



UNIVERSITI PUTRA MALAYSIA

**INFLUENCE OF PLANT GROWTH-PROMOTING RHIZOBACTERIA AND
ARBUSCULAR MYCORRHIZAL FUNGI ON GROWTH AND YIELD OF
STRAWBERRY (*Fragaria x ananassa* Duchesne)**

NUR LAILI BINTI SAMSURRIJAL

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By

NUR LAILI BINTI SAMSURRIJAL

**Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in
fulfilment of the Requirements for the Degree of Master of Science**

May 2019

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

INFLUENCE OF PLANT GROWTH-PROMOTING RHIZOBACTERIA AND ARBUSCULAR MYCORRHIZAL FUNGI ON GROWTH AND YIELD OF STRAWBERRY (*Fragaria x ananassa* Duchesne)

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May 2019

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Strawberry is one of the main crops in Cameron Highlands, Pahang, Malaysia, which contribute significantly to the economy of local people. It was being grown all year round in substrate media under fertigation system. This system consumes high chemical fertilizer that can increase input cost and may lead to environmental pollution. The application of plant growth-promoting rhizobacteria (PGPR) and arbuscular mycorrhizal fungi (AMF) could be the solution for better plant performance with lesser chemical input. The following studies aimed to 1) characterize the growth-promoting traits of bacteria isolated from strawberry roots, 2) determine the effects of PGPR and AMF on growth and fruit quality and 3) observe the bacterial colonization on plant roots under controlled condition. In Study 1, bacterial isolates obtained from rhizosphere of three strawberry cultivars (Camarosa, Festival and Chandler) grown in Cameron Highlands, were differentiated based on phenotypic characterization, evaluated for various beneficial traits and identified using molecular technique. The selected bacterial isolates were then evaluated for plant growth promoting performance. A fertigation study was conducted with five treatments consisting of four bacterial isolates and non-inoculated which was served as control. All plants were applied with 70% nutrient strength. The study was arranged in randomized complete block design (RCBD) with three replications (three plantlets per replication). In Study 2 the following treatments were; 1) control – non-inoculated (100% strength); 2) control - uninoculated (70% strength); 3) *G. mosseae* (70% strength); 4) *M. oxydans* (70% strength); and 5) dual inoculation (70% strength). Plant growth, berry yield production, fruit quality and plant root colonization were determined. In Study 3, colonization of bacteria on plant roots was observed using Scanning Electron Microscope (SEM). Results in Study 1 showed a total of 80 PGPR were isolated from different strawberry cultivars. Fifty isolates were positive for biological N₂-fixation and 26 showed potassium solubilizing activity. Five of the isolates showed phosphate solubilizing activity and the solubilizing efficiency ranged from 13.2 to 42.3%. Only few produced hydrolyzing enzyme. Most isolates produced phytohormone which ranged from 1.6 to 42.4 µg mL⁻¹. Four of the selected bacteria were identified as *Microbacterium oxydans* (STUPM01), *Bacillus cereus* (STUPM12),

Leclercia adecarboxylata (STUPM20) and *Pseudomonas umsongensis* (STUPM 25). Plants inoculated with *M. oxydans* showed better root development and highest in leaf surface area and leaf dry weight. In Study 2, microbial treatments enhanced root dry weight compared to non-inoculated control (70% strength). *M. oxydans* inoculation resulted in higher shoot and root dry weights than both controls. Leaf surface area also increased 13% - 21% with *G. mosseae* and *M. oxydans*, respectively. Microbial treatments resulted in increment of berry yield from 11% - 20% compared to non-inoculated control of the same nutrient strength. The number of fruits produced increased to 15% in *M. oxydans* treatment. Higher average weight of fruits was obtained in plant treated with *M. oxydans* and dual inoculation. Microbial treatments enhanced the amount of sugars (glucose, sucrose, fructose) as well organic acids (ascorbic, malic, citric) in the strawberry fruit. Mycorrhizal roots harvested were observed with the presence of vesicles and hyphae in the roots. In Study 3, the colonization of *M. oxydans* on strawberry roots was visually observed with SEM technique and bacterial cells were found to colonize the roots. These studies showed that beneficial PGPR were successfully isolated from strawberry roots, and growth and yield of strawberry was significantly increased when inoculated with PGPR and AMF.

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

PENGARUH RHIZOBAKTERIA PENGALAK TUMBESARAN POKOK DAN KULAT MIKORIZA ARBUSKUL PADA PERTUMBUHAN DAN HASIL STRAWBERI (*Fragaria x ananassa* Duchesne)

Oleh

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Strawberi adalah salah satu tanaman utama di Cameron Highlands, Pahang, Malaysia, yang memberi sumbangan besar kepada ekonomi penduduk tempatan. Ia ditanam sepanjang tahun menggunakan media substrat di bawah sistem fertigasi. Sistem ini menggunakan baja kimia yang tinggi yang dapat meningkatkan kos input dan boleh membawa kepada pencemaran alam sekitar. Penggunaan rhizobakteria penggalak tumbesaran pokok (PGPR) dan kulat mikoriza arbuskul (AMF) boleh menjadi penyelesaian untuk prestasi tumbuhan yang lebih baik di samping mengurangkan penggunaan input kimia. Kajian berikut bertujuan untuk 1) mengenalpasti ciri-ciri penggalak tumbesaran pada PGPR yang diasingkan dari akar strawberi, 2) menentukan kesan PGPR dan AMF terhadap tumbesaran pokok dan kualiti buah dan 3) membuat pemerhatian pada kolonisasi rhizobakteria terhadap akar tumbuhan di bawah penanaman terkawal. Dalam kajian 1, bakteria diasingkan dari rhizosfera tiga kultivar strawberi (Camarosa, Festival dan Chandler) yang ditanam di Cameron Highlands. Ianya dibezakan berdasarkan pencirian fenotip, kemudian ditentukan ciri-ciri bermanfaat dan dikenalpasti menggunakan teknik molekuler. Bakteria yang dikenalpasti telah dipilih untuk dinilai prestasinya pada tumbesaran pokok. Satu kajian fertigasi dijalankan dengan lima rawatan yang terdiri daripada empat jenis bakteria, manakala rawatan tanpa inokulasi bakteria berfungsi sebagai kawalan. Kesemua tumbuhan menggunakan kekuatan nutrien sebanyak 70% daripada kekuatan nutrien yang biasa digunakan. Kajian ini disusun dalam rekabentuk blok rawak penuh lengkap (RCBD) dengan tiga replikasi (tiga tanaman setiap replikasi). Rawatan bagi kajian 2 pula adalah; 1) kawalan – tanpa inokulasi (100% nutrien); 2) kawalan - tanpa inokulasi (70% nutrien); 3) *G. mosseae* (70% nutrien); 4) *M. oxydans* (70% nutrien); dan 5) dwi inokulasi (70% nutrien). Tumbesaran pokok, pengeluaran hasil beri, kualiti buah dan kolonisasi mikoriza pada akar tanaman telah ditentukan. Dalam kajian 3, kolonisasi bakteria pada akar tumbuhan telah diperhatikan dengan menggunakan mikroskop electron pengimbas (SEM). Keputusan diperolehi dalam kajian 1 menunjukkan bahawa sejumlah 80 PGPR telah diasingkan dari kultivar strawberi yang berbeza. Lima puluh PGPR adalah positif bagi pengikatan nitrogen dan 26 PGPR menunjukkan berlakunya aktiviti pelarutan kalium.

Lima PGPR menunjukkan aktiviti pelarutan fosforus dengan kadar pelarutan antara 13.2 hingga 42.3% manakala hanya beberapa PGPR menghasilkan enzim hidrolisasi. Kebanyakan PGPR dapat menghasilkan fitohormon dengan kadar antara 1.6 hingga 42.4 $\mu\text{g mL}^{-1}$. Empat jenis bakteria yang dipilih telah dikenalpasti sebagai *Microbacterium oxydans* (STUPM01), *Bacillus cereus* (STUPM12), *Leclercia adecarboxylata* (STUPM20) dan *Pseudomonas umsongensis* (STUPM 25). Pokok yang diinokulasi *M. oxydans* menunjukkan prestasi yang baik dalam perkembangan akar, juga paling tinggi luas permukaan daun dan berat kering daun. Manakala dalam kajian 2, berat kering akar bagi semua rawatan mikrob telah meningkat berbanding kawalan tanpa inokulasi (70% nutrien). Inokulasi *M. oxydans* pula menunjukkan luas permukaan daun dan berat kering akar yang tinggi berbanding kedua-dua rawatan kawalan. *G. mosseae* dan *M. Oxydans* masing-masing telah meningkatkan luas permukaan daun sebanyak 13% - 21%. Dari segi pengeluaran hasil beri, semua rawatan mikrob menunjukkan peningkatan sebanyak 11% - 20% berbanding kawalan tanpa inokulasi yang diberikan kekuatan nutrien yang sama. Bilangan buah beri yang dihasilkan pula meningkat kepada 15% dalam rawatan *M. Oxydans* dan purata berat beri yang lebih tinggi telah diperolehi dalam dua rawatan mikrob iaitu rawatan *M. oxydans* dan rawatan dwi inokulasi. Kandungan gula (glukosa, sukrosa, fruktosa) dan asid organik (askorbik, malik, sitrik) yang terdapat di dalam buah strawberi juga menunjukkan peningkatan dalam semua rawatan mikrob. Akar pokok yang dirawat mikoriza pula dituai dan dilihat kesan kolonisasi di mana menunjukkan kehadiran vesikel dan hifa di dalamnya. Dalam kajian 3, kolonisasi *M. oxydans* pada akar strawberi dilihat secara visual dengan teknik SEM dan sel-sel bakteria dijumpai pada permukaan akar. Seluruh kajian ini telah membuktikan bahawa beberapa jenis bakteria bermanfaat telah berjaya diasingkan daripada akar strawberi dan mempengaruhi tumbesaran dan hasil strawberi dengan memberi peningkatan secara signifikan apabila diinokulasi PGPR dan AMF.

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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 Master of Science. The members of the Supervisory Committee were as follows:

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LIST OF ABBREVIATIONS

AMF	Arbuscular Mycorrhizal Fungi
cfu	colony forming unit
cv	cultivar
DAI	Days After Inoculation
DAT	Days After Transplanting
EC	electrical conductivity
HDTMA	hexadecyltrimethylammonium
IAA	Indole-3 Acetic Acid
PBS	Phosphate Buffer Saline
PGPR	Plant Growth-promoting Rhizobacteria
RCBD	Randomized Completely Block Design
rpm	revolutions per minute
SEM	Scanning Electron Microscopy
TSA	Tryptic Soy Agar
TSB	Tryptic Soy Broth

CHAPTER 1

INTRODUCTION

Strawberries (*Fragaria x ananassa* Duchesne) are commonly found in its original cold sub-tropical and temperate region. In tropical region of Malaysia, they are widely grown all-year round at the high-latitude part known as Cameron Highlands. It is highly valued fruit crop and one of the most important species of berry fruits in the world (Pesakovic *et al.*, 2013). Strawberry gives the quickest profit in a short time and it is among the popular attraction in Cameron Highlands. They are mostly grown under greenhouse system and using cocopeat as a substrate of planting medium. Few disadvantages of using substrate are it consumes high inputs of water and fertigation, nearly tripled the inputs than that of field grown and increase in cost as well. Too much fertigation might damage the crop and can cause environmental pollution (Landis *et al.*, 2010).

Compared to soils, substrate used in fertigation lacks of advantageous microorganisms like plant growth-promoting rhizobacteria (PGPR) and arbuscular mycorrhizal fungi (AMF) (Boyer *et al.*, 2016). The application of microorganisms is essential as an alternative to achieve sustainable agriculture and yet able to lessen the amount of fertigation requirement (Jeffries *et al.*, 2003). The idea of applying microorganisms on plant to lower the amount of chemical fertilizers being used is not new. The utilization to partially or maybe fully replacing fertilizers is however not much taken into the attention (Hungria *et al.*, 2013). The use of these beneficial microorganisms are always known to boost plant growth and help in sustaining the environmental health (Mitter *et al.*, 2013). Roots excrete nutrients-rich exudates which make them as a perfect factory that convey some plant-microbes interaction. The efficient use of microbial inoculant is the vital plan towards a sustainable agriculture and environmentally friendly with the purpose to reduce the chemical fertilizers, pesticides or the artificial growth regulator (Adesemoye *et al.*, 2009; Hungria *et al.*, 2010, 2013).

Studies have revealed most of bacterial species related with plant rhizosphere are beneficial for growth of plant, crop quality and yield, and they were known as PGPR. PGPR are beneficial whether for forage crops, cash crops or field production (Cvijanovic *et al.*, 2007; Pesakovic *et al.*, 2013). The AMF are obligate symbionts for most plant. The interaction of AMF and plant is also mutual and there is a bidirectional nutrient transfer between them (Bonfante and Andrea, 2008). In a study done by Borkowska (2002), commercial AMF inoculum was reported to enhance the growth of root, leaf area and crown of strawberry plant and even tolerance to water stress. Few other studies also showed better performance of strawberry plant with the addition of AMF (Castellanos-Morales *et al.*, 2010). Meanwhile the rhizospheric microbial consortium with mineral fertilization showed improvement in nutrient use efficiency in plant (Meena *et al.*, 2017). There are potential of soil microorganisms in contributing positive effects to plant. The use of beneficial microorganisms instead of synthetic chemical in field will lead to sustainable fruit production. The use of the beneficial microbes in plants such as strawberry is very uncommon but their introduction into growth media substrate might improve plant growth and fruit quality. Since the beneficial microbes such as PGPR and

AMF in growth media substrate are almost absent, application of these microorganisms can potentially achieve better plant growth performance and yield of strawberry.

The study aimed to examine the effects of strawberry plant inoculated with PGPR and AMF. The ability of these microorganisms in creating healthy rhizosphere will improve the growth, yield and quality of strawberry. Therefore, the objectives of the study were:

1. To characterize the growth-promoting traits of bacteria isolated from strawberry roots from Cameron Highlands and their performances on plant growth.
2. To determine the effects of PGPR and AMF on growth, quality of strawberry production and mycorrhizal colonization.
3. To observe bacterial colonization on plant roots under controlled condition.

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LIST OF PUBLICATIONS

Journal

Zakiah, M., Radziah, O., **Laili N. S.**, Nashriyah, M., Jamil, A. Z. and Hasima N. M. Determination of nitrogen fixing capacity of bacteria isolated from the rhizosphere of *Acacia mangium* from the BRIS soil of Tembila, Besut, Terengganu, Malaysia. *International Journal of Engineering & Technology* 7 (4.43): 140-144.

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Proceedings

Laili, N. S., Radziah, O. and Zaharah S. S. "Influence of Arbuscular Mycorrhizal Fungi and Plant Growth-promoting Rhizobacteria on Root Development and Yield of Strawberry (*Fragaria ananassa*) in Cameron Highlands", "Soil Science Conference of Malaysia 2016". TH Hotel & Convention Centre, Kuala Terengganu, Terengganu, 5 – 7 April 2016.

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