

**IDENTIFYING POTENTIAL FUNGI SPECIES THROUGH MOLECULAR
APPROACH AND ITS BIOSURFACTANTS PRODUCTION**

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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*, *Penicillium 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.

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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 & Soyong, 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.