UNIVERSITI PUTRA MALAYSIA

DISTRIBUTION AND BIOCHEMICAL AND GENOTYPIC COMPARISON OF PHOSPHATE SOLUBILIZING BACTERIA IN OIL PALM SOILS

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By

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Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, In Fulfilment of the Requirement for the Degree of Master of Science

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DEDICATION

Thanks to Allah

To my beloved family

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

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Chair person: Associate Professor Halimi Mohd Saud, PhD

Faculty: Agriculture

Phosphorus (P) is one of the most important elements in crop production. Deficiency of P may occur in crop plant growing in soils, even in the soil containing adequate phosphates. This may be partly due to fact that plants are able to absorb phosphate only in an available form and unfortunately most of the P in soil is not available. Furthermore to increase the production with limited land size, forced the farmers to optimize land use through application of more chemical phosphate fertilizers. Although the phosphate fertilizers applied to the soils is rapidly immobilized after application and become unavailable to the plant. Soil phosphate is rendered available either by plants root or by phosphate solubilizing bacteria (PSB) through secretion of organic acids. Therefore, phosphate solubilizing soil bacteria' play some part in correcting P deficiency of plants. In this regard, Population distribution of PSB in the soil is one the important factor taken for consideration for application of chemical fertilizers in the field and producing biofertilizers which more environmental friendly .Currently, there is insufficient information on the occurrence of phosphate solubilizing bacteria (PSB) in soil under oil palm cultivation. The objectives of the study was to isolate phosphate solubilizing bacteria (PSB) from the oil palm rhizosphere, determine the distribution and biochemical characterization of PSB, evaluated their ability to solubilize different forms of insoluble phosphate and found the genetic profile comparison of different isolates from different area.. Study was carried out to determine the population distribution of PSB in fertile oil palm soils by Standard Plate Count. The distribution of PSB strains in the rhizosphere of three oil palm field were 3.41×10^{8}, 3.30×10^{7} and 6.73×10^{7} c.f.u.mL^{-1} in UPM, Dengkil and Semenyih respectively. The distribution of PSB in UPM, Dengkil and Semenyih were 3.37×10^{7}, 1.97×10^{7} and 2.58×10^{7} c.f.u.mL^{-1} respectively in the non rhizosphere fractions. Overall the PSB population in the UPM area was higher(3.41×10^{8} , 3.37×10^{7})than another location and followed by Semenyih.

The percentage of PSB population compared to total bacterial was higher (91.67%) in the rhizosphere of Dengkil. In terms of population distribution, the PSB in Dengkil showed the highest(91.67%) proportion from the total bacteria count of the rhizosphere fraction and 53.69% in the non rhizosphere fraction compared to the other soil locations which have a lower percentage of PSB. The least percentage of PSB was in the non rhizosphere fraction of UPM constituting only 25.34% of the total bacteria population. Distribution of PSB in Semenyih was 40.54% and 34.96% in rhizosphere and non-rhizosphere, compared to total bacteria population, respectively. Generally the percentage of PSB was lower in the non rhizosphere of all three soils compared to the rhizosphere fraction.

16DNR, 23DR and 12DNR were the most effective phosphate solubilizer isolates after eight days of inoculation in National Botanical Institute Phosphate Medium (NBRIP), respectively.

The results of culturing the isolates in NBRIP indicated that among all carbon sources, glucose was the best for phosphate solubilization and KNO_3 was less effective compared to $(NH_4)NO_3$ when it is used as a source of nitrogen. All PSB isolates were acid producers and among isolates, 16DR had the lowest pH after eight days incubation (pH=4.16).

The REP–PCR pattern of isolates designated 3DNR, 16DNR, 8DNR, 2UPMR, 31SR, were found to be highly related to one another (>75%) but very distinct from 6UPMR, 8DR, 5DR, 31UPMR. The cluster analysis identified four major groups of A, B, C and D at genetic distance=0.05. Cluster A contained 14 isolates. Cluster B contained eight isolates and cluster C and D is made-up of two isolates 8DR and 6UPMR respectively. Cluster A and B together formed a main cluster at genetic distance of 0.1. Cluster analysis showed great variability of PSB genetic resources in oil palm soils which can be utilized for selection and improvement of the PSB isolates for use in biofertilizers.

In conclusion, it could be postulated that the most effective PSB strain (31UPMR) within the population of microorganism in three different oil palm soils was identified by the cluster analysis of REP-PCR pattern and biochemical trait and phosphate solubilizing bacteria from different soil type have different molecular characteristic.

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

PERBANDINGAN TABURAN DAN BIOKIMIA DAN GENETIK KEATAS BAKTERA PELARUT FOSFORUS DALAM TANAH KELAPA SAWIT

Oleh

Mohammad Bagher Javadi Nobandegani

March 2008

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Fosforus merupakan salah satu elemen terpenting dalam penghasilan produk tanaman. Kekurangan P boleh berlaku pada tanaman yang ditanam di atas tanah, walaupun tanah tersebut mengandungi P yang mencukupi. Ini mungkin terjadi akibat tumbuhan hanya mampu menyerap P dalam bentuk tertentu dan malangnya kebanyakan bentuk P tidak mampu diserap oleh tumbuhan. Tambahan lagi, untuk meningkatkan penghasilan tanaman dengan keluasan tanah yang terhad, petani terpaksa mengoptimakan penggunaan tanah melalui penggunaan baja kimia P. Walaupun baja fosfat yang diletakkan pada tanah dengan cepatnya tidak bergerak selepas penambahan dan tidak boleh diserap oleh tumbuhan. Fosfat di dalam tanah mampu diserap samaada oleh akar atau bakteria pelarut fosfat (PSB) melalui pembebasan asid organik. Oleh itu, PSB memainkan peranan mengurangkan kekurangan P pada tumbuhan. Dari itu, taburan populasi PSB di dalam tanah merupakan faktor penting yang perlu diambil kira untuk penggunaan baja kimia di ladang dan menghasilkan baja organik yang lebih mesra alam. Pada masa kini, maklumat mengenai kewujudan PSB di dalam tanah pada tanaman kelapa sawit tidak mencukupi. Objektif kajian ini ialah memencilkan PSB

daripada rizosphera kelapa sawit, menentukan taburan dan pencirian biokimia PSB , menilai kebolehan PSB untuk melarutkan pelbagai bentuk fosfat tidak larut dan mengkaji perbandingan profil genetik pencilan yang berbeza daripada kawasan yang berbeza. Kajian telah dilakukan untuk menentukan taburan populasi PSB dalam tanah kelapa sawit yang subur menggunakan Pengiraan Plat Piawai. Taburan strain PSB dalam rhizosphera daripada tiga ladang tanaman kelapa sawit iaitu di UPM, Dengkil dan Semenyih masing-masing ialah 3.41×10^{8}, 3.30×10^{7} dan 6.73×10^{7} c.f.u. mL^{-1}. Taburan PSB di UPM, Dengkil dan Semenyih iaitu 3.37×10^{7}, 1.97×10^{7} dan 2.58×10^{7} c.f.u. mL^{-1} masing-masing adalah dalam bahagian bukan rhizosphera. Secara keseluruhanya populasi PSB di kawasan UPM merupakan yang tertinggi (3.41×10^{8}) berbanding lokasi lain dan diikuti oleh Semenyih.

Peratusan populasi PSB berbanding daripada jumlah keseluruhan bakteria adalah lebih tinggi (91.67%) dalam rhizosphera dari Dengkil. Daripada aspek taburan populasi, PSB dari Dengkil menunjukkan pecahan PSB yang tertinggi (91.67%) daripada jumlah keseluruhan bakteria yang dikira dalam bahagian rhizosphera dan 53.96% dalam bahagian bukan rhizosphera berbanding daripada kawasan tanah lain dimana ia mempunyai peratusan PSB yang lebih rendah. Peratusan PSB terendah adalah dalam bahagian bukan rhizosphera dari UPM menunjukkan hanya 25.34 % daripada jumlah keseluruhan populasi bakteria. Taburan PSB di Semenyih ialah 40.54 % dan 34.96 % dalam rhizosphera dan bahagian bukan rhizosphera, berbanding daripada jumlah keseluruhan populasi bakteria masing-masing. Secara am peratusan PSB adalah rendah dalam bahagian bukan rhizosphere daripada ketiga-tiga tanah berbanding dengan bahagian rhizosphera. 16DNR, 23DR dan 12DNR adalah pencilan pelarut fosfat yang paling efektif selepas lapan hari masing-masing dinokulasi dalam Media Fosfat Institut Botani Nasional (NBRIP).

Hasil daripada pengkulturan pencilan di dalam NBRIP menunjukkan bahawa di anatara semua sumber karbon, glukos merupakan sumber yang terbaik bagi pelarutan fosfat dan KNO3 adalah kurang efektif berbanding dengan $(NH_4)NO_3$ apabila ia digunakan sebagai sumber bagi nitrogen. Kesemua pencilan PSB merupakan penghasil asid dan dan di antara semua pencilan, 16DR mempunyai pH terendah selepas lapan hari inkubasi (pH=4.16).

Corak REP-PCR, hasil daripada pencilan 3DNR, 16DNR, 8DNR, 2UPMR, 7SR menunjukkan perkaitan yang tinggi diantara satu sama lain (>75%) tetapi sangat ketara daripada 6UPMR, 8DR, 5DR, dan 31UPMR. Analisis kluster menunjukkan empat kumpulan utama A, B, C, dan D pada jarak genetik = 0.05. Kluster A mengandungi 14 pencilan. Kluster B mengandungi 8 pencilan dan kluster C dan D masing-masing terdiri daripada dua pencilan 8DR dan 6UPMR. Kluster A dan B bergabung membentuk kluster utama pada jarak genetik 0.1. Analisis kluster menunjukkan variasi genetik PSB yang ketara dalam tanah tanaman kelapa sawit dimana ia boleh digunakan untuk pemilihan dan penambahbaikan keatas pencilan PSB untuk digunakan dalam baja organik.

Sebagai kesimpulan, boleh dipostulatkan bahawa strain PSB yang paling efektif (31UPMR) diantara populasi mikroorganisma di dalam tiga jenis tanah tanaman kelapa sawit yang berbeza dikenalpasti melalui analisis kluster corak REP-PCR dan ciri biokimia dan bakteria pelarut fosfat daripada jenis tanah yang berbeza mempunyai pencirian molekular yang berbeza.

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I certify that an Examination Committee has met on 17th March 2008 To conduct the final examination of Mohammad Bagher Javadi Nobandegani on his Master of Science thesis entitled "Distribution and Biochemical and Genotypic Comparison 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 degree of Master of Science.
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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 Supervisory Committee were as follows:

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Date: 12 June 2008

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.

MOHAMMAD BAGHER JAVADI NOBANDEGANI

Date: 28 April 2008

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LIST OF ABBREVIATIONS

AFLP	Amplified Fragment Length Polymorphism
Al	Aluminum
ARDRA	Amplified Ribosomal DNA Restriction Analysis
ATP	Adenosine Triphosphate
B	Bur
C	Carbon
ºC	Degree Centigrade
Ca	Calcium
C.F.U	Colony Forming Unite
C-P	Calcium Phosphate
DNA	Deoxyribonucleic Acid
DNase	Dexoyribonuclease
Fe	Ferric
ERIC	Entrobacterial Repetitive Intergenic Consensus
H	Hydrogen
$H_2PO_4^-$	Hydrogen Phosphate
ISR	Intergenic Spacer Region
K	Potassium
Kg	Kilogram
LB	Luria Bertoni
LMW	Low Molecular Weight

Mg	Magnesium
Mg.Kg	Milligram. Kilogram
Mn	Manganese
Mps+	Mineral Phosphate Solubilizer
Min	Minute
mL	Milliliter
MLST	Multilocus Sequence Typing
MPN	Most Probable Number
N	Nitrogen
NA	Nutrient Agar
NaCl	Sodium Chloride
NaOH	Sodium Hydroxide
NBRIP	National Botanical Institute Phosphate Medium
NR	Non Rhizosphere
P	Phosphorus
PCR	Polymerase Chain Reaction
PDYA	Potato Dextrose Yeast Agar
PFGE	Pulsed Field Gel Electrophoresis
pH	Potential Hydrogen
Pi	Phosphin
PGPR	Plant Growth Promoting Rhizobacteria
ppm	Part Per Million
PSB	Phosphate Solubilizing Bacteria

PVK	Pikovskaya
RAPD	Random Amplification of Polymorphic DNA
REP	Repetitive Exteragenic Palindromic
Rep-PCR	Repetative Exteragenic Palindromic- PCR
RNA	Ribonucleic Acid
RNase	Ribonuclease
R	Rhizosphere
Rpm	Round Per Minute
16S rDNA	16s Ribosomal Deoxyribo Nucleic Acid
TP-RAPD	Two Primer Random Amplified
TSA	Tryptic Soy Agar
U	Unit
UPGMA	Unweighted Pair Group Method Average
UPM	Universiti Putra Malaysia
VAM	Vascular Arbuscular Mycorrhiza
μm	Micromole

CHAPTER 1

INTRODUCTION

Phosphorus is one of the least available of all essential nutrients in the soil and its concentration is generally below that of many other micronutrients. Many soils around the world are deficient in Pi, and even in fertile soils, available Pi seldom exceeds 10 μM(Raghothama,1999) .In most soils, the concentration (2 μM) of available Pi in soil solution is several orders of magnitude lower than that in plant tissues (5–20 mM). Phosphorus availability is of particular concern in the highly weathered and volcanic soils of the humid tropics and subtropics, and in many sandy soils of the semiarid tropics, where crop productivity is severely compromised through lack of available Pi. Aluminum ions, which predominate in acid soils of the world, and iron interact strongly with Pi and render it unavailable to plants. Acid conditions exist in approximately 30% of soils worldwide, and are found in all continents. In addition, a considerable fraction (20–80%) of Pi in soils is found in the organic form (Jungk et at., 1993), which has to be mineralized to the inorganic form before it becomes available to plants. Observations suggest that the low concentration of Pi in the soil solution is a major factor limiting growth in many natural ecosystems (Raghothama, 1999).

Phosphorus is considered to be the most limiting nutrient for growth of leguminous crops in tropical and subtropical regions (Ae et al., 1990). The non renewable nature of Pi resources results in continuous depletion of terrestrial Pi in the absence of added fertilizers or organic matter, a very common condition in many developing countries.

Because of the unique interaction of Pi with other elements, up to 80% of applied Pi may be fixed in the soil (Holford ,1997), forcing farmers to use up to four times the fertilizer necessary for crop production (Goldstein et al., 1987). At the current rate of usage of P fertilizer, readily available sources of phosphate rocks will be depleted over the next 60 to 90 years (Ae et al., 1990). At present, many tropical regions are faced with excessive mining of nutrients, including P, whereas some temperate regions with intensive, animal-based agricultural systems have, ironically, to deal with excessive soluble P in the soil that is threatening the ecosystem. In many parts of the United States and Europe, where enormous quantities of nutrient-rich manure are spread on the soils, the soluble Pi levels often exceed the crop requirement. Under these conditions, there is a significant potential for Pi movement. Excess soil Pi not removed by crops can enter surface water by erosion of Pi-rich soil particles, runoff, and leaching to field drain tiles. Increased Pi concentration in aquatic systems results in eutrophication and degradation of the environment. In this regard oil palm as one of the industrial crop in Malaysia with very fast increasing industry have great attention not just for country even for the world. Oil palm was introduced to Malaysia as an ornamental plant from the West Africa in 1911. The African oil palm is classified as *Elaeis guineensis* which belongs to the palmaea family. The first Malaysian commercial field of oil palm was set up in 1912 at Tennamaran estate, Selangor (Hartley, 1988).The oil palm industry grow very fast and need more and more chemical phosphate fertilizers .

Soil is a complex habitat where a large number of different microorganisms including bacteria, fungi, protozoa and algae interact. Only 1% to 10% of the soil microbiota can be cultured so there is still much to learn about soil as an environment for microbial life. Bacteria are by far the most numerically abundant soil microorganisms. They can be found free-living or attached to the surface of soil particles in bulk soil, but a large number of soil bacteria also interact with the roots of plants, in what is termed the rhizosphere. The rhizosphere is frequently divided into the endorhizosphere, the rhizoplane and the ectorhizosphere (Lynch ,1990). These respective compartments encompass the root tissues, the root surface and associated soil. Soil further away from the rhizosphere is often termed as bulk soil. Observations have shown that the concentration of bacteria found around the roots of plants is generally much greater than in the surrounding soil and that the rhizosphere supports higher microbial growth rates and activities as compared to the bulk soil (Söderberg and Bååth 1998). One of the main reasons for these higher growth rates is the increased availability of soluble organic compounds that results from plant root exudation. These are typically carbohydrate monomers, amino acids and sugars, but the composition and quantity of root exudates varies depending on plant species (Smith 1986) and abiotic conditions such as water content and temperature (Martin and Kemp 1980). In turn, rhizosphere microorganisms increase root exudation through production of plant hormones (Grayston et al. 1996). In general, the nutrient-rich rhizosphere is naturally colonized by many beneficial or pathogenic bacteria and fungi which may have a considerable impact on plant growth, development and productivity. The numerous interactions between bacteria, fungi and roots may have beneficial, harmful or neutral effects on the plant, the outcome being dependent on the type of symbiont interaction and the soil conditions (Smith and Read 1997).