IDENTIFYING POTENTIAL FUNGI SPECIES THROUGH MOLECULAR APPROACH AND ITS BIOSURFACTANTS PRODUCTION

WONG YIENG YONG

DISSERTATION SUBMITTED IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE OF BACHELOR OF BIOTECHNOLOGY (HONOURS)

FACULTY OF HEALTH AND LIFE SCIENCES
INTI INTERNATIONAL UNIVERSITY
PUTRA NILAI, MALAYSIA

AUGUST 2017

NON-PLAGIARISM DECLARATION

By this letter I declare that I have written this thesis completely by myself, and that I have used no other sources or resources than the ones mentioned.

I have indicated all quotes and citations that were literally taken from publications, or that were in close accordance with the meaning of those publications, as such. All sources and other resources used are stated in the references.

Moreover, I have not handed in a thesis similar in contents elsewhere.

In case of proof that the thesis has not been constructed in accordance with this declaration, the Faculty of Health and Life Sciences has the right to consider the research thesis as a deliberate act that has been aimed at making correct judgment of the candidate's expertise, insights and skills impossible.

I acknowledge that the assessor of this item may, for the purpose of assessing this item,

- reproduce this assessment item and provide a copy to another member of the University; and/or,
- communicate a copy of this assessment item to a plagiarism checking service (which may then retain a copy of the assessment item on its database for the purpose of future plagiarism checking).

In case of plagiarism the examiner has the right to fail me and take action as prescribed by the rules regarding Academic Misconduct practiced by INTI International University.

WONG YIENG YONG	*Wong Yieng Yong*
Name	Signature
I14005464	20th Nov 2017
ID. Number	Date

DECLARATION

I hereby declare that the work in this thesis is my own except for quotations and summaries which have been duly acknowledged, and completed under the supervision of Dr. Ong Ghim Hock.

Wong Yieng Yong	*Dr. Ong Ghim Hock*
I14005464	(SUPERVISOR)
20th / NOVEMBER / 2017	

ACKNOWLEDGEMENTS

The completion of this thesis could not have been possible without the guidance and encouragement of so many people. First of all, I would like to say thank to my supervisor Dr. Ong Ghim Hock who have always been patient in guiding, encouraging, commenting and suggesting me throughout the whole project and thesis writing for the past two semesters. Secondly, I would like to express my sincere gratitude to laboratory assistants Ms. Kuah who assisting and facilitating me during the practical experiments. Next, I would like to express my gratitude to all my friends who have assisted and supported me throughout the unexpected challenges during experiment and hardships faced throughout whole project. Last but not least, I would like to express my deepest appreciation to my family members who have always given me mental support during the obstacles. The skills and the knowledge which I have gained throughout my project and thesis writing as really valuable in my future career developments. I have no valuable words to express my gratitude to every person too, but in my heart, was still full of favors received from every person.

ABSTRACT

There are approximately 1.5-5million of fungal species present worldwide. However, actually only 5% of the fungi have been classified accordingly. Basic classification of fungi species was based on morphology characteristics identification but this is an inaccurate approach due to certain fungi species only presence of limited morphological characteristics. With the development of sciences and new technologies nowadays, researchers are able to discover molecular identification approach of the fungi based on DNA extracted. Hence, identification of the fungi species would be more accurate. Biosurfactant was well known on bioremediation on the polluted environments such as heavy metal polluted soil. The aims of this experiment were to determine the fungal species obtained from Aeyna Metal Trading in Shah Alam through molecular identification approach and to identify the ability of those fungal species to produce the biosurfactant. Twelve different fungal species were identified. Unfortunately, the DNA was only extracted from eight fungal samples and identified by molecular approach. The query sequence that compared by using bioinformatics tool BLAST consisted of up to 99% of identities were chosen namely *Trichoderma erinaceum, Trichoderma longibrachiatum, Penicillum simplicissimum, Aspergillus niger, Hypocrea koningii, Aspergillus ustus and Gongronella butleri.* Another four fungi species were identified through macroscopic and microscopic identification by previous senior namely *Penicillium chrysogenum, Aspergillus nidulans, Fusarium oxysporum* and *Aspergillus flavus.* Three methods were carried out to detect the production of biosurfactants by each of these fungal species. Oil Spreading method and Drop Collapse method showed negative results for all species while Emulsifying Index method showed positive results for all fungal species except for *Gongronella butleri.* Among these species, *Penicillium chrysogenum* produced most biosurfactants while *Gongronella butleri* produced no biosurfactant. Identifying the exact species can identify the potential of the fungi species to produce biosurfactant.

TABLE OF CONTENT

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

PCR	Polymerase Chain Reaction
PDA	Potato Dextrose Agar
PDB	Potato Dextrose Broth
BHB	Bushnell Haas Broth
ITS	Internal Transcribed Spacer
°C	degree Celsius
mL	milliliter
g	gram
TAE	Tris-Acetate-EDTA
UV	Ultraviolet
M	Mole
EDTA	Ethylenediaminetetraacetic Acid
NaCl	Sodium Chloride
CTAB	Cetyltrimethyl Ammonium Bromide
LAF	Laminar Air Flow
HCl	Hydrochloric acid
NaOH	Sodium Hydroxide
µL	microliter
rpm	revolutions per minute
V	Voltage
s	second
bp	base pair

Cu	Copper
Cd	Cadmium
Sp.	Species
Pb	Lead
Ni	Nickel
≥	Greater than or equal to
%	Percentage

CHAPTER 1

INTRODUCTION

Fungi are eukaryotic organisms that are different from animal, plant and the smaller kingdoms. The common fungi include yeasts, molds, mushrooms and corals. Fungi are very unique among microorganisms as they produce variety of extracellular enzymes that can degrade different hazardous chemicals. Several types of fungi that were identified by previous researchers that have shown the ability for bioremediation include filamentous fungi. Some fungi have the ability on bioremediation towards heavy metals or hydrocarbons was identified but unfortunately the fungal species was not known. Identification of the exact fungal species will allow researchers to gain more information and other researcher can search details of the specific fungi species in the future research (Salvamani, Baskaran & Nawawi, 2014).

Species of fungi can be identified using the traditional method known as morphological method which are based on the phenotype or colony-producing characteristics formed as a means of identifying fungal species (Hyde, Elsalam & Cai, 2010). Sometimes, there are only limited morphology characteristics that can be used for identification hence it is challenging to identify the exact fungal species. For instance, endophytes or endolithic fungi which are usually used for separating secondary metabolites do not sporulate frequently hence they do not provide any morphological characters for identification (Hyde & Soytong, 2008).

Fungal species can be identified by the conventional morphology method but it is not an accurate identification method and often lead to error in interpretation. Hence, the main focus in this research is on the molecular identification which can accurately identify the exact fungal species through various steps. Molecular techniques are the technologies using DNA extraction and polymerase chain reaction and sequence analysis to identify the exact species. Database is used as a source with the help of bioinformatics tools to compare and identify the species (Youusuf & Kerstin, 2010). Molecular approach is a simple and easy method that can efficiently extract fungal DNA and use as a template for PCR amplification thus the quantity and quality of samples can be taken for molecular assays. This can be applied to the fungi from many environmental sources as well as filamentous fungi that obtained from the soil.

This allows the characterization and identification of fungi communities in this project (Salvamani et al.,2014)

Also, in this research we are looking for the fungi that able to produce biosurfactants (Bhardwaj, Cameotra & Chopra, 2013). This is because some of the biosurfactant can be alternatively uses as synthetic medicine for therapeutic usage (Banat, Teixeira & Oliveira, 2006). The advantages of producing biosurfactant from fungi are that it is not costly, surfactants have lower toxicity and work well in extreme temperature and pH value. This research is mainly focused in identifying fungal species thus we can clearly know which fungi can be used for bioremediation in polluted environments.

The objective for this research project are as follows:

1. To identify fungal species using molecular identification such as DNA Extraction, PCR Amplification, Gel Electrophoresis and Sequence Analysis.
2. To identify fungal species that has the ability to produce biosurfactants.