UNIVERSITI PUTRA MALAYSIA

BIOCHEMICAL AND MOLECULAR CHARACTERIZATION OF PHOSPHATE- SOLUBILIZING BACTERIA ISOLATED FROM OIL PALM SOIL

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By
MOHAMMAD BAGHER JAVADI

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

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DEDICATION

Thanks to Allah

To my beloved family

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

BIOCHEMICAL AND MOLECULAR CHARACTERIZATION OF PHOSPHATE- SOLUBILIZING BACTERIA ISOLATED FROM OIL PALM SOILS

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Chairman: Associate Professor Halimi Mohd Saud, PhD

Institute: Tropical Agriculture

Bacterial isolates from Malaysian oil palm soils were examined and screened for capability to solubilize calcium phosphate. In the present study, Pikovskaya (PVK) and NBRIP were chosen as the media for isolating the PSB from different Malaysian oil palm soils. Isolated bacteria were able to solubilize calcium phosphate. The phosphate solubilization ability and microbial growth were dependent on the species of bacteria. The results indicated that maximum solubilization was found up to 7.56 ppm and related to 35dr (*S. marcescens*). The bacterial isolates exhibited different level of phosphate solubilization when the media containing different carbon or nitrogen sources. 10upmr and 7sr bacterial isolates could solubilize insoluble phosphate with all carbon sources. In all cases, insoluble phosphate solubilization was accompanied by decreasing the pH value. All bacteria isolated were identified using 16S rRNA molecular technique except 32dr, a bacterial isolate which was isolated from Dengkil rhizosphere. The identification analysis confirmed *Proteobacteria* as the most abundant group in Malaysian oil palm soils. The comparison of the 16S rRNA gene sequences of phosphate solubilizing bacteria allowed differentiation between isolates at the species level across *Proteobacteria*

and *Firmicutes* phylum but it couldn't classify isolates at strain. The desired experimental resolution for the differentiation of closely-related isolates of *Proteobacteria* and *Firmicutes* phylum was achieved by using the Rep-PCR techniques. With this approach, the polymorphism of 31 PSB from different area were observed. In the case of the PSB isolates examined here, cluster analysis of *Pseudomonas* and *Alcaligenes* species could reveal better differentiation by BOX-PCR and REP-PCR primers respectively. By using NBRIP media, 16S rDNA and Rep-PCR technique found a very fast and precise method for evaluating Phosphate solubilizing ability, finding the most effective isolates for conducting the molecular process required for creating the strain-base biofertilizer.

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

PENCIRIAN BIOKIMIA DAN MOLEKUL BAKTERIA PELARUT FOSFAT PADA TANAH KELAPA SAWIT

Oleh

MOHAMMAD BAGHER JAVADI

Disember 2012

Pengerusi: Prof Madya Halimi Mohd Saud, PhD

Institut: Pertanian Tropika

Isolat bakteria daripada tanah minyak sawit Malaysia telah diperiksa dan disaring untuk keupayaan untuk melarutkan kalsium fosfat. Dalam kajian ini, Pikovskaya (PVK) dan NBRIP telah dipilih sebagai media untuk mengasingkan PSB dari tanah minyak sawit yang berbeza. Bakteria yang telah diasingkan boleh melarutkan kalsium fosfat. Keupayaan untuk melarutkan fosfat dan pertumbuhan mikrob adalah bergantung kepada spesies bakteria. Keputusan menunjukan bahawa kelarutan maksimum adalah sehingga 7.56ppm dan berkaitan dengan 35dr (*S. marcescens*). Bakteria isolates menunjukkan tahap untuk melarutkan fosfat yang berbeza apabila media mengandungi karbon yang berbeza atau sumber nitrogen. 10upmr dan isolat bakteria 7sr boleh melarutkan fosfat tidak larut dengan semua sumber karbon. Dalam semua kes, kelarutan fosfat tidak larut akan diikuti dengan pengurangan nilai pH. Kesemua bakteria yang diasingkan telah dikenal pasti menggunakan teknik molecular 16S rRNA kecuali 32dr, satu isolat bakteria yang telah diasingkan dari Dengkil rhizosphere. Analisis pengenalan mengesahkan bahawa *Proteobacteria* sebagai kumpulan yang paling banyak di tanah minyak sawit Malaysia. Perbandingan gen jujukan 16S rRNA bakteria yang melarutkan fosfat membenarkan

pembezaan antara isolat-isolat pada peringkat sepsis bagi *Proteobacteria* and *Firmicutes* filum tetapi ia tidak dapat mengklasifikasikan isolate pada peringkat strain. Resolusi eksperimen yang diingini bagi pembezaan isolate yang berkait rapat dengan *Proteobacteria* and *Firmicutes* filum telah dicapai dengan mengunakan teknik REP-PCR. Dengan pendekatan ini, polimorfisme 31 PSB daripada kawasan berbeza telah diperhatikan. Dalam kes PSB isolat yang diperiksa di sini, analisis kelompok sepsis *Pseudomonas* and *Alcaligenes* boleh mendedahkan pembezaan yang lebih baik oleh primer-primer BOX-PCR dan REP-PCR masing-masing. Dengan menggunakan media NBRIP, 16s rDNA dan teknik Rep-PCR mendapati kaedah yang cepat dan tepat untuk menilai keupayaan melarutkan fosfat, mencari isolate yang paling berkesan untuk menjalankan process molekular yang diperlukan untuk mewujudkan biobaja strain-asas.

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I certify that an Examination Committee has met on July 2011 to conduct the final examination of Mohammad Bagher Javadi Nobandegani on his Doctor of philosophy thesis entitled "Molecular Characterization of Phosphate Solubilizing Bacteria in Oil Palm Soils" in accordance with Universiti Pertanian Malaysia (Higher Degree) Act 1980 and Universiti Pertanian Malaysia (Higher Degree) Regulations 1981. The committee recommends that the student be awarded the Doctor of philosophy.
Member of the 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 Doctor of philosophy. The members of Supervisory Committee were as follows:

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DECLARATION

I declare that the thesis is my original work except for quotations and citations which have been duly acknowledged. I also declare that it has not been previously, and is not concurrently, submitted for any other degree at Universiti Putra Malaysia or any other institution.

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MOHAMMAD BAGHER JAVADI NOBNDEGANI

Date: 7 December 2012

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