UNIVERSITI PUTRA MALAYSIA

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FP 2012 4

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2012

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By

STELLA MATTHEWS

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

January 2012

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

MOLECULAR CHARACTERIZATION AND ORGANIC ACID PRODUCTION OF MINERAL PHOSPHATE SOLUBILIZING BACTERIA FOR MALAYSIAN SOIL

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January 2012

Chair : Prof. Madya Halimi b Mohd Saud, PhD

Faculty : Faculty of Agriculture

The present study emphasizes on the isolation and characterization of bacteria with the ability to solubilize mineral phosphates based on the consistency to form clear zone on National Botanical Research Institute's Phosphate Growth Medium agar medium (NBRIP) and the ability to release high amount of orthophosphates from insoluble mineral phosphates. Ten bacteria were identified as efficient mineral phosphate solubilizers namely 7 strains of *Klebsiella pneumoniae*, 2 strains of *Enterobacter aerogenes* and 1 strain of *Pseudomonas aeruginosa*. All the ten strains were able to dissolve calcium phosphate (Ca-P), ferric phosphate (Fe-P) and aluminium phosphate (Al-P) efficiently. Ca-P solubilization and the time of incubation were well correlated (correlation = 0.708, p = 0.000). The best Ca-P solubilizer was STMPSB 8 (*Klebsiella penumoniae*) which could solubilize 1772.5±112.4 mg/L orthophosphate. The best Fe-P solubilizer was STMPSB 9 (*Klebsiella pneumoniae*) which could release 1679.11±8.43 mg/L of orthophosphate. The best Al-P solubilizer was STMPSB 8 which has recorded 1198.57±14.04 mg/L of orthophosphate release. STMPSB 8 (*Klebsiella pneumoniae*) could be designated

as the best mineral P solubilizer for all the three insoluble mineral phosphates as it has exhibited high solubilization capacity for Ca-P, Fe-P and Al-P. Consequently, multiple organic acids such as gluconic acid, 2- ketogluconic acid, malic acid, pyruvic acid, acetic acid, propoanoic acid, glutaric acid, lactic acid, succinic acid, citric acid and fumaric acid were detected by Gas Chromatograph–Mass Spectrometer (GC-MS) which could have contributed to the solubilization of mineral phosphates. These organic acids may also function as chelators and involve in biocontrol activity as well. Phytohormones such as indole acetic acid (IAA) and gibberellic acid were also detected in some bacteria. High Performance Liquid Chromatography (HPLC) analysis was able to quantify gluconic acid and 2-ketogluconic acid produced by these bacterial strains. Gluconic acid was detected in all the bacterial isolates but 2-ketogluconic acid was only detected in seven of them. The correlation between gluconic acid production and solubilization of mineral phosphates was 0.795, significant at the 0.01 level. The highest amount of gluconic acid was produced by STMPSB 6 (199.51±36.56 mg/ml) followed by STMPSB 4 (197.04±24.67 mg/ml) where both strains were *Enterobacter aerogenes*. Finally, the detection of *pqq* C gene in eight of the bacterial isolates indicates that direct oxidation pathway was used during biosynthesis of gluconic acid with the aid of PQQ cofactor. Partial sequences of the *pqq C* gene obtained in the present study were deposited in the GenBank. The accession numbers were *JF683614*, *HQ727983*, *JF683615*, *JF683626*, *JF683617*, *HQ727985*, *JF683613* and *JF683618*. Based on this study it was concluded that the majority of the Gram negative bacteria produce multiple organic acids particularly gluconic acid to facilitate the mineral phosphate solubilization. These strains could be explored further for the production of biofertilizer.

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

PENCIRIAN MOLEKULAR DAN PENGHASILAN ASID ORGANIK BAKTERIA PELARUT FOSFAT MINERAL UNTUK TANAH MALAYSIA

Oleh

STELLA MATTHEWS

Januari 2012

Pengerusi : Prof. Madya Halimi b Mohd Saud, PhD

Fakulti : Fakulti pertanian

Kajian ini memberi penumpuan kepada pengasingan dan pencirian bakteria yang berupaya melarutkan fosfat mineral tak larut berdasarkan ketetapan (konsisten) untuk membentuk zon jernih pada medium agar National Botanical Research Institute's Phosphate Growth Medium (NBRIP) serta kebolehan membebaskan kuantiti ortofosfat yang tinggi daripada fosfat mineral tak larut. Sepuluh bakteria telah dikenalpasti sebagai pelarut fosfat yang cekap yang terdiri daripada 7 strain *Klebsiella pneumoniae*, 2 strain *Enterobacter aerogenes* dan 1 strain *Pseudomonas aeruginosa*. Semua strain berupaya melarutkan kalsium fosfat (Ca-P), ferum fosfat (Fe-P) dan aluminium fosfat (Al-P) dengan berkesan. Perlarutan Ca-P dan masa inkubasi menunjukkan korelasi yang baik (korelasi = 0.708, p = 0.000). Pelarut kalsium fosfat terbaik adalah STMPSB 8 (*Klebiella penumoniae*) yang berupaya melarutkan 1772.5±112.4 mg/L ortofosfat. Pelarut ferum fosfat terbaik adalah STMPSB 9 (*Klebsiella pneumoniae*) yang berupaya membebaskan 1679.11±8.43 mg/L ortofosfat. Pelarut aluminium fosfat terbaik adalah STMPSB 8 (*Klebsiella pneumoniae*) yang telah merekodkan 1198.57±14.04 mg/L pembebasan fosfat larut. STMPSB 8 (*Klebsiella pneumoniae*) dapat dicalonkan sebagai pelarut fosfat mineral

terbaik bagi ketiga-tiga fosfat tak larut dimana ia telah menunjukkan kebolehan perlarutan tertinggi bagi Ca-P, Fe-P dan Al-P. Seterusnya, pelbagai asid organik seperti asid glukonik, asid 2-ketoglukonik, asid malik, asid piruvik, asid asetik, asid propanoik, asid glutarik, asid laktik, asid suksinik, asid sitrik dan asid fumarik telah dapat dikesan melalui Gas Chromatograph–Mass Spectrometer (GC-MS) yang berkemungkinan telah menyumbang dalam perlarutan fosfat mineral. Semua asid organik mungkin juga berfungsi sebagai pengangkut ion (chelator) dan juga terlibat dalam kawalan biologi. Fitohormon seperti asid indol asetik (IAA) dan asid giberelik juga telah dikesan dalam beberapa isolat bakteria. Analisis dengan menggunakan 'High Performance Liquid Chromatography' (HPLC) berupaya mengkuantifikasikan asid glukonik dan asid 2-ketoglukonik yang dihasilkan oleh semua strain bakteria tersebut. Asid glukonik telah dikesan dalam semua kultur bakteria tetapi asid 2-ketoglukonik hanya dapat dikesan antara tujuh daripadanya. Korelasi antara penghasilan asid glukonik dan perlarutan fosfat adalah 0.795, bererti pada tahap 0.01. Kepekatan tertinggi asid glukonik dihasilkan oleh STMPSB 6 (199.51±36.56 mg/ml) diikuti oleh STMPSB 4 (197.04±24.67 mg/ml) dimana kedua-dua strain merupakan *Enterobacter aerogenes*. Akhirnya, pengecaman gen *pqq* C dalam 8 isolat bakteria menunjukkan bahawa laluan oksidasi secara langsung telah digunakan semasa biosintesis asid glukonik dengan bantuan kofaktor PQQ. Sebahagian daripada urutan gen *pqq* C yang didapati daripada kajian ini telah disimpan di 'GenBank'. Nombor sirinya adalah *JF683614*, *HQ727983*, *JF683615*, *JF683626*, *JF683617*, *HQ727985*, *JF683613* and *JF683618*. Berdasarkan kajian ini dapat disimpulkan bahawa kebanyakan bakteria Gram negatif menghasilkan pelbagai asid organik terutamanya asid glukonik untuk membantu perlarutan fosfat mineral. Kesemua strain tersebut dapat dieksploitasi lebih lanjut lagi untuk penghasilan baja bio.

ACKNOWLEDGEMENTS

First and foremost I would like to express my gratitude to THE ALMIGHTY GOD for HIS grace and mercy.

I am very grateful to my supervisor Professor Madya Dr. Halimi Mohd Saud and member of my supervisory committee, Professor Madya Datin Dr. Siti Nor Akmar Abdullah for enlightening suggestions, guidance and for the support given throughout my study. I also would like to extend my gratitude to the internal and external examiners who evaluated my thesis thoroughly and gave constructive comments to improve the quality of my thesis.

I would like to extend my sincere appreciation and heartfelt gratitude to my employer, Malaysian Agriculture Research and Development Institute (MARDI) for providing financial support and funding my study throughout these years.

Profound gratitude is also extended to Universiti Putra Malaysia (UPM), especially Faculty of Agriculture and Department of Agriculture Technology for the facilities available to conduct experiments and to acquire knowledge.

My special thanks to the members of Pesticide Laboratory, Bioprocess Laboratory and Soil and Microbiology Laboratory in Strategic Resource Research Center, MARDI for the assistance provided to use the laboratory facilities.

I also would like to convey my appreciation to all my friends in Department of Agriculture Technology, Faculty of Agriculture, UPM for their advices in conjunction to my study.

Last but not least, my heartfelt thanks to my parents, husband and sisters for being very supportive to complete my thesis.

I certify that a Thesis Examination Committee has met on **13th January 2012** to conduct the final examination of Stella Matthews on her thesis entitled "**Molecular Characterization and Organic Acid Production of Mineral Phosphate Solubilizing Bacteria for Malaysian Soil**" in accordance with the Universities and University College 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 Master of Science Degree.

Members of the Examination Committee were as follows:

Mihdzar b Abdul Kadir, PhD
Professor
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Maheran bt Abd Aziz, PhD
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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 the Supervisory Committee were as follows:

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BUJANG BIN KIM HUAT, PhD
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Date:

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 at any other institution.

STELLA MATTHEWS

Date: 13 January 2012

TABLE OF CONTENTS