UNIVERSITI PUTRA MALAYSIA

EFFECTS OF PHOSPHATE SOLUBILIZING BACTERIA, PHOSPHATE SOURCES AND ORGANIC ACID ON GROWTH PROMOTION OF AEROBIC RICE

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EFFECTS OF PHOSPHATE SOLUBILIZING BACTERIA, PHOSPHATE SOURCES AND ORGANIC ACID ON GROWTH PROMOTION OF AEROBIC RICE

By

QURBAN ALI PANHWAR

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

April 2011

DEDICATION

This work is dedicated to my beloved father and mother, who had a great dream for my success in education but passed away at early age without seeing any of those long journeys. I also dedicate this humble effort to my wife and my son who did their best to support me.

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

EFFECTS OF PHOSPHATE SOLUBILIZING BACTERIA, PHOSPHATE SOURCES AND ORGANIC ACID ON GROWTH PROMOTION OF AEROBIC RICE

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Chairman: Associate Professor Radziah Othman, PhD

Faculty : Agriculture

Phosphate-solubilizing bacteria (PSB) are frequently used as plant growth promoters and known to be able to solubilize different forms of inorganic phosphates. A series of experiments were conducted in laboratory and glasshouse conditions with the following objectives; i) to isolate and characterize the PSB strains from aerobic rice, ii) to evaluate the colonization of PSB on aerobic rice roots, iii) to evaluate the efficacy of PSB for production of organic acid from different sources of phosphate (P) in aerobic rice, iv) to determine the effect of combined PSB strains with organic acids on solubilization of P from phosphate rock and v) to determine the efficiency of P uptake and plant growth promotion of aerobic rice by PSB from phosphate rock using ^{32}P technique. Forty three PSB strains were isolated from aerobic rice (*Oryza sativa* L.). Sixteen potential PSB strains tested and characterized in *in vitro* condition for P solubilization activity. Root colonization of the PSB strains was visually observed by using Scanning Electron Microscope (SEM) and Transmission Electron Microscope (TEM). Glasshouse studies were conducted to evaluate the ability of strains *Bacillus* spp. (PSB9 and PSB16) to produce organic acids from different sources of

P fertilizers and the effect of diffrerent rates of dominant organic acids with PSB inoculation on solubilization of Christmas Island Phosphate Rock. The ^{32}P isotopic dilution technique was used to determine the efficiency of plant P uptake. The population of isolated PSB strains from aerobic rice was significantly ($P<0.05$) higher in rhizosphere than in non-rhizosphere soil. The P solubilizing activity of strains differed in different phosphate media used with the highest P solubilization observed in NBRIP broth containing calcium phosphate, while comparatively lower P observed in CIPR, and in PDYA-AlP containing aluminum phosphate. The P solubilization rate of different phosphate forms followed the first order kinetics. The bacterial strains were able to produce different organic acids (oxalic, malic, succinic and propionic acids) and in addition produce indole acetic acid (IAA), and enzymes (phosphatase and phytase). In general, the P solubilization by the different bacterial strains were significantly ($P<0.05$) influenced by the P sources used. The SEM and TEM micrographs proved that the PSB strains were able to colonize on surfaces and interior of rice roots. The PSB strains inoculated to rice plants under glasshouse conditions were able to produce organic acids and enhance P solubilization from CIPR. Exogenous oxalic acid application solubilized higher P (31.51%) of CIPR than the malic acid and this significantly affected growth of aerobic rice. The isotopic ^{32}P dilution studies proved that applied PSB strains were able to release P ions either from the added CIPR or from the less available indigenous P in soil resulting in higher plant P uptake and higher grain yield (37.87% over control). Inorganic fractionations of P in soil were ranked in the descending order of Fe-P > Al-P > Ca-P > soluble P. This study proved that PSB inoculation enhanced P solubilization from the different P sources through production of organic acids which enhanced P acquisition for growth improvement of aerobic rice.

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

KESAN BAKTERIA PELARUT FOSFORUS, SUMBER FOSFAT DAN ASID ORGANIK KE ATAS PENGGALAKAN PERTUMBUHAN PADI AEROBIK

Oleh

QURBAN ALI PANHWAR

April 2011

Pengerusi: Profesor Madya Radziah Othman, PhD

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Bakteria fosfat-pelarut (PSB) sering digunakan sebagai penggalak pertumbuhan tanaman dan diketahui dapat melarutkan pelbagai bentuk fosfat tak organik dalam bentuk yang berbeza. Beberapa siri eksperimen telah dilakukan di dalam makmal dan rumah kaca dengan tujuan seperti berikut; i) untuk mengasingkan dan mengkarakterisasi PSB dari padi aerobik, ii) untuk menilai pengkolonian PSB pada akar padi aerobik, iii) untuk menilai keberkesanan PSB untuk pengeluaran asid organik dari pelbagai sumber fosfat (P) pada padi aerobik, iv) untuk menentukan kesan gabungan PSB dengan asid organik ke atas solubilisasi P dari batuan fosfat, dan v) untuk menentukan kecekapan pengambilan P dan pertumbuhan tanaman penggalak padi aerobik oleh PSB dari batuan fosfat dengan menggunakan teknik pencairan ^{32}P. Empat puluh tiga PSB strain diisolasi dari padi aerobik (*Oryza sativa* L.). Enam belas strain berpotensi PSB diuji dan dicirikan di dalam keadaan *in vitro* untuk solubilisasi P. Pengkolonian akar oleh PSB dilihat secara visual dengan menggunakan mikroskop imbasan elektron (SEM) dan Mikroskop Elektron Transmisi (TEM). Kemampuan strain PSB (PSB9 dan PSB16) untuk menghasilkan asid organik dari tri-superfosfat (TSP) dan Fosfat Batuan Pulau Krismas (CIPR) pada padi aerobik dinilai. Kesan asid organik dominan dengan PSB pada kadar

yang berbeza ke atas solubilisasi dari CIPR ditentukan. Teknik pencairan isotop ^{32}P digunakan untuk menentukan pengambilan P oleh tanaman. Keputusan kajian menunjukkan bahawa populasi strain PSB diisolasi dari padi aerobik adalah secara signifikan ($P < 0.05$) lebih tinggi pada rhizosfera daripada bukan rhizosfera. Aktiviti strain P solubilisasi adalah berbeza pada media berbeza yang digunakan dengan pertumbuhan tertinggi adalah pada piring media NBRIP. Strain bakteria juga mampu menghasilkan asid organik yang berbeza (asid oksalik, malik, suksinik dan propionik), asid asetik Indol (IAA), dan enzim (fosfatase dan fitase). Semua strain bakteria yang telah dikenalpasti sebagai Bacillus spp dan P solubilisasi oleh strain bakteria yang berbeza ($P < 0.05$) dipengaruhi secara signifikan oleh sumber P yang digunakan. Solubilisasi P tertinggi diperhatikan dalam larutan NBRIP yang mengandungi kalsium fosfat, manakala P dilarutkan lebih rendah pada CIPR, dan di PDYA-AlP yang mengandungi aluminium fosfat. Kadar solubilisasi P oleh bentuk P yang berbeza adalah mengikut hukum kinetik pertama. Mikrograf SEM dan TEM membuktikan bahawa strain mampu membuat pengkolonian pada permukaan akar primer dan akar lateral interior tanaman padi. Eksperimen rumah kaca menunjukkan bahawa strain PSB diinokulasi pada tanaman padi berkebolehan menghasilkan asid organik dan meningkatkan P dari PR. Aplikasi asid oksalik menunjukkan bahawa solubilisasi P pada PR lebih tinggi (31.51%) dari asid malik dan secara signifikan mempengaruhi pertumbuhan padi aerobik. Kajian pencairan isotop ^{32}P membuktikan bahawa strain PSB berkebolehan melepaskan ion P samada dari penambahan PR atau dari P ketersediaan yang kurang dalam tanah. Inokulasi pada tanaman menunjukkan pengambilan P dan hasil tanaman adalah lebih tinggi (37.87%) berbanding kawalan. Pembahagian P tidak organik disusun mengikut urutan Fe-P> Al-P> Ca-P> P

terlarut. Kajian ini membuktikan bahawa inokulasi PSB meningkatkan kadar keterlarutan P dari pelbagai sumber P tidak larut dengan penghasilan asid organik yang berupaya untuk menggalakkan pengambilan P dan seterusnya meningkatkan pertumbuhan padi aerob.

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I certify that a Thesis Examination Committee has met on 29th of April, 2011 of viva voce to conduct the final examination of Qurban Ali Panhwar on his Doctor of Philosophy thesis entitled "Effects of phosphate-solubilizing bacteria, phosphate sources and organic acids on the growth promotion of aerobic rice" in accordance with the Universities and Universtity 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.

Members of the Thesis 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 partial requirement of the degree of Doctor of Philosophy. The members of the 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 other institutions.

QURBAN ALI PANHWAR

Date: 29 April 2011

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