
**UNIVERSITI PUTRA MALAYSIA**

# *CHARACTERIZATION OF PLANT GROWTH-PROMOTING BACTERIA FROM KACIP FATIMAH (Labisia pumila BENTH. & HOOK. F.) AND ITS EFFECTS ON GROWTH OF THE PLANT*

**YASMI BINTI AWANG**

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**By**

**YASMI BINTI AWANG**

**Thesis submitted to the school of Graduate Studies, Universiti Putra Malaysia, in fulfillment of the Requirement for the Degree of Master of Science**

**December 2015**

*DEDICATED TO MY HUSBAND, MY BELOVED PARENTS AND MY SIBLINGS WHO HAVE SHOWERED ME WITH UNCONDITIONAL LOVE AND UNENDING SUPPORT*

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfillment of the requirement for the Degree of Master of Science

**CHARACTERIZATION OF PLANT GROWTH-PROMOTING BACTERIA FROM KACIP FATIMAH (*Labisia pumila* BENTH. & HOOK. F.) AND ITS EFFECTS ON GROWTH OF THE PLANT**

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**YASMI BINTI AWANG**

**December 2015**

**Chairman: Associate Professor Radziah Othman, PhD**
**Faculty: Agriculture**

Kacip Fatimah (*Labisia pumila*) is one of the major medicinal plant species in Malaysia. Mass production of this plant is difficult as it grows very slow under cultivated condition. Plant growth-promoting bacteria (PGPB) are associated with many plant species and commonly present in many environments. These beneficial rhizosphere organisms in Kacip Fatimah could improve the plant growth and its phytochemical properties. A series of experiments were conducted in laboratory and glasshouse conditions with the following objectives; (i) to isolate and characterize the beneficial traits of plant growth-promoting bacteria (PGPB) isolated from Kacip Fatimah (*Labisia pumila*) established under natural forest, (ii) to evaluate the effects of PGPB on growth and chemical properties of Kacip Fatimah (*Labisia pumila*) under glasshouse conditions, and (iii) to determine the effects of PGPB on Kacip Fatimah (*Labisia pumila*) root colonization. Bacterial populations from Kacip Fatimah plants sampled from two natural forest sites were determined on nutrient agar (NA) media plates and screened for plant growth promoting traits. Two promising bacterial isolates were subjected to partial sequence polymerase chain reaction (PCR) identified as *Stenotrophomonas* sp. and *Exiguobacterium* sp. were chosen for the plant inoculation study in glasshouse condition. Two varieties of 2 months old Kacip Fatimah plants were planted in 14' x 14' polybag containing mixed cocopeat, rice husk and chicken dung at the ratio of 5:5:1. The experiment was laid out in a Completely Randomized Design (CRD) with five replications. The bacterial populations differed among plant parts and varieties. In *L. pumila* var. *alata*, high bacterial population was found in the phyllosphere and low in root endosphere. In contrast, *L. pumila* var. *lanceolata*, showed higher population in root endosphere and low in phyllosphere. The Gram reaction showed that 12 isolates were Gram-negative and 25 were Gram-positive. All bacteria were able to grow on N-free solid malate medium except two of the isolates. Thirteen of the isolates were able to solubilize calcium phosphate. Six of the isolates were positive for carboxymethylcellulase (CMC) and citrus pectin test. Only one of the isolate showed positive for both carboxymethylcellulase (CMC) and negative for citrus pectin. Most of strains were able to produce indole-acetic acid (IAA). Twenty-four of

the isolates showed positive result for catalase test and 22 isolates showed positive result for starch hydrolysis. There were some differences in the plant growth parameters with bacterial inoculation. Inoculation of PGPB seems able to affect the growth of Kacip Fatimah for both of var. *lanceolata* and var. *alata* under glasshouse. *Exiguobacterium* sp.increased bacterial populations in rhizosphere and stem endosphere of var. *alata*, increased flavonoid content and enhanced stomatal conductance of var. *lanceolata. Stenotrophomonas* sp. influenced transpiration rate and stomatal conductance and bacterial population in non-rhizosphere of var. *alata*, improved leaf width of var. *lanceolata* and increased stem diameter and bacterial population in root endosphere of both varieties. Dual inoculation influenced Kacip Fatimah growth as it increased leaf length and leaf width of var. *alata*, transpiration rate and water vapor deposit of var. *lanceolata*, total phenolic content and photosynthesis rate for both varieties. There were no significant difference in root biomass, shoot biomass and leaf area index of Kacip Fatimah plants with the bacterial inoculations. Scanning Electron Microscope (SEM) observations revealed the ability of the bacterial isolates to colonize the root surface. In general, plant growth-promoting bacteria isolated from Kacip Fatimah were able to give effects on the growth of the plant through several growth promoting traits.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Master Sains

**KARAKTERISTIK BAKTERIA PENGGALAK TUMBESARAN TANAMAN DARIPADA POKOK KACIP FATIMAH (*Labisia pumila* BENTH. & HOOK. F.) DAN KESANNYA KE ATAS TUMBESARAN POKOK**

Oleh

**YASMI BINTI AWANG**

**Disember 2015**

**Pengerusi: Profesor Madya Radziah Othman, PhD**
**Fakulti: Pertanian**

Kacip Fatimah (*Labisia pumila*) adalah salah satu spesis tumbuhan ubatan utama di Malaysia. Pengeluaran besar-besaran tumbuhan ini adalah sukar kerana ia tumbuh dengan perlahan dalam keadaan yang bukan semulajadi. Bakteria penggalak tumbesaran didapati berkait dengan banyak spesis tumbuhan dan biasa dijumpai di kebanyakan tempat. Organisma yang berfaedah di rizosfera pokok Kacip Fatimah ini diharap dapat meningkatkan dan membantu tumbesaran dan fitokimia pokok . Beberapa kajian telah dijalankan di makmal dan rumah kaca berdasarkan objektif berikut; (i) untuk mengasingkan bakteria penggalak tumbesaran yg diisolasi daripada pokok Kacip Fatimah yang diperolehi daripada hutan dan mengenalpasti ciri-ciri berfaedah, (ii) untuk menilai kesan bakteria penggalak tumbesaran ke atas tumbesaran dan ciri-ciri kimia pokok Kacip Fatimah (*Labisia pumila*) di dalam rumah kaca, dan (iii) untuk memerhati kesan bakteria penggalak tumbesaran ke atas kolonisasi akar Kacip Fatimah (*Labisia pumila*). Populasi bakteria daripada pokok Kacip Fatimah yang diambil daripada dua kawasan hutan semulajadi ditentukan di atas agar nutrisi (NA) media dan dipilih untuk ciri-ciri penggalak tumbesaran. Dua isolat bakteria yang berpotensi telah dikenalpasti dengan kaedah reaksi rantaian polymerase (PCR) sebagai *Stenotrophomonas* sp. dan *Exiguobacterium* sp. telah dipilih untuk diinokulasi ke atas tumbuhan kajian di dalam rumah kaca. Dua varieti pokok Kacip Fatimah (*L. pumila* var. *alata* and *L. pumila* var. *lanceolata*) yang berumur 2 bulan telah ditanam di dalam polibeg bersaiz 14’ x 14’ mengandungi campuran habuk kelapa, sekam padi dan tahi ayam dengan nisbah 5:5:1. Eksperimen telah dijalankan dengan Rekabentuk Rawak Sepenuhnya (CRD) dengan 5 replikasi. Populasi bakteria yang diperolehi adalah berbeza di bahagian pokok dan varieti pokok yang berlainan. Populasi bakteria dijumpai lebih banyak di filosfera dan rendah di endosfera akar untuk *L. pumila* var. *alata.* Sebaliknya populasi bakteria dijumpai lebih banyak di endosfera akar dan rendah di filosfera untuk *L. pumila* var. *lanceolata*. Tindakbalas Gram telah menunjukkan 12 isolat adalah Gram negatif manakala 25 isolat adalah Gram positif. Semua bakteria boleh hidup di atas media pepejal malat tanpa N kecuali 2 isolat. Tiga belas daripada isolat berupaya melarutkan kalsium fosfat. Enam daripada isolat menunjukkan

keputusan positif untuk kedua-dua ujian carboxymethylcellulase (CMC) dan 'citrus pectin'. Hanya satu daripada isolat menunjukkan keputusan positif untuk ujian carboxymethylcellulase (CMC) dan negatif untuk 'citrus pectin'. Kebanyakan isolat berupaya untuk merembeskan asid indol-asetik (IAA). Dua puluh empat daripada isolat menunjukkan keputusan positif untuk ujian katalis dan 22 isolat menunjukkan keputusan positif untuk hidrolisis kanji. Terdapat beberapa perubahan pada parameter tumbesaran dengan inokulasi bakteria. Inokulasi dengan bakteria penggalak tumbesaran didapati boleh meningkatkan tumbesaran Kacip Fatimah bagi kedua-dua var. *lanceolata* dan var. *alata* di dalam eksperimen rumah kaca. *Exiguobacterium* sp. meningkatkan populasi bacteria di rhizosfera dan endosfera batang var. *alata*, kandungan flavonoid dan konduksi stomata var. *lanceolata*. *Stenotrophomonas* sp. mempengaruhi kadar transpirasi, konduksi stomata dan populasi bakteria di non-rhizosfera var. *alata*, meningkatkan lebar daun var. *lanceolata* serta meningkatkan diameter batang dan populasi bakteria di endosfera akar bagi kedua-dua varieti. Dwi inokulasi mempengaruhi tumbesaran Kacip Fatimah dengan meningkatkan panjang daun dan lebar daun var. *alata*, kadar transpirasi dan deposit pemeluapan air var. *lanceolata*, kandungan fenolik dan kadar fotosintesis bagi kedua-dua varieti. Pemerhatian di bawah mikroskop pengimbas electron (SEM) menunjukkan kebolehan bakteria isolat untuk mengkolonisasi kawasan permukaan akar. Secara amnya, bakteria penggalak tumbesaran yang diisolasi daripada Kacip Fatimah dapat mempengaruhi pertumbuhan pokok melalui beberapa sifat penggalak tumbesaran.

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I certify that a Thesis Examination Committee has met on 1 December 2015 to conduct the final examination of Yasmi binti Awang on her thesis entitled "Characterization of Plant Growth-Promoting Bacteria from Kacip Fatimah (*Labisia pumila* Benth. & Hook. f.) and its Effects on Growth of the Plant" in accordance with the Universities and University Colleges Act 1971 and the Constitution of the Universiti Putra Malaysia [P.U.(A) 106] 15 March 1998. The Committee recommends that the student be awarded the Master of Science.

Members of the Thesis Examination Committee were as follows:

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This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfillment of the requirement for the degree of Master of Science. The members of the Supervisory Committee were as follows:

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| | |
|---|---|
| BAP | Benzylaminopurine |
| BLAST | Basic Local Alignment Search Tool |
| CEC | Cation Exchange Capacity |
| Cfu | Colony forming unit |
| CMC | Carboxymethylcellulase |
| CRD | Completely Randomized Design |
| ECERDC | East Coast Economic Region Development Council |
| GAE | Gallic acid equivalent |
| GNP | Gross National Product |
| IAA | Indole-acetic Acid |
| IMR | Institute for Medical Research |
| LAI | Leaf area index |
| NA | Nutrient Agar |
| NB | Nutrient broth |
| NBRIP | National Botanical Research Institute's phosphate |
| NCBI | National Center for Biotechnology Information |
| NOA | No-adverse-effect-level |
| OD | Optical Density |
| PBS | Phosphate Buffered Saline |
| PGPB | Plant Growth Promoting Bacteria |
| PGPR | Plant Growth Promoting Rhizobacteria |
| PSB | Phosphate solubilizing bacteria |
| SAS | Statistical Analysis System |
| SEM | Scanning Electron Microscope |

# CHAPTER 1

## INTRODUCTION

Kacip Fatimah (*Labisia pumila*) is one of major medicinal plant species in Malaysia. It has a tremendous potential to be cultivated and developed into various value added natural products. Kacip Fatimah has been widely studied for its usage, bioassay and chemical content, but most of these plants are being taken from the wild. This is of public concern as over harvesting, destructive collection techniques and conversion of habitats to crop-based agriculture will lead to extinction. The demand for Kacip Fatimah has increased over the years but the effort to propagate this herb has not been too encouraging as it is difficult to culture away from its natural habitat.

It is well-known that 80-90% of the processes in the soil are reactions mediated by microbes (Nannipieri and Badalucco, 2003). The indigenous microorganisms are present in the soil but the community varies with plant species, plant age and location on the root and soil properties (Louise, 2004). Determining the community structure depends on the diversity of rhizosphere microbial communities, the heterogeneity of the root environment and the importance of environmental and biological factors (Baudoin et al., 2002; Berg et al., 2002).

A beneficial plant-associated bacteria or plant growth-promoting bacteria (PGPB) have been known to affect the plant growth directly or indirectly either through phytostimulation, biofertilization, bioremediation or biocontrol of plant pathogens (Bashan and de-Bashan, 2005). It can directly enhance the plant growth through the biologically fixation of atmospheric nitrogen and produce plant growth-promoting substances such as siderophores that chelate, solubilize and sequester iron (Timmusk, 2003). It also make the substance available to the plant root by increased the local availability of nutrients through phosphate solubilizing activity, synthesized and modulated phytohormones (auxins, cytokinins and gibberellins) and inhibit the growth of phytophatogens via competition for nutrients or colonization sites and synthesis of antibiotics/lytic enzymes (Lodewyckx et al., 2002). The indirect mechanism includes the ability to synthesize anti-fungal metabolites such as antibiotics which inhibit or suppress the growth of fungal pathogens, the ability to successfully compete with pathogens for nutrients or specific niches on the root, reduce the pathogen infection, as well as biotic and abiotic plant stress and the ability to induce systemic resistance (Persello et al., 2003; Compant et al., 2010). PGPB offer a safe and environmental friendly approach to increase the plant growth, yield production and also manage plant health. Currently there is insufficient information on microorganism associated with Kacip Fatimah (*Labisia pumila*). Thus, the following studies were conducted with the objectives as below:

To isolate and characterize the beneficial traits of plant growth-promoting bacteria (PGPB) from Kacip Fatimah (*Labisia pumila*) established under natural forest.

i) To evaluate the effects of selected PGPB on growth and some of the phytochemical of Kacip Fatimah (*Labisia pumila*) under glasshouse conditions.

ii) To determine the effects of selected PGPB on root colonization of Kacip Fatimah (*Labisia pumila*).

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## LIST OF PUBLICATIONS

### Published

Yasmi, A., Radziah, O., Hawa, Z.E.J., Zainal Abidin, M.A. and Panhwar, Q.A. (2016) Characterisation of Plant Growth-Promoting Bacteria from Kacip Fatimah (*Labisia pumila*) under Natural Tropical Forest. *Pertanika J. Trop. Agric. Sci*. 39 (4): 557 – 575.

### Proceeding

Yasmi, A., Radziah, O., Hawa, Z. E. J. and Zainal, A. M. A. 2011. Isolation and characterization of indigenous bacteria from Kacip Fatimah (Labisia pumila). In Soil Fertility and Plantation Productivity. Proceedings of 10th Malaysian Soil Science Society (MSSS)'s Conference on Soil (SOILS 2011), pp. 309-313. Kota Kinabalu, Sabah, Malaysia.

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