UNIVERSITI PUTRA MALAYSIA

ISOLATION, SCREENING AND CHARACTERIZATION OF BIOSURFACTANT PRODUCING MICROORGANISMS

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FBSB 2015 43

ISOLATION, SCREENING AND CHARACTERIZATION OF BIOSURFACTANT PRODUCING MICROORGANISMS

By:

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Thesis Submitted to the Faculty of Biotechnology and Biomolecular Sciences, Universiti Putra Malaysia, in fulfilment of the requirement for the Degree of Bachelor of Science (Honours) Biotechnology

2015

FACULTY OF BIOTECHNOLOGY AND BIOMOLECULAR SCIENCES
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Date:

LETTER OF PERMISSION

It is thereby to state that I, SITI ZULAIKHA SURYATI HALIM (Matric No: 162562) have done a final year project entitled "**Isolation, Screening and Characterization of Biosurfactant Producing Microorganisms**" under supervision of Professor Dr. Suraini Abd. Aziz from the Department of Bioprocess Technology, Faculty of Biotechnology and Biomolecular Sciences, Universiti Putra Malaysia, Serdang, Selangor, Malaysia.

I hereby give permission to my supervisor to write and prepare manuscript from the results of this research to be published in any form, if I do not do so in six (6) months from the date above, in condition that my name is also added as one of the article's authors. The arrangement of the name depends on the supervisor herself.

Yours sincerely,

……..
(SITI ZULAIKHA SURYATI HALIM)

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APPROVAL SHEET

This thesis entitled "**Isolation, Screening and Characterization of Biosurfactant Producing Microorganisms**" is submitted by SITI ZULAIKHA SURYATI HALIM (Matric No: 162562) in fulfilment of the requirement for the Degree of Bachelor of Science (Honours) Biotechnology in Faculty of Biotechnology and Biomolecular Sciences, Universiti Putra Malaysia, Serdang, Selangor, Malaysia.

Approved by,

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ABSTRACT

Abstract of thesis presented to the Faculty of Biotechnology and Biomolecular Sciences in fulfilment of the requirement for the Degree of Bachelor Science of (Honours) Biotechnology

ISOLATION, SCREENING AND CHARACTERIZATION OF BIOSURFACTANT PRODUCING MICROORGANISMS

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June 2015

Supervisor: Professor Dr. Suraini Abd. Aziz

Faculty: Faculty of Biotechnology and Biomolecular Sciences

Biosurfactants are amphiphilic compounds comprising of a non-polar (hydrophobic) group and a polar (hydrophilic) group which are produced by microorganisms. Biosurfactants play an important role in bioremediation processes due to their effectiveness as dispersion and bioremediation agents as well as their environmentally characteristics, such as low toxicity and high biodegradability than chemical surfactants. However, the high production cost of biosurfactants is a major drawback in the industry. Used cooking oil is one of the alternative cheap substrate for biosurfactant production. The aim of this study is to isolate, screen and characterize biosurfactant-producing bacteria from the sources of used cooking oil, contaminated soil, and palm oil mill effluent (POME). Four strains which designated as HIO 1, HIP 1, HIP 2 and HIP 3 were able to produce biosurfactant from used cooking oil. Identification using 16S rRNA reveals that HIP 1 and HIP 2 are *Lysinibacillus* strain while HIP 3 and HIO 1 are strains of *Bacillus*. HIP 3 has the biggest oil displacement area with 92.12 cm^2 when tested using oil spreading assay. All the bacterial strains were identified as the biosurfactants producer.

ABSTRAK

Abstrak tesis yang dikemukakan kepada Fakulti Bioteknologi dan Sains Biomolekul sebagai memenuhi sebahagian daripada keperluan untuk Bacelor Sains (Kepujian) Bioteknologi

PENGASINGAN, PENYARINGAN DAN PENCIRIAN MIKROORGANISMA YANG MENGHASILKAN BIOSURFAKTAN

Oleh:

SITI ZULAIKHA SURYATI HALIM

Jun 2015

Penyelia: **Professor Dr. Suraini Abd. Aziz**

Fakulti: **Fakulti Bioteknologi dan Sains Biomolekul**

Biosurfaktan adalah sebatian amfifilik yang terdiri daripada kumpulan bukan kutub (hidrofobik) dan kumpulan kutub (hidrofilik) yang dihasilkan oleh mikroorganisma. Biosurfaktan memainkan peranan penting dalam proses bioremediasi disebabkan oleh keberkesanannya sebagai agen penyebaran dan bioremediasi dan juga disebabkan oleh ciri-ciri alam seperti kurang toksik dan tinggi biodegredasi berbanding surfaktan kimia. Namun, kos penghasilan biosurfaktan yang tinggi menjadi kelemahan utama untuk diaplikasikan di dalam industri. Minyak masak terpakai adalah satu substrat alternatif yang murah untuk penghasilan biosurfaktan. Tujuan kajian ini adalah untuk mengasing, menyaring dan mencirikan bakteria yang menghasilkan biosurfaktan dari sumber minyak masak terpakai, tanah yang tercemar dan efluen kilang minyak kelapa sawit (POME). Empat strain yang dinamakan sebagai HIO 1, HIP 1, HIP 2 dan HIP 3 mampu menghasilkan biosurfaktan dari minyak masak terpakai. Pengenalpastian menggunakan 16S rRNA menunjukkan bahawa HIP 1 dan HIP 2 adalah strain *Lysinibacillus* manakala HIP 3 dan HIO 1 adalah strain *Bacillus.* HIP 3 menunjukkan kawasan minyak tersesar yang paling besar, iaitu 92.12 cm^2 apabila diuji dengan penilaian minyak merebak. Semua strain bakteria dikenal pasti sebagai penghasil biosurfaktan.

ACKNOWLEDGEMENTS

Alhamdulillah, praise to Allah SWT, whom with His willing I was able to complete this final year project. In this opportunity, I would like to express my insightful appreciation to my project supervisor, Professor Dr. Suraini Abd. Aziz for her non-stop guidance, observing and constant encouragement throughout the course of this thesis. A mountain of thanks to my co-supervisor, Miss Nurul Hanisah Md Badrul Hisham for her genuine support, valuable information and guidance in which helping me in completing this task through various phases. I am grateful to all Master and PhD students of Biomass Technology Centre (BTC) and laboratory assistants of Makmal Pengajaran Pusat, who had given full cooperation and assistance in using the equipments and apparatus in the laboratory. I am also grateful for their readiness to assist me in improving my laboratory skills and laboratory management practices. Lastly, I would like to thank all my colleagues and family for their absolute support from the beginning until the end of the project.

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LIST OF ABBREVIATIONS

µL	Microlitre
πr^2	Pi Radius Squared
BLAST	Basic Local Alignment Search Tool
BS	Biosurfactant
BTC	Biomass Technology Centre
CAGR	Compound Annual Growth Rate
CMC	Critical Micelle Concentration
dH_2O	Distilled Water
DNA	Deoxyribonucleic acid
g	Gram
g/cm	Gram per Centimetre
g/L	Gram per Litre
h	Hour
HLB	Hydrophilic and Lipophilic Balance
IgG	Human Immunoglobin G
min	Minute
kg	Kilogram
MEOR	Microbial Enhanced Oil Recovery
MES	Methyl Ester Sulfonate
mg	Milligram
mg/L	Milligram per Litre
mL	Millilitre

MSM	Mineral Salt Medium
NCBI	National Center for Biotechnology Information
nm	Nanometre
OD	Optical Density
OOME	Olive Oil Mill Effluent
PAHs	Polycyclic Aromatic Hydrocarbons
PCR	Polymerase Chain Reaction
PDA	Potato Dextrose Agar
POME	Palm Oil Mill Effluent
RoW	Rest of the World
rRNA	Ribosomal Ribonucleic Acid
sp.	Species
USD	United States Dollar
UPM	Universiti Putra Malaysia
v/v	Volume over Volume
YPD	Yeast Potato Dextrose

CHAPTER 1

INTRODUCTION

Oil spillage and oil pollution in marine environment have been the main risk to the ecology including the ocean life as well as to the human being through the transmission of toxic organic materials including polycyclic aromatic hydrocarbons (PAHs) into the food chain (Dasgupta *et al*., 2013). Deziel *et al.* (1996) stated that most of PAHs are carcinogenic, thus giving a potential threat to the marine animals and also human health. To encounter this problem, bioremediation technology has become a global phenomenon. Mueller *et al.* (1996) defines bioremediation as the process of degrading organic wastes biologically under controlled conditions to a harmless state, or to the points below concentration limits recognized by regulatory authorities. From the definition, bioremediation is the utilization of living organisms, predominantly microorganisms, to reduce the environmental contaminants into less toxic forms.

Principally, Vidali (2001) describes that bioremediation uses naturally occurring bacteria and fungi or plants in order to degrade or detoxify hazardous substances that can endanger human health and the environment. As being studied by Saravanan & Vijayakumar (2012), hydrocarbons degradation using microorganisms has a really important role in the environmental pollution treatment because most of the hydrocarbons are insoluble in water. Hydrocarbon degrading microorganisms produce biosurfactants which are surface active compounds with different chemical nature and molecular size. This compound has a very unique property in which it can reduce the surface tension of the hydrophobic water-insoluble substrates, thus improve their bioavalability as well as the rate of bioremediation.

Nitschke & Costa (2007) found that the world attention is triggered to microbial-derived surface-active compounds basically because of their low toxicity and biodegradable nature compared to synthetic surfactants which are produced chemically derived from petroleum that possess toxic property. Biosurfactants are amphiphilic compounds, consisting of two parts, a non-polar (hydrophobic) group and a polar (hydrophilic) moiety which are produced by microorganisms, such as

bacteria, yeast and fungi. The hydrophobic moiety compromises of saturated, unsaturated, and hydroxylated fatty acids whereas hydrophilic group contains mono-, oligo-, or polysaccharides, proteins or peptides.

From the previous studies, many potential microorganisms have been found to be producing biosurfactants. However, the abundant part of biosurfactants is produced by bacteria and only few are produced by fungi and yeast (Kitamoto *et al.*, 2002). Microbial surfactants are categorized by their chemical composition and microbial origin (Nitschke & Costa, 2007) and as being suggested by Rosenberg & Ron (1999), biosurfactants can be grouped into low sub-atomic mass particles which proficiently lower surface and interfacial strain; and high sub-atomic polymers which are more compelling as emulsion-balancing out agents. Cameotra *et al.* (2010) and Desai & Banat (1997) described that low-sub-atomic mass particles are including glycolipids, lipopeptides and phospholipids, and fatty acids whereas high-mass surfactants incorporate polymeric surfactants and particulate surfactants.

Generally, Makkar & Cameotra (1999) stated that hydrocarbons have been the substrates of choice for the production of biosurfactants. As being stated by Carey (2014), hydrocarbons are any class of organic chemical compounds that comprised of carbon (C) and hydrogen (H) elements only. The carbon atoms grouped to form the compound framework while the hydrogen atoms attached to them in diverse configurations. The examples of hydrocarbons include alkanes, alkenes, alkynes and arenes. However, many industries such as cosmetics, food and pharmaceutical industries cannot accept hydrocarbon as the substrate. Therefore, the usage of non-hydrocarbon substrates such as oils, fats, carbohydrates and glycerol are explored widely. Cameotra & Makkar (1998) suggested that the success of biosurfactant production relies on the improvement of economical processes and the utilization of cheaper raw materials in which make up for 10–30% of the overall cost. As being reported by Makkar & Cameotra (1999), some research had been conducted in finding the best way to reduce the cost and the solutions found is by using wastes either free or carry a cost credit to the environmental benefit.

Vegetable oils are used in the worldwide abundantly for many cooking processes, for instance, in Europe, about 17 million tons of vegetable oils are used annually and for each passing year, the amount raises approximately by 2% (Agriculture and Food Development, 2000). Hanisah *et al*. (2013) reported that the waste cooking oil produced by Europe was approximately 700,000-1,000,000 tonnes per annum, inclusive of the oils from snack food and French fries. As being studied by Ismail (2005), it was estimated that Asia countries such as China, Malaysia, Indonesia, Thailand, Hong Kong and India produced 40,000 tonnes waste cooking oil per year. Based on these facts, it can be concluded that there are a huge amount of waste frying oil resources will be produced in the next few years.

From the study conducted by Khalisanni *et al*. (2008), used cooking oils possess high fixed carbon about 99.92%, indicating that it is rich in carbon content. The high carbon content of used cooking oils makes it the best candidate to be the raw material in the production of biosurfactants. Other than that, used cooking oils possess long chain of palmitic acid and oleic acid which is potential to be cracked by thermal cracking or catalytic cracking for possible formation of hydrocarbon chain. As from these chemical characteristics of used cooking oil, it is greatly potential to be used as the sole substrate for the production of biosurfactant that can reduce the overall production cost because the substrate can be obtained without any charge. Besides, used cooking oil is chosen because it is a renewable feedstock as being proposed by Mulligan *et al.* (2014).

The objectives of the study are:

1. To isolate and screen the potential biosurfactants producing microorganisms
2. To characterize the potential biosurfactants producing microorganisms

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