. Four strains with high activity on BAP selective medium which suggested its potential as good rhamnolipid producer were screened out using combination of modified drop-collapse test, oil spreading and emulsification index (E_{24}) test. A strain with an outstanding performance and demonstrated good activity in all the above mentioned screening methods were successfully isolated and showed comparable results against control samples such as Triton-X 100 and sodium n-dodecyl sulfate (SDS), the chemical surfactants. Using morphological, Gram staining, Biolog Gen III MicroPlate analysis and 16s rRNA sequence analysis, the strain was identified as *Pseudomonas aeruginosa* and it was designed as the RS6 strain. *P. aeruginosa* RS6 produced rhamnolipid optimally at pH 7.0 when supplemented with 2.5% (v/v) palm oil (10.17 g/L) and palm olein (8.65 g/L). It reduced the surface tension ranging from 26 to 28 mN/m and showed emulsifying capability up to 62.0%. High performance liquid chromatography (HPLC) and fourier transform infrared spectroscopy (FT-IR) confirmed that biosurfactants produced by this strain was rhamnolipid in nature. In this study, an efficient and simple protocol to screen out rhamnolipid-biosurfactant producing bacteria was used and this finding will also help to add novel members to the biosurfactants group and expanded current knowledge regarding the diversity and productive capability biosurfactants from a single specific strain. Therefore, the biosurfactants produced by this strain might be useful as an alternative to chemical surfactants for a wide range of potential applications.

Keywords Biosurfactants; Characterization; Isolation; *Pseudomonas aeruginosa*; Rhamnolipid

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Master Sains

PENGHASILAN DAN PENGELASAN BIOSURFAKTAN OLEH PEMENCIL *Pseudomonas aeruginosa* RS6 STRAIN

Oleh

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Biosurfaktan menjadi alternatif penting kepada surfaktan kimia dalam hampir setiap sektor dalam industri moden kerana ciri-cirinya lebih baik berbanding dengan bahan-bahan kimia lain. Rhamnolipid adalah antara biosurfaktan glikolipid yang terbaik dengan sifat-sifat permukaan yang efektif dan produktiviti yang tinggi. Kajian ini bertujuan untuk pemencilan dan pencirian bakteria yang dipencilkankan daripada pelbagai persekitaran yang berbeza dengan kemampuan untuk menghasilkan rhamnolipid-biosurfaktan apabila ditumbuhkan di atas piring agar biru (BAP) media terpilih. Empat strain dengan aktiviti yang tinggi di atas BAP media terpilih disaringkan dengan menggunakan gabungan ujian titisan-runtuh yang telah diubahsuai, teknik sebaran minyak dan ujian indeks emulsifikasi (E_{24}). Satu strain yang berprestasi cemerlang dan menunjukkan aktiviti yang baik dalam semua ujian saringan yang dinyatakan di atas telah berjaya dipencilkan dan menunjukkan keputusan yang setanding dengan sampel kawalan seperti Triton X-100 dan sodium n-dodecyl sulfate (SDS), iaitu surfaktan kimia. Menggunakan morfologi, Gram pewarnaan, Biolog Gen III analisis MicroPlate dan 16s rRNA analisis turutan, strain itu dikenal pasti sebagai *Pseudomonas aeruginosa* dan dinamakan sebagai strain RS6. *P. aeruginosa* RS6 menghasilkan rhamnolipid secara optimum pada pH 7.0 apabila ditambah dengan 2.5% (v/v) minyak sawit (10.17 g/L) dan olein sawit (8.65 g/L). Ia mengurangkan ketegangan permukaan dari 26 hingga 28 mN/m dan menunjukkan keupayaan mengemulsi sehingga 62.0%. Kromatografi cecair berprestasi tinggi (HPLC) dan fourier transform infrared spektroskopi (FT-IR) mengesahkan bahawa biosurfaktan yang dihasilkan oleh strain ini adalah rhamnolpid secara semulajadi. Dalam kajian ini, protokol yang cekap dan mudah untuk menyaring bakteria yang menghasilkan rhamnolipid-biosurfaktan telah digunakan dan kajian ini juga akan membantu untuk menambah ahli baru dalam kumpulan biosurfaktan dan menambahkan pengetahuan semasa mengenai kepelbagaian dan keupayaan produktif biosurfaktan dari strain tertentu yg spesifik. Oleh itu, biosurfaktan yang dihasilkan oleh strain ini mungkin berguna sebagai alternatif kepada surfaktan kimia untuk pelbagai aplikasi yang berpotensi.

Katakunci Biosurfaktan; Mencirikan; Pemencilan; *Pseudomonas aeruginosa*; Rhamnolipid

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I certify that a Thesis Examination Committee has met on 11 February 2016 to conduct the final examination of Nordiyana binti Nordin on her thesis entitled "Production and Characterization of Biosurfactant by Isolated *Pseudomonas aeruginosa* RS6 Strain" in accordance with the Universities and University Colleges Act 1971 and the Constitution of the Universiti Putra Malaysia [P.U.(A) 106] 15 March 1998. The Committee recommends that the student be awarded the Master of Science.

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LIST OF ABBREVIATIONS

BAP	Blue agar plate
CTAB	Cetyltrimethylammonium bromide
MB	Methylene blue
MSA	Mineral salts agar
CMC	Critical micelle concentration
Rpm	Revolutions per minute
OD	Optical Density
BSM	Basal salt medium
NMR	Nuclear magnetic resonance
HPLC	High Performance Liquid Chromatography
LC-MS	Liquid chromatography-mass spectrophotometry
S.D	Standard deviation
R_f	Retention factor
LASs	Linear alkylbenzene sulfonates
HOC	Hydrophobic organic compound
CAGR	Compound Annual Growth Rate
dNTP	Deoxyribose nucleoside triphosphate
FT-IR	Fourier Transform Infrared Spectroscopy
ATR	Attenuated total reflection
IR	Infrared spectroscopy
SAA	Surface active agent
SDS	Sodium n-dodecyl sulfate
BASF	Badische Anilin und Soda Fabrik
CN	Cetrimide, nalidixate

CFC	Cetrimide, fucidin, cephalosporin
dTDP-L-rhamnose	L-rhamnose deoxythymidine diphosphate
NaCl	Sodium Chloride
NaOH	Sodium Hydroxide
NiO	Nickel oxide
$CaCl_2$	Calcium Chloride
$MgSO_4$	Magnesium Sulfate
ZnS	Zinc sulfide
µL	Microliter
µg	Microgram
g	Gram
h	Hour
L	Litre
m	Mili
min	Minute
mg	Miligram
ml	Mililiter
mN	Mili Newton
s	Second
v/v	volume/volume
w/v	weight/volume

CHAPTER 1

INTRODUCTION

1.1 General Background

Surfactants or surface active agents intervene in almost every products and every aspect of human daily life, making them one of the most important molecules of industrial bulk chemicals. They are extensively used as formulation aid to promote solubilisation, emulsification and dispersion of other molecules for a wide variety of applications (Reznik *et al*., 2010). In 2009, our government had introduced the National Green Policy to promote the development of the Green Industry in Malaysia. The Ministry of Energy, Green Technology and Water (KeTTHA) defined 'Green technology' as the development and application of products, equipment and systems used to conserve the natural environment and resources, as well as minimizing and reducing the negative impact of human activities. With the increasing awareness in the communities towards safe and eco-friendly products, the exotoxicity, bio-accumulation and biodegradability of these chemically synthesized surfactants has become a major concern (Marchant and Banat, 2012; George and Jayachandran, 2012).

Through modern technology, biosurfactants have come to light as a green alternative to chemical surfactants to meet the ever-increasing demand for effective and environmentally compatible specialty products. Biosurfactants are amphiphilic molecules containing hydrophilic head and hydrocarbon tail moieties that reduce surface and interfacial tension by accumulating at the interface between immiscible fluids (Banat *et al*., 2010). Their applications have been widely spread in the past five decades as they provide improved properties which chemical surfactant is lacking. They are considered as a very safe alternative to chemical surfactants due to their low toxicity, good biodegradability, effectiveness in extreme conditions and better environmental compatibility (Pornsunthorntawee *et al*., 2008; Abdel-Mawgoud *et al*., 2009).

Biosurfactants are generally grouped into glycolipids, lipopeptides, phospholipids, fatty acid salts and polymeric biosurfactants. Of all currently known biosurfactants, rhamnolipid (a glycolipid-type biosurfactants) are the well-studied biosurfactants and have the highest potential for becoming the next generation of biosurfactants introduced on the market (Henkel *et al.,* 2012). These compounds are characterized by the presence of one or two rhamnose molecules bonded to a hydrophobic fatty acid moiety. They have been applied in a wide range of applications, ranging from biological control, cosmetics, pharmaceuticals, and detergents, to environmental cleanup and petroleum recovery (Maier and Saberon-Chavez 2000; Haba *et al*., 2003; Mulligan, 2009).

Although biosurfactants have shown some industrious advantages but commercial biosurfactants are still far from being economically competitive compared to chemical surfactants. The main concern for large scale production and commercial application of biosurfactants are due to the high cost associated with their production using microorganism and their low yield. This is due to the poor strain improvement, inefficient bioprocessing methodologies available and the use of expensive substrate (Aparna *et al.*, 2012; Shafiei *et al.*, 2014).

The discovery and evaluation of new biosurfactants-producing microorganism and economical biosurfactants production are necessary for expanding the commercial utilization of biosurfactants. In view of the above, this present work aimed to screen and isolate efficient biosurfactants-producing bacteria from various environments. Different water-soluble and water-insoluble carbon sources were used for better production of biosurfactants. The biosurfactants were extracted and its physiochemical properties were characterized using HPLC and FT-IR spectroscopy. The results from this study can provide excellent materials and resources to expand current knowledge on biosurfactants.

1.2 Objectives of Study

The objectives of this research were:

a) To screen, isolate and characterize an efficient biosurfactants producing bacteria from local environments and to characterize the isolated bacterium using phenotypic and molecular studies.
b) To obtain efficient biosurfactants production technique by isolated bacterium in shake flask culture and to characterize the biosurfactants produced.

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