



UNIVERSITI PUTRA MALAYSIA

***ANTIBACTERIAL ACTIVITY AND CHARACTERIZATION OF
ENDOPHYTIC BACTERIA FROM SELECTED MEDICINAL PLANTS OF
MALAYSIA***

MOHAMED IKHTIFAR BIN MOHAMED RAFI

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BACTERIA FROM SELECTED MEDICINAL PLANTS OF MALAYSIA**

MOHAMED IKHTIFAR BIN MOHAMED RAFI

**Thesis Submitted to the School of Graduate Studies, Universiti Putra
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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

ANTIBACTERIAL ACTIVITY AND CHARACTERIZATION OF ENDOPHYTIC BACTERIA FROM SELECTED MEDICINAL PLANTS OF MALAYSIA

By

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November 2018

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Malaysia is blessed with rich biodiversity and her people have harnessed this virtue in many ways such as for medicinal uses. However, not all of the plants could be easily grown or harvest for its bioactive natural products for use. Endophytes which are microorganisms that live in plants could be the key in solving this conundrum. Many past studies have shown that endophytes could have the same properties as the host and proven to produce anti-bacterial or anti-cancer compounds. The aims of this study is to characterize the endophytic bacteria isolates which has been isolated from known medicinal plants such as *Senna obtusifolia* (SK 2283/13), *Leea indica* (SK 2285/13), *Pereskia sacharosa* (SK 2286/13) and *Isotoma longiflora* (SK 2288/13) according their phenotypic traits and genotypic traits. The phenotypic traits that were taken into consideration were the isolation media, the parts of the plants that the isolates were isolated from, the growth conditions such growth rate and temperature, type of gram, antibiogram profile of the isolates, anti-bacterial susceptibility test against bacterial pathogens. The genotypic traits were deduced by using Random Amplification of Polymorphic DNA (RAPD). This study have found that the highest number of isolates were isolated from *Senna obtusifolia* and the highest number of isolates that were isolated are from the roots of the plants. The best isolation media to be used for isolating the isolates is Starch-Casein Agar. 36 isolates were gram negative and 27 isolates were gram positive in nature. In this study, it was discovered that most of the isolates were susceptible towards Chloramphenicol and resistant towards Gentamicin. The dichloromethane extracts of 28 bacterial isolates shown inhibition towards 9 bacterial pathogens which are *Acinobacter baumanii*, *Bacillus cereus*, *Bacillus subtilis*, *Enterobacter cloacae*, *Klebsiella pneumonia*, *MRSA*, *Proteus vulgaris*, *Salmonella Paratyphi A* and *Staphylococcus aureus*. For the genotypic traits, the genomic DNA of the isolates were amplified successfully by using Primers OPO 10 and OPO 16. In conclusion, the endophytic bacteria isolates were characterized by their phenotypic and genotypic traits and it was found that 9

single isolates clusters which were (ATC)DB3-B, (PDA)IB(B1)-B, (PDA)IF(B1)-B, (SCA)FSe(B1)-B, (PDA)IS(B1)-B, (SCA)IL(B1)-B and (ATC)IF(B1)-B were unique based on both phenotypic and genotypic traits of the isolates.



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AKTIVITI ANTIBAKTERIA DAN KARAKTERISTIK BAKTERIA ENDOFIT DARIPADA TUMBUHAN PERUBATAN TERPILIH DI MALAYSIA

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Malaysia adalah sebuah negara yang diberkati dengan kepelbagaiannya biodiversiti yang kaya dan rakyatnya memanfaatkan kebaikan ini dalam pelbagai cara seperti dalam penggunaan ubat. Tetapi, bukan semua tumbuhan yang mempunyai kegunaan sebagai ubatan boleh ditanam dan tidak senang untuk menpencarkan sebatian yang mempunyai sifat bioaktif. Endofit adalah mikroorganisma yang tinggal di dalam tumbuh-tumbuhan boleh menjadi kunci dalam menyelesaikan masalah ini.. Banyak kajian terdahulu telah menunjukkan bahawa endofit boleh mempunyai nilai perubatan yang sama dengan tuan rumahnya dan telah terbukti dapat menghasilkan sebatian anti-bakteria atau anti-kanser. Tujuan kajian ini adalah untuk mencirikan isolat bakteria endofit yang telah dipencarkan daripada tumbuh-tumbuhan perubatan yang dikenali seperti *Senna obtusifolia* (SK 2283/13), *Leea indica* (SK 2285/13), *Pereskia sacharosa* (SK 2286/13) dan *Isotoma longiflora* (SK 2288/13) dari segi sifat fenotip dan sifat genotipnya. Ciri-ciri fenotip yang telah dipertimbangkan adalah media pengasingan yang digunakan, bahagian-bahagian tumbuhan yang isolat diasingkan dari, keadaan pertumbuhan seperti kadar pertumbuhan dan suhu, jenis gram, profil antibiogram isolat, ujian kerentanan terhadap bakteria terhadap patogen bakteria. Ciri-ciri genotip telah disimpulkan dengan menggunakan Random Amplification of Polymorphic DNA (RAPD). Kajian ini mendapati bahawa bilangan isolat tertinggi telah dipencarkan dari *Senna obtusifolia* dan bilangan isolat tertinggi yang telah terpencil adalah dari akar tumbuhan. Media pengasingan yang terbaik untuk digunakan untuk mengasingkan isolat adalah Starch-Casein Agar. 36 isolat adalah gram negatif dan 27 isolat adalah gram positif. Dalam kajian ini, didapati bahawa kebanyakan isolat mempunyai kerintangan rendah kepada Chloramphenicol dan rintang terhadap Gentamicin. Ekstrak diklorometana daripada 28 isolat bakteria menunjukkan perencutan terhadap 9 bakteria bakteria iaitu *Acinobacter baumanii*, *Bacillus cereus*, *Bacillus subtilis*, *Enterobacter cloacae*, *Klebsiella pneumonia*, *MRSA*, *Proteus vulgaris*, *Salmonella Paratyphi A* dan *Staphylococcus aureus*. Bagi ciri genotip,

DNA genomik isolat telah berjaya diamplifikasi dengan menggunakan Primer OPO 10 dan OPO 16. Kesimpulannya, bakteria endophytic telah berjaya dicirikan oleh ciri-ciri fenotip dan genotip mereka dan didapati bahawa 9 kumpulan isolat tunggal iaitu (ATC (B1) -B, (PDA) IF (B1) -B, (SCA) FSe (B1) -B, (PDA) IS (B1) -B, (SCA) IL (B1) -B dan (ATC) IF (B1) -B adalah unik berdasarkan sifat fenotip dan genotip isolat.



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CHAPTER 1

INTRODUCTION

1.1 General Introduction

Malaysia is a country which is blessed with high biodiversity and rich in ethnopharmacological knowledge due to the melting pot of its people. Each of the races in the country has their own understanding of ethnopharmacology and have different usages of the same plant. This increase the importance of the plants and making it a valuable source for bio prospecting.

However, with the rapid pace of development and deforestation, the plants which were highly prized as medicine are threatened with extinction which causes to lose a valuable source for drug discovery for various diseases. Even though, most chemists have successfully synthetized compounds that were isolated from Nature and other new compounds were synthetized in laboratories, some of the synthetized compounds do not have the same effects nor have the same chirality as natural compounds. Also, the chemists had a few challenges which are 1) no suitable starter materials 2) synthetized compounds have high toxicity and 3) hard to synthetize (Bankova, 2007; Hong, 2014). Therefore, an alternative producer or source is needed to replace the plants. Endophytes are microorganisms that lives in the plants without causing any harm to the plants. Endophytes could divided into three categories which are fungi, bacteria and algae. A study done by Strobel et al. (1996) have shown that endophytes could produce the same compounds as the host plant. Following his study, many other researchers had found similar results.

This study aims to characterize the previously isolated bacterial endophytes and determine if the isolates could show anti-microbial properties similar to their host plants.

1.2 Problem Statement

Due to emergence of multi-resistant strains of bacteria and the lack of antibiotic research in recent years have prompted researchers especially chemists and biologists to address the problem. The emergence of multi-resistant strains of bacteria is caused by various factors such as air travel, improper prescription of antibiotics, lack of hygiene and the ability of the microorganisms to evolve (Unemo & Nicholas., 2012). The lack of antibiotic research is caused by the shift of focus in drug discovery to focus on cancer. This shift of focus is due to multi factors such as the availability of grants, lack of incentives and policies (Mossialos & Morel, 2010). The need to explore new sources of bioactive compounds as anti-microbial drug especially from natural products of bacterial

endophytes are evident as natural products and its bioactive properties are largely untapped. The plants which were selected for the isolation of the bacterial endophytes are due to their antibacterial properties (Rahman, Imran & Islam, 2003; Chung et al., 2004; Doughari, El-Mahmood & Tyoyina, 2008; Philip et al., 2009; Rosidah, Lestari & Astuti, 2014).

1.3 Significance of Study

The significance of the study is to characterize the bacterial endophytes according to their phenotypic and genotypic characterization and also to identify bacterial endophytes which have anti – bacterial properties which would be useful for future study and therefore aims to achieve 2 Global Sustainable Development Goals which are 1) Good health and promote well-being for all at all ages and 2) Life on land (www.un.org). The goals will be achieved by discovering new sources for anti-bacterial compounds other than the plants itself and therefore will reduce the need to harvest the plants for anti-bacterial compounds in the future.

1.4 Conceptual Framework

Figure 1.1 shows the conceptual framework for this study. Previously, the bacterial endophytes were isolated by Dr Elaine Chin Jinfeng and stored in -20°C freezer. The isolates were revived and phenotypic and genotypic characterization were done on the isolates. For the characterization of phenotypic traits of the isolates, the growth rate, growth temperature, antibiogram and anti-bacterial susceptibility test were done. While the characterization of genotypic traits of the isolates, Random Amplified Polymorphic DNA (RAPD) was used. Finally, based on these phenotypic and genotypic traits, the isolates were characterized using Bionumerics 6.6.

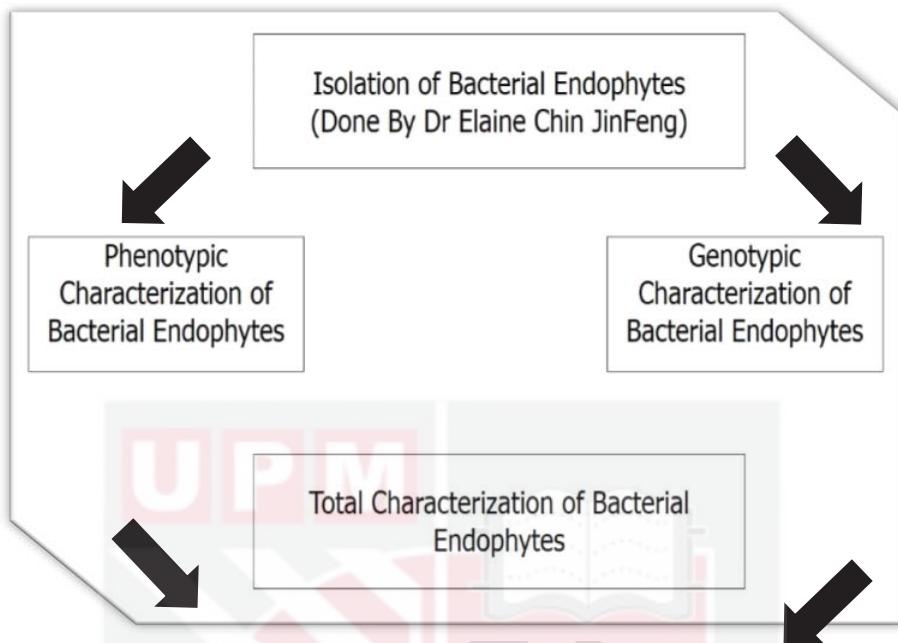


Figure 1.1 : Conceptual Framework of the Research

1.5 Research Questions

The following research questions guided this study:

- 1) What are the phenotypic or genotypic characteristics of the previously isolated endophytic bacteria?
- 2) Do the endophytic bacteria produce bioactive compounds which have anti-bacterial properties towards gram positive or gram negative pathogens?
- 3) Can the endophytic bacteria be clustered by their phenotypic and genotypic characteristics?

1.6 Objectives of Study

1.6.1 General Objective

To characterize the isolates from the previously isolated endophytic bacteria of selected Malaysian medicinal plants according to their phenotypic and genotypic traits and elucidate potential isolates that have bioactive extract(s)

1.6.2 Specific Objectives

- 1) To characterize the phenotypic and genotypic traits of endophytic bacteria
- 2) To screen the anti-bacterial properties of the endophytic bacteria towards gram positive and gram negative pathogens
- 3) To identify the endophytic bacteria which has anti-bacterial properties by using 16S rDNA PCR

1.7 Hypothesis of Study

The previously isolated endophytic bacteria from selected Malaysian medicinal plants could be characterize according to their phenotypic traits such as growth rate, antibiogram and morphology. It is also hypothesized that the endophytic bacteria could produce bioactive extract(s) which could have anti-bacterial properties.

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