



UNIVERSITI PUTRA MALAYSIA

***POTENTIAL USE OF FUNCTIONAL BACTERIA FROM TROPICAL
REHABILITATED FOREST AS BIO-FERTILIZER***

AMELIA TANG

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**POTENTIAL USE OF FUNCTIONAL BACTERIA FROM TROPICAL
REHABILITATED FOREST AS BIO-FERTILIZER**

By

AMELIA TANG

**Thesis Submitted to the School of Graduate Studies, Universiti Putra
Malaysia, in Fulfilment of the Requirements for the Degree of Doctor of
Philosophy**

August 2017

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Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfillment of the requirement for the degree of Doctor of Philosophy

POTENTIAL USE OF FUNCTIONAL BACTERIA FROM TROPICAL REHABILITATED FOREST AS BIO-FERTILIZER

By

AMELIA TANG

August 2017

Chair: Ahmed Osumanu Haruna, PhD
Faculty: Agriculture and Food Sciences (Bintulu)

Rehabilitated forests are established in an effort to overcome the threat of major soil degradation and erosion. Soil cellulolytic, nitrogen-fixing (N-fixing), and phosphate-solubilizing microorganisms are very important functional microbial groups in regulating the elemental cycle, and plant nutrition in forest soil. Bacteria, reflected by their major abundance among microbial groups, make up a large constitution of biodiversity in soils, and they participate in soil key processes which eventually impact the whole terrestrial ecosystems operations. Relationship between soil cellulolytic, N-fixing, and phosphate-solubilizing bacterial counts, and forest stand ages of 4, 9, 14, and 19 were assessed as means to evaluate soil health of planted forests. All cellulolytic, N-fixing, and phosphate-solubilizing populations showed linear relationships with forest age. These functional bacterial populations demonstrated good potential to be adopted as soil health indicators in the rehabilitated forest. Assessment of functional bacterial population sizes should be ongoing with forest stand succession for better understanding their relationships, and growth trends. Out of 15 functional isolates, cellulolytic isolate *Serratia nematodiphila* C46d, N-fixing isolate *Burkholderia nodosa* NB1, and phosphate-solubilizing isolate *Burkholderia cepacia* PB3 with outstanding plant growth-promoting (PGP) activities were selected for a pot trial. The treatments evaluated in the pot trial were: 1) control with no bacterial inoculation, and no chemical fertilizer (Control); 2) no bacterial inoculation but with compost, and chemical fertilizer combination at 50% fertilization level (OM); 3) inoculation with C46d, and with compost and chemical fertilizer combination at 50% fertilization level (TC); 4) inoculation with NB1, and with compost, and chemical fertilizer combination at 50% fertilization level (TN); 5) inoculation with PB3, and with compost, and chemical fertilizer combination at 50% fertilization level (TP); 6) inoculation in consortium of C46d, NB1, and PB3, with compost, and chemical fertilizer combination at 50% fertilization level (TCNP); and 7) control with no bacterial inoculation, and no compost, but only with chemical fertilizer at 100% fertilization level (Chemical Fertilizer). Cellulolytic population was highest at all

bacterial treatments; N fixers dominant in TN, TP, and TCNP whereas phosphate-solubilizers flourished in TP and TCNP. Only N fixers demonstrated significant effects on plant biomass, from positive relationships with plant N and P uptake. This present study indicated presence of some efficient and effective cellulolytic, N-fixing, and phosphate-solubilizing bacterial species among the natural rhizobacterial community in soils of tropical rehabilitated forest at Universiti Putra Malaysia Bintulu Campus. These attributes are indispensable growth promoting factors for the planted trees, and natural vegetation thriving amid ongoing threat of soil degradation. Treatment with N-fixing species *Burkholderia nodosa* NB1 alone resulted in higher root and total N use efficiencies, besides leaves, roots, and total P efficiencies by multiple folds than full chemical fertilizer treatment. Incorporating PGPR isolates with compost and chemical fertilizer proved sustainable in integrated nutrient management (INM) system with minimized usage of chemical fertilizers. *Burkholderia nodosa* NB1 demonstrates good potential in future field trials before application as bio-fertilizer. Future studies should include more thorough characterization of this particular isolate prior to practical field applications.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia
sebagai memenuhi keperluan untuk ijazah Doktor Falsafah

POTENSI KEGUNAAN BAKTERIA BERFUNGSI DARI HUTAN DIPULIHARA TROIKA SEBAGAI BIO-BAJA

Oleh

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Hutan dipulihara untuk menangani ancaman degradasi dan hakisan tanah yang menular. Mikroorganisma tanah yang terdiri daripada selulolitik, pengikat-nitrogen, dan pelarut-fosfat adalah kumpulan berfungsi yang amat penting dalam pengawalan kitaran elemen dan nutrisi tumbuhan dalam tanah hutan. Bakteria, melalui bilangannya yang terbesar di antara kumpulan-kumpulan mikrob membentuk badan biodiversiti terbesar di tanah, dan mengambil bahagian dalam proses-proses utama tanah yang akhirnya memberi impak ke atas keseluruhan operasi ekosistem daratan. Hubungan antara bilangan populasi bakteria tanah selulolitik, pengikat-nitrogen, dan pelarut-fosfat dan hutan berusia 4, 9, 14, dan 19 tahun telah dinilai untuk tujuan mengevaluasi kesihatan tanah hutan dipulihara. Populasi ketiga-tiga kumpulan bakteria iaitu selulolitik, pelarut-fosfat, dan pengikat-nitrogen menunjukkan hubungan linear dengan usia hutan. Ketiga-tiga kumpulan berfungsi bakteria ini menunjukkan potensi yang baik untuk diterima sebagai petunjuk kesihatan tanah hutan dipulihara. Penilaian saiz populasi bakteria berfungsi disarankan untuk dijalankan secara berterusan seiring dengan peningkatan usia hutan untuk lebih memahami perhubungan di antara mereka, beserta dengan aliran pertumbuhan mereka. Daripada 15 isolat berfungsi, isolat selulolitik *Serratia nematodiphila* C46d, isolat pengikat-nitrogen *Burkholderia nodosa* NB1, dan isolat pelarut-fosfat *Burkholderia cepacia* PB3 yang memberikan aktiviti meningkatkan pertumbuhan pokok terbaik telah dipilih dalam eksperimen pasu yang mempunyai tujuh rawatan: 1) kawalan, tanpa inokulasi bakteria, dan tanpa baja kimia (Kawalan); 2) tanpa inokulasi bakteria, dan ditambah campuran kompos dengan baja kimia dalam tahap pembajaan sebanyak 50% (OM); 3) inokulasi dengan isolat C46d, dan ditambah campuran kompos, dan baja kimia dalam tahap pembajaan sebanyak 50% (TC); 4) inokulasi dengan isolat NB1, dan ditambah campuran kompos, dan baja kimia dalam tahap pembajaan sebanyak 50% (TN); 5) inokulasi dengan isolat PB3, dan ditambah campuran kompos, dan baja kimia dalam tahap pembajaan sebanyak 50% (TP); 6) inokulasi dengan konsortia bakteria yang terdiri daripada isolat C46d,

NB1, dan PB3 dan ditambah campuran kompos, dan baja kimia dalam tahap pembajaan sebanyak 50% (TCNP); dan 7) kawalan, tanpa inokulasi bakteria, dan tanpa kompos, tetapi hanya ditambah baja kimia dengan tahap pembajaan sebanyak 100% (Chemical Fertilizer). Populasi bakteria selulolitik adalah tertinggi dalam keseluruhan rawatan bakteria; populasi bakteria pengikat-nitrogen adalah dominan dalam rawatan TN, TP, dan TCNP, manakala populasi bakteria pelarut-fosfat adalah tertinggi dalam rawatan TP dan TCNP. Hanya bakteria pengikat-nitrogen memberikan kesan signifikan ke atas biojisim pokok hasil hubungan positif dengan pengambilan nitrogen dan fosforus pokok. Kajian ini menunjukkan kehadiran beberapa spesies bakteria selulolitik, pengikat-nitrogen, dan pelarut-fosfat yang efisien dan efektif di antara komuniti rhizobakteria semula jadi dalam tanah hutan dipulihara tropika di Universiti Putra Malaysia Kampus Bintulu. Sifat-sifat ini adalah faktor peningkatan pertumbuhan yang penting untuk pokok-pokok yang ditanam dan tumbuhan asli yang bertumbuh dengan pesat di tengah-tengah ancaman kemerosotan tanah yang berterusan. Rawatan dengan spesies pengikat-nitrogen *Burkholderia nodosa* NB1 sahaja memberikan kecekapan penggunaan nitrogen akar, dan keseluruhan penggunaan nitrogen yang lebih tinggi, selain meningkatkan kecekapan penggunaan fosforus daun, akar, dan keseluruhan penggunaan fosforus sebanyak berkali ganda daripada rawatan penuh menggunakan baja kimia. Gabungan penggunaan isolat rhizobakteria yang meningkatkan pertumbuhan pokok dengan kompos dan baja kimia membuktikan kemampunan sistem pengurusan nutrien bersepadu dengan penggunaan baja kimia yang minima. *Burkholderia nodosa* NB1 menunjukkan potensi yang baik untuk ujian lapangan di masa hadapan sebelum diaplikasi sebagai bio-baja. Kajian masa hadapan disarankan untuk menerapkan lebih banyak pencirian yang lebih teliti ke atas isolat ini sebelum aplikasi lapangan praktikal.

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

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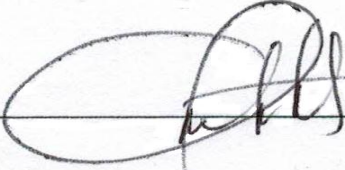
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LIST OF ABBREVIATIONS

BNF	Biological nitrogen fixation
PSB	Phosphate-solubilizing bacteria
PSM	Phosphate-solubilizing microorganisms
PGPR	Plant growth-promoting rhizobacteria
PGPB	Plant growth-promoting bacteria
PGP	Plant growth-promoting
16S rDNA	16S ribosomal deoxyribonucleic acid
IAA	Indole-3-acetic acid
OD	Optical density
SVI	Seedling vigor index
C/N	Carbon/nitrogen ratio
CEC	Cation exchange capacity
OM	Organic matter
INM	Integrated nutrient management
MOP	Muriate of potash or KCl
ERP	Egyptian Rock Phosphate
DAS	Days after sowing
AAS	Atomic absorption spectrophotometer
SAS	Statistical Analysis System



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CHAPTER 1

INTRODUCTION

Soil, being one of the basic natural resources, apart from air and water, has long been acknowledged for its forest production roles, as well as its notable functions in conservation, and improvement of ecosystem services. Tropical forests, being a continuous flux and self-restructuring system are indispensable regulator of C cycle, soil, water, and biological preservation, as well as protection from erosion, drought, and flood, besides woody and non-woody production, *via* the establishment of forest plantations, and agroforestry programme (Karam *et al.*, 2012; Aquilar-Amuchastequi and Henebry, 2007; González-Iturbe *et al.*, 2002; Food and Agriculture Organization, 1995).

Soil microbial populations affect soil fertility and elemental cycle. According to Anderson (2003), the associations between functional microbial communities, and the cycles of crucial elements in particularly carbon (C), nitrogen (N), phosphorus (P), and sulfur (S), as well as their effects on development of plants could serve as key signs of soil health level in view of disturbances. Soil microorganisms, albeit their miniature volume, reflect the dominant populations in soil, and hence, play essential roles in soil processes involving transformation of nutrients, and rates of litter decomposition, which are vital elements of the biological balance in soils (Arias *et al.*, 2005; Zak *et al.*, 1994). Bacteria make up a large constitution of microbial diversity in soils, and participate in soil key processes that eventually impact the whole terrestrial ecosystems operations, by being involved in completing nutrient, and geochemical cycles (Bardgett *et al.*, 2008; Fulthorpe *et al.*, 2008; Roesch *et al.*, 2007).

The forest floor is amassed with dead plant residue primarily consisting of plant cell wall constituents which include cellulose, the most abundant biopolymer, making up 20-30% of plant litter mass (Berg and Laskowski, 2006). Soil microorganisms are mainly responsible for the decomposition of cellulose by reducing the complex polysaccharides into simple sugars, in which they assimilate (Baldrian and Valášková, 2008; Lynd *et al.*, 2002). With bacteria known to be more prolific over saprotrophic fungi with their capability to consume inherently dilute nutrients in tropical soils (Baldrian *et al.*, 2012), the study of cellulolytic bacteria in tropical soils is significantly important.

Being one of the key elements for plant development and essentially soil fertility, plant requirement for N are supplied by processes of organic matter decomposition, atmospheric N deposition, and BNF, in which 97% of natural N input is attributed to BNF (Galloway *et al.*, 2008; Dreyfus *et al.*, 1987) by *Bacteria* and *Archaea* (Eady, 1992; Young, 1992). The N-fixing microorganisms

(diazotrophs) mostly prevail at free-living condition, and as much as 12.2 to 36.1 kg N ha⁻¹ year⁻¹ could be produced by them in the N budget in tropical ecosystems (Cleveland *et al.*, 1999), making them one of the most indispensable causes for studies, in particularly on their occurrence in tropical rehabilitated forests, which remains very lacking.

Phosphorus is one of the most commonly limiting macronutrients in most tropical soils with highest P fixation capacity area occupied 1,018 million hectares (ha) (Sanchez and Logan, 1992). The tropical soils are usually highly acidic, typically characterized by high Al and/or Fe contents which fix P as insoluble mineral complexes, rendering P to be unavailable for root uptake (Rengel and Marschner, 2005). The incorporation of microorganisms has been used over the years to enhance nutrient availability in soils (in particularly P) and reduce Al toxicity (Panhwar *et al.*, 2014), apart from the application of chemical treatment. The acidic tropical soils contain low amount of total microorganisms, which differ substantially, based on vegetation type and soil management. There are only limited acid-tolerant plant species and microbes that are able to thrive in these soils, which warrant studies.

Major drawbacks of chemical fertilizer application alone in terms of its expenses, availability and environmental impacts in particularly from N fertilizers have created the urgent need for alternative and sustainable strategies. Harmful influences of conventional N and P fertilization alone could be mitigated with application of INM system. This approach boosts little chemical usage but enhances nutrient-use efficiency with natural and man-made origin complement of plant nutrients for improved crop yield in a productive and environmentally friendly way (Gruhn *et al.*, 2000). The use of microbial inoculants (bio-fertilizers) possessing plant growth promotion traits is considered as an environmental-friendly alternative to further applications of chemical based fertilizers. Some of particular functional bacteria could have cross-functional abilities besides their principal functional ability, which could have other beneficial effects such as production of PGP phytohormones, polysaccharides and organic acids that are essential to plants and improve their growths (Panhwar *et al.*, 2014).

A complex network of plants and microbes is embodied in soils in an uneven solid medium, which is with differing chemical and physical conditions at molecular and cellular level. Microorganisms influence the environment and *vice versa*; hence, the understanding obtained from both chemical and biological approaches are needed in comprehending the changes in soils. Despite the need of soil physical, chemical, and biological properties to be investigated concurrently in order to assess sustainability/unsustainability of varying management practices, numerous studies in developing countries have merely delved into physical and chemical properties only (Laishram *et al.*, 2012).

The general objectives of this study were to: 1) evaluate functional bacterial populations in selected forest age as means to determine soil health of rehabilitated forest, and in understanding the association of the functional bacterial populations with selected soil properties in regulating forest soil ecology dynamics, as well as to: 2) characterize and identify selected functional bacteria, and assessing their potential use as bio-fertilizer.

This study was conducted to determine the: 1) relationship between cellulolytic, N-fixing, and phosphate-solubilizing bacterial populations with selected tropical rehabilitated forest stand age, as means to evaluate soil health of planted forest, 2) relationship between functional bacterial populations with some selected soil physico-chemical properties, with the intent of better understanding how these functional bacterial populations are associated or related to these soil properties in regulating the forest soil ecology dynamics, 3) isolate and identify beneficial cellulolytic, N-fixing, and phosphate-solubilizing bacteria from tropical rehabilitated forest soils, 4) characterize selected bacterial isolates for some phenotypic and biochemical properties, as well as PGP activities such as their respective functional activities, IAA production, and early plant growth promotion, and 5) potential bio-fertilizer effects of cellulolytic *Serratia nematodiphila* C46d, N-fixing *Burkholderia nodosa* NB1, and phosphate-solubilizing *Burkholderia cepacia* PB3, on functional rhizobacterial interactions, nutrient uptake and use efficiency of maize, and properties of Bekenu series by means of pot trial.

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