Isolation, Identification and Characterization of Bacterial Community in Different Agricultural Land Uses in Betong, Sarawak

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Isolation, Identification and Characterization of Bacterial Community in Different Agricultural Land Uses in Betong, Sarawak

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A thesis submitted in partial fulfilment of the Requirement of The Degree Bachelor of Science with Honours
(Resource Biotechnology)

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ABSTRACT

The increase in land usage for agricultural purposes may influence the soil biota. The present study aims to isolate soil bacteria from different agricultural land uses in Betong, identify them, and characterize them to determine their possible effects on agriculture and farm workers. Soil samples from four different crops area which where oil palm, rubber, cocoa, and pepper were analyzed. A sum of 15 isolates were cultured on nutrient agar and Mueller Hinton Agar, identified using 16S rRNA Sequencing, characterized using AGE and Gram Stain, and analysed using BLAST tool. The antibiotic susceptibility of these isolates were also tested against eight antibiotics. Results showed that most of the isolates could be categorized under four genera which were *Bacillus*, *Enterobacter*, *Pseudomonas*, and *Burkholderia*. Most of the isolates were found to be PGPRs that were beneficial to crop growth and health. The MAR index result also showed that 100 % of the isolates have a high risk of contamination and could be a potential hazard to people who are in direct contact with the soil in the area. Twelve isolates showed highest susceptibility to the antibiotic Imipenem making it a possible tool to counter harmful soil bacteria. Further research on Sarawak's soil bacteria should be conducted because of its significant environmental and human health implications.

Key words: Agricultural Land Use, PGPR, Antibiotic Susceptibility, Bacterial Communities

ABSTRAK

Peningkatan penggunaan tanah bagi tujuan pertanian berpotensi mempengaruhi biota tanah. Kajian ini bertujuan untuk mengasingkan bakteria tanah daripada kegunaan tanah pertanian yang berbeza di Betong dan mengenal past serta mencirikan mereka untuk menentukan kesan bakteria tanah tersebut kepada ladang dan pekerja pertanian. Sampel tanah daripada empat kawasan tanaman berbeza iaitu kelapa sawit, getah, koko dan lada telah dianalisis. Sejumlah 15 isolat telah dikultur pada agar nutrien dan agar Mueller-Hinton, dikenal pasti menggunakan Penjujukan rRNA 16S, dicirikan menggunakan AGE dan Gram Stain serta dianalisis menggunakan alat BLAST. Kerentanan antibiotik bagi isolat juga telah diuji menggunakan lapan antibiotik. Keputusan menunjukkan bahawa kebanyakkan isolat boleh dikategorikan di bawah empat genera iaitu Bacillus, Enterobacter, Pseudomonas, dan Burkholderia. Kebanyakan isolat didapati merupakan PGPR yang bermanfaat untuk pertumbuhan dan kesihatan tanaman. Keputusan indeks MAR juga menunjukkan bahawa 100 % daripada isolat mempunyai risiko pencemaran yang tinggi dan berpotensi untuk membahayakan orang yang bersentuhan langsung dengan tanah di kawasan tersebut. Dua belas isolat menunjukkan kerentanan tertinggi terhadap antibiotik Imipenem dan boleh dijadikan alat untuk menentang isolat tanah berbahaya. Kajian lanjut mengenai bakteria tanah Sarawak perlu dijalankan kerana kesannya yang penting terhadap alam sekitar dan kesihatan manusia.

***Kata kunci**: Guna Tanah Pertanian, PGPR, Kecenderungan Antibiotik, Komuniti Bakteria*

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LIST OF ABBREVIATIONS

°	Degree
℃	Degree celsius
%	Percentage
µg	Microgram
µl	Microliter
AGE	Agarose gel electrophoresis
bp	Base pair
BLAST	Basic local alignment search tool
cm	Centimetre
ddH_2O	Double distilled water
DNA	Deoxyribonucleic acid
dNTP	Deoxyribonucleoside triphosphates
g	Gram
mA	Milliampere
$MgCl_2$	Basic local alignment tool
ml	Milliliter
mm	Millimeter
rRNA	Ribosomal ribonucleic acid

RNA	Ribonucleic acid
rpm	Revolutions per minute
NaCl	Sodium chloride
NCBI	National Center for Biotechnology Information
PCR	Polymerase chain reaction
UV	Ultra violet
V	Volt

CHAPTER 1: INTRODUCTION

1.1 Study Background

The agriculture sector in Malaysia plays an essential part in the country's economic development. In the year 2009, the sector was shown to have contributed to 4% of the country's gross national income (GNI) which amounts to approximately RM20 billion (Matahir et al., 2013). Approximately 4.06 million hectares of Malaysian land have been set aside for agricultural purposes, with 80% of the aforementioned land being used to cultivate industrial crops of economic importance such as pepper, palm oil and rubber (Murad et al., 2008). Land use related to agricultural intensification is the focal point of global change in tropical countries such as Malaysia. Malaysia has shown great economic growth over the years, owing a huge part of it particularly to the palm oil industry. In relation to the global market, Malaysia and Indonesia contributed to 90% of the palm oil export trade and is predicted to continue being the key participants in the palm oil business (Sumathi et al., 2008).

Although intensive agriculture is important to cater to the global food supply, these anthropogenic disturbances may affect the soil biota (De carvalho et al., 2016). According to Swift (2001), the systematic usage of land for agriculture purposes have a significant effect on the macrofauna of their respective soils. Commercial land usage may alter the properties of the soil and consequently lead to difference in microbial community present in each soil. Conventional agriculture have resulted in the increased use of pesticides and fertilizers in the soils. According to Singh et al. (2011), the increased usage of chemicals will eventually lead to reduced soil vitality and loss of useful microbes. To feed an ever-increasing global population, agricultural approaches that allow for a sustainable rise in productivity are becoming increasingly important.

According to De Oliveira-Longatti et al. (2013), some soil bacteria exhibit plant growth enhancing abilities. Interactions between crops and their surrounding microbes can bring agronomic benefits including improved nutrient absorption and nitrogen fixation (Liu et al., 2017). These bacteria act as growth promoters via Biological Nitrogen Fixation (BNF) mechanism that directly impacts plant development. The *Burkholderia* genus, which thrives in a free-living condition within the soil, is a well-known example of nitrogen-fixing bacteria. Agronomic interest is raised for the biological nitrogen fixation ability of soil bacteria since it is beneficial to both the agriculture system and the natural ecology.

Although soil bacteria can be potential growth promoters for industrial crops, the soil environment may also harbour potential pathogenic bacteria that are harmful to humans. According to Baumgardner (2012), pathogenic soil microbes can be transferred to human hosts through direct contact with open wounds or indirect ingestion via contaminated food. These bacteria may damage the nervous or respiratory system. Thus, the soil ecosystem in which conventional agricultural practices take place may harbour pathogens that can possibly harm farmers working on the site.

Hence, the soil bacteria present in a certain soil ecosystem may vary depending on their land use and development. Due to a wide variation of soil bacteria, they may either be beneficial for agriculture or harmful for the workers in the field. Unfortunately, not enough research has been done to determine the bacterial composition of soil bacteria in the agricultural land uses of Sarawak. As such, not enough published data is available regarding these soil bacteria and their biological and chemical effects to Sarawak's

agriculture and manpower. This thesis aims to isolate, identify, and subsequently characterize soil bacteria composition of different agricultural land uses in Betong, Sarawak to determine whether the bacterial pool is beneficial for Sarawak's agriculture sector or vice versa.

1.2 Research Objectives

The objectives of this thesis are as follows:

1. To isolate, identify and characterize soil bacteria from different agricultural land uses in Betong.

2. To analyse the environmental roles of soil bacteria and its effects on Sarawak's agriculture and manpower.

3. To determine the presence of soil bacteria that can cause potential harm and identify which antibiotics can negate their negative effects.

CHAPTER 2: LITERATURE REVIEW

In this section, several important terms related to the research topic are introduced and elaborated to touch on the important aspects revolving around the subject matter which is the bacterial community present within the top soils of agricultural land uses in Betong, Sarawak. Additionally, several principles involved to efficiently analyse the bacterial community was elucidated.

2.1 Bacterial Community

A bacterial community can be roughly defined as a variety of bacteria living in the same location. Bacterial communities may differ from each other in terms of species composition, niche inhabited, and environmental impact. According to Stubbendieck et al. (2016), due to these intricacies, bacterial communities cannot be defined by a single basic description. These bacteria exist in intricate multi-species groups in the environment. Kusai and Ayob (2020) stated that bacteria are adaptable creatures that can survive in different habitat and climatic zones. Many bacterial dispersion studies have been conducted in severe soil niches. Some examples of severe soil niches include permafrost soil and saline soil. Soil bacteria play a significant part in organic matter mineralization, carbon and nitrogen biogeochemical cycling, and a range of other soil processes (Lin et al., 2019). The bacterial community existing in different locations may vary depending on its respective environmental conditions. Several aspects of the environment such as water table, temperature, pH, as well as organic matter composition may influence the bacterial communities in soils.

2.1.1 Beneficial Soil Bacteria

According to Hayat et al. (2010), plant growth promoting rhizobacteria (PGPRs) are soil bacteria that contribute significantly in the enhancement of crop growth. Hayat et al. stated that PGPRs are linked to the rhizosphere which is a crucial biological habitat for soil microbe-plant interactions. Soil bacteria involved in nitrogen fixation can either be free-living or have symbiotic relationships with their host plant. Nitrogen-fixing bacteria that are symbiotic in nature include soil bacteria from the genus *Rhizobium*, *Allorhizobium*, and *Bradyrhizobium*. Soil bacteria that are free living include bacteria from genus *Bacillus*, *Enterobacter*, *Pseudomonas*, and *Burkholderia*. PGPRs are capable of aiding in the promotion of sustainable crop growth. In general, PGPRs work in three ways. Firstly, PGPRs help manufacture specific chemicals vital for the growth of crops such as auxin. Next, PGPRs enable the uptake of specific nutrients present within the soil. Thirdly, PGPRs are capable of mitigating plant diseases that can affect the production of crops.

2.1.2 Pathogenic Soil Bacteria

Soil bacteria have long been associated with serious human diseases such as botulism and tetanus (Baumgardner, 2012). Both botulism and tetanus can be caused by soil bacteria from the genus *Clostridium*. The transmission of soil bacteria to human patients can be via direct contact with soil pathogens in highly contaminated areas, ingestion of contaminated food and drinks, or even inhalation of bioaerosols. Open wounds or injuries in areas contaminated with soil pathogens can also cause soil minerals to enter which in turn increases infection by decreasing the defenses of the local host. Ingestion of pathogen may be direct or indirect and lead to symptoms such as vomiting and diarrhoea. Pathogenic soil bacteria are more likely to effect immunologically compromised individuals.

2.2 Land Use

Land use is the conversion of the natural environment into land used for economic and cultural purposes. Land use related to agricultural intensification is the focal point of global change in tropical countries such as Malaysia. A major part of the Malaysian greenery is either labeled as protected areas or transformed into plantations for crops of economic importance such as oil palm and rubber. The reduction of biodiversity above the ground due to deforestation to make way for agriculture activities may cause animal and plant variety to dwindle (De carvalho et al., 2016). Conversely, converting forests to agricultural systems can boost mineral nutrient availability and minimize soil acidity and potentially enhance soil bacteria growth (De souza et al., 2009). According to Swift (2001), the systematic usage of land for agriculture purposes have a significant effect on the macrofauna of their respective soils. Commercial land usage may alter the properties of the soil and consequently lead to difference in microbial community present in each soil.

2.3 Spreading Plate Method

According to Sanders (2012), the spreading plate technique is often applied for separation of different bacteria colonies present within a sample. The separation is done by spreading the samples across the surface of a culture medium using an L-shaped spreader. This technique will enable the formation of prominent colonies that are equally scattered across the culture medium. The spreading plate method is usually used for procedures that involve enrichment, screening, as well as selection processes. Sanders stated that the spread plate method is preferably used for experiments that require isolation of colonies in order to conduct additional studies since colonies from spread plate technique can be accessed

easily. In contrast, it is difficult to access cells obtained from methods such as the pour plate because the colonies get buried in the agar. Figure 1 shows the difference between pour plate method and spread plate method.

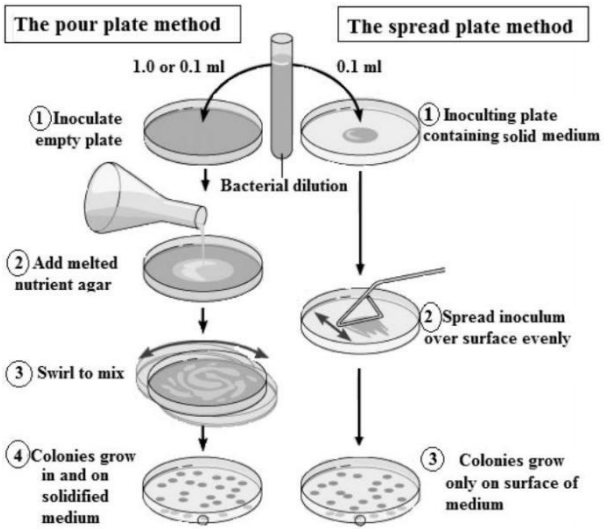

Figure 1 : Differences Between Pour Plate Method and Spread Plate Method.

Note. This figure was produced by Alnaimat and Abushattal in 2012, showing the differences between pour plate method and spread plate method. From "Techniques for isolation of pure culture.", by Alnaimat, S. M., & Abushattal, S. (2012). Techniques for isolation of pure culture. *Laboratory Manual inGeneral Microbiology For Undergraduate Students.*, 15–17.

2.4 Streaking Plate Method

The streak plate method is often utilized for the separation of pure bacterium culture from mixed microbe populations (Sanders, 2012). A single colony can be formed when millions of cells cluster together in a culture medium in which agar plates are usually used as the culture medium. In contrast to a single cell of bacteria, a bacterial colony may be visible with the naked eyes. Theoretically, all the cells present in the same colony are derived from one bacterium so they are all identical in terms of genetics. The streaking plate method is done by dispersing a mixture of cells across a culture media surface using the metal loop. The quadrant method is applied when streaking the cells in order to "thin out" the bacterial cells. By doing so, the density of bacterial cells will decrease with each streak

and the remaining cells will be distributed evenly on the culture media. These single cells will eventually developed into colonies following an incubation period. Figure 2 illustrates the spreading plate process.

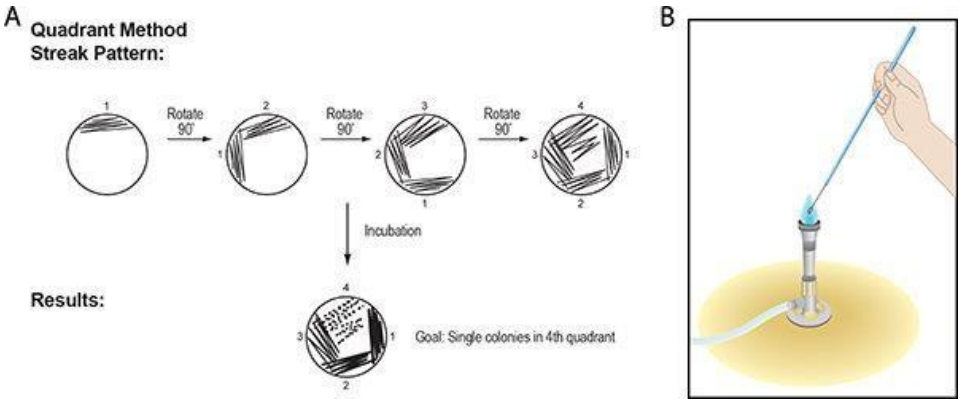

Figure 2 : Streaking Plate Method.

Note. This figure was produced by Sanders in 2012, demonstrating the quadrant method of streaking plate technique. From "Aseptic Laboratory Techniques: Plating Methods.", by Sanders, E. R. (2012). Aseptic Laboratory Techniques: Plating Methods. Journal of Visualized Experiments, (63). doi:10.3791/3064.

2.5 16S rRNA Sequencing

The 16S rRNA Sequencing method is a cutting-edge molecular method of identifying bacterial isolates living in a complex community (Osman et al., 2018). This method targets the 16S small subunit ribosomal gene that exists inside prokaryotes. These 16S rRNA genes functions to assemble ribosomes which is important for bacteria and have variable regions that may be utilized as fingerprints to identify specific bacteria species. Because of these characteristics, the 16S rRNA gene has become a popular genetic segment for bacterial identification. Prior to sequencing, a Polymerase Chain Reaction (PCR) machine will be used for amplification of a selected variable region of the targeted bacteria isolate. A universal PCR primer is commonly used to target the conserved part of the 16S component, enabling the possible amplification of a wide variety of different soil bacteria within a single soil sample (Mignard et al., 2006).

2.6 Agarose Gel Electrophoresis (AGE)

According to Mesapogu et al. (2013), agarose gel electrophoresis techniques are utilized for the process of separating fragments of DNA based on their sizes. These DNA fragments can range between a mere 50 bp to multiple megabases. “Gel” is a term usually used to indicate a matrix that is used to hold and differentiate molecules according to their respective sizes. The specific composition and weight of a target molecule can be used to determine the porosity and composition of a gel since it is typically a cross-linked form of polymer. Gels made up of various concentrations of acrylamide and a cross linker are used to separate proteins and small nucleic acids like DNA or RNA, resulting in varied sized polyacrylamide mesh networks. Gel visualization will be done under an ultra-violet (UV) light to ascertain the existence of single DNA fragments.

2.7 Basic Local Alignment Search Tool (BLAST)

Computational techniques with biological data have become an important aspect of biology in recent years, notably in studies involving protein or DNA sequences (Eric et al., 2014). The field of bioinformatics have played a significant part in increasing our understanding for biology by evaluating the similarities found between different biological sequences. Basic Local Alignment Search Tool (BLAST) is commonly used to compare two distinct sequence pairs based on areas with local similarity and is easily accessible online in the NCBI Genbank. Nowadays, the BLAST program is used to accomplish a variety of goals such as identification of species, domain location, mapping of DNA and DNA annotation.