UNIVERSITI PUTRA MALAYSIA

IN VITRO AND *IN SILICO* INVESTIGATION OF THE PLANT GROWTH-PROMOTING RHIZOBACTERIA FROM PEPPER (*Piper nigrum* L.)

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"IN THE NAME OF ALLAH THE MOST GRACIOUS AND THE MOST MERCIFUL"

3) "And it is He Who spread out the earth, and placed therein firm mountains and rivers and of every kind of fruits He made Zawjain Ithnaîn (two in pairs - may mean two kinds or it may mean: of two sorts, e.g. black and white, sweet and sour, small and big, etc.) He brings the night as a cover over the day. Verily, in these things, there are Ayât (proofs, evidences, lessons, signs, etc.) for people who reflect."

4) "And in the earth are neighbouring tracts, and gardens of vines, and green crops (fields etc.), and date-palms, growing out two or three from a single stem root, or otherwise (one stem root for every palm), watered with the same water, yet some of them We make more excellent than others to eat. Verily, in these things, there are Ayât (proofs, evidences, lessons, signs) for the people who understand."

(Al-Qur'an: Ar-Rad: 3-4)

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By

ZAKRY FITRI BIN AB. AZIZ

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

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IN VITRO AND _IN SILICO_ INVESTIGATION OF THE PLANT GROWTH-PROMOTING RHIZOBACTERIA FROM PEPPER (_Piper nigrum_ L.)

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September 2013

Chairman: Associate Professor Halimi Mohd. Saud, PhD

Faculty: Agriculture

The increase in awareness of the public to use inexpensive, safe, organic and environmentally friendly agricultural inputs makes plant growth-promoting rhizobacteria as one of the more attractive biological applications used to reduce or complement the use of synthetic agricultural inputs. In the present study, diligent investigation has been conducted in search of the most promising plant growth-promoting rhizobacteria from pepper (*Piper nigrum* L.) rhizosphere. As a result of the search, 14 rhizobacteria were isolated from the rhizosphere of the pepper plant (*Piper nigrum* L.). *In vitro* analyses showed that the isolates possessed many traits beneficial to agronomy, *viz*. biological nitrogen fixation, phosphate solubilisation and indole acetic acid (IAA) production. Analyses by 16S rRNA sequencing indicated that the isolates belonged to *Acinetobacter radioresistens* (UPMLH19), *Bacillus cereus* (UPMLH1, UPMLH13, UPMLH24, UPMLH41 and UPMLH42), *Bacillus megaterium* (UPMLH3 and UPMLH22), *Bacillus subtilis* (UPMLH5), *Bacillus* spp. (UPMLH8, UPMLH23, UPMLH34 and UPMLH43) and *Leclercia* sp. (UPMLH2).

Phylogenetic analyses indicated that the newly isolated rhizobacteria from *P. nigrum* were from Firmicutes and Proteobacteria, with the former being more common. In the present study, computational methods were used to predict information within the small subunit 16S rRNA gene from *B. cereus* strain UPMLH24. The computational methods employed revealed that the small subunit 16S rRNA gene sequence from *B. cereus* strain UPMLH24 contained several open reading frames that encoded gamma-polyglutamic acid protein, lipoprotein, cytoplasmic protein and ribosomal protein S10. Computational predictions based on protein-protein identity classified seven phyla of organisms associated with small open reading frames of novel *B. cereus* strain UPMLH24. Plant bioassay technique was used to select the most promising isolates. Three vegetable crop species were used as test plants, namely mustard (*Brassica juncea*), mung bean (*Vigna radiata*) and shallot (*Allium ascalinocum*). Of the 14 inocula, *Bacillus* spp. strains (UPML23, UPMLH34 and UPMLH43) and *B. cereus* UPMLH24 improved early plant growth of mustard significantly. Early development of mung bean seedlings showed significant improvement after inoculation with *Bacillus* sp. strain UPMLH8, *B. megaterium* strains UPMLH3 and UPMLH22, and *B. cereus* strain UPMLH24. Only *B. cereus* strain UPMLH1, significantly promoted root number and shoot height of shallot plants. *B. cereus* strains UPMLH1 and UPMLH24 inoculum were selected and used in further investigation after showing to be the most promising in enhancing the early development of mustard, bean and shallot plants. The selected strains of *B. cereus* were then used to evaluate the effect of inoculation on their original host, *P. nigrum* stem cuttings of Kuching variety, studied for root formation under nursery conditions. Results showed that *B. cereus* UPMLH24 inoculation on fresh pepper stem cuttings stimulated root number (55% increase over control), length of longest

root (25% increase over control), total root length (87% increase over control), root fresh weight (28% increase over control) and root dry weight (112% increase over control). Present study recommends *Bacillus cereus* UPMLH24 as a potential candidate in a formulation of a biostimulant for organic and sustainable nursery for pepper production.

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

PENYIASATAN *IN VITRO* DAN *IN SILICO* RIZOBAKTERIA PENGGALAK-TUMBESARAN TUMBUHAN DARI LADA (*Piper nigrum* L.)

Oleh

ZAKRY FITRI BIN AB. AZIZ

September 2013

Pengerusi: Profesor Madya Halimi Mohd. Saud, PhD

Fakulti: Pertanian

Peningkatan kesedaran awam ke atas penggunaan input pertanian yang murah, selamat, organik dan mesra alam telah menjadikan rizobakteria penggalak-tumbesaran satu daripada aplikasi biologi yang menarik untuk mengurangkan atau mengganti input pertanian sintetik. Satu kajian terperinci telah dijalankan untuk mencari rizobakteria penggalak-tumbesaran dari rizosfera lada (*Piper nigrum* L.) yang berpotensi tinggi. Hasil dari pencarian ini, 14 rizobakteria berjaya dipencilkan dari rizosfera tanaman lada (*Piper nigrum* L.). Analisis *in vitro* telah menunjukkan asingan itu mempunyai beberapa ciri berfaedah dari segi agronomi, seperti pengikatan nitrogen biologi, penguraian fosfat dan penghasilan asid asetik indol. Analisis penjujukan 16S rRNA menunjukkan isolat terdiri dari *Acinetobacter radioresistens* (UPMLH19), *Bacillus cereus* (UPMLH1, UPMLH13, UPMLH24, UPMLH41 dan UPMLH42), *Bacillus megaterium* (UPMLH3 dan UPMLH22), *Bacillus subtilis* (UPMLH5), *Bacillus* spp. (UPMLH8, UPMLH23, UPMLH34 dan UPMLH43) dan *Leclercia* sp. (UPMLH2). Analisis filogenetik menunjukkan

rizobakteria dari *P. nigrum* yang baru dipencilkan ini adalah terdiri dari Firmicutes dan Proteobacteria, dengan Firmicutes mempunyai bilangan terbanyak. Dalam kajian ini, kaedah-kaedah pengkomputeran digunakan bagi meramal maklumat di dalam subunit kecil gen 16S rRNA dari *Bacillus cereus* UPMLH24. Kaedah-kaedah pengkomputeran yang dijalankan membuktikan subunit kecil gen 16S rRNA dari *B. cereus* UPMLH24 mengandungi beberapa "*open reading frames*" yang mengekod protein asid poliglutamik, lipoprotein, protein sitoplasmik dan protein ribosom S10. Teknik bioasai tumbuhan telah digunakan untuk membuat pilihan isolat yang paling berpotensi. Tiga tanaman sayuran telah dijadikan tumbuhan ujian, iaitu sawi (*Brassica juncea*), kacang hijau (*Vigna radiata*) dan bawang merah (*Allium ascalinocum*). Dari 14 inokula, *Bacillus* spp. (UPML23, UPMLH34 dan UPMLH43) dan *B. cereus* UPMLH24 telah meningkatkan pertumbuhan awal tanaman sawi secara bererti. Perkembangan awal tanaman kacang hijau menunjukkan perkembangan yang bererti setelah inokulasi dengan *Bacillus* sp. UPMLH8, *B. megaterium* (UPMLH3 dan UPMLH22) dan *B. cereus* UPMLH24. Hanya *B. cereus* UPMLH1, merangsang secara bererti ke atas bilangan akar dan tinggi pucuk pertumbuhan tanaman bawang merah. Inokulum *B. cereus* UPMLH1 dan UPMLH24 telah dipilih dan digunakan dalam kajian lanjutan setelah menunjukkan peningkatan ke atas perkembangan awal tanaman sawi, kacang hijau dan bawang merah. Isolat *B. cereus* yang terpilih kemudiannya dinilai kesan inokulasinya ke atas hos asalnya, iaitu keratan batang *P. nigrum* varieti Kuching, untuk mengkaji pembentukan akar di nurseri. Keputusan kajian menunjukkan inokulasi *B. cereus* UPMLH24 ke atas keratan-keratan batang lada merangsang bilangan akar (peningkatan 55% berbanding kawalan), panjang akar terpanjang (peningkatan 25% berbanding kawalan), jumlah panjang akar (peningkatan 87% berbanding kawalan), berat basah akar (peningkatan

28% berbanding kawalan) dan berat kering akar (peningkatan 112% berbanding kawalan). Kajian ini mencadangkan *Bacillus cereus* UPMLH24 adalah calon yang berpotensi di dalam formulasi biopenggalak bagi pengeluaran nurseri tanaman lada organik dan lestari.

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I certify that a Thesis Examination Committee has met on 6th September 2013 to conduct the final examination of Zakry Fitri bin Ab. Aziz on his thesis entitled "*In vitro* and *In silico* Investigation of the Plant Growth-Promoting Rhizobacteria from Pepper (*Piper nigrum* L.)" 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 Doctor of Philosophy.

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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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