UNIVERSITI PUTRA MALAYSIA

APPLICATION OF RHIZOBIA AND PLANT GROWTH PROMOTING RHIZOBACTERIA FOR INCREASED GROWTH, N2 FIXATION AND YIELD OF RICE

TAN KEE ZUAN

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APPLICATION OF RHIZOBIA AND PLANT GROWTH PROMOTING RHIZOBACTERIA FOR INCREASED GROWTH, N_2 FIXATION AND YIELD OF RICE

By

TAN KEE ZUAN

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

March 2015

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

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TAN KEE ZUAN

March 2015

Chair : Professor Zulkifli Hj. Shamsuddin, PhD

Faculty : Agriculture

Rice is one of the major foods consumed by people in most countries and especially from the Asian region, including Malaysia. Due to the ever-increasing demand, rice farmers tend to over-apply chemical fertilizers as the solution to increase the crop yield but this indiscriminate practice has a toll on the environment such as eutrophication due to fertilizer losses through leaching, volatilization and surface runoff. Biofertilizers have become prominent in the agricultural sector as an alternative source of nitrogen fertilizer and can substantially supplement the N requirement while enhancing the uptake of water and mineral nutrients of crop plants. Plant growth-promoting rhizobacteria (PGPR) have been reported to be beneficial to various crops including rice, while rhizobia are known for their symbiotic associations with legumes. There were reports on the benefits of rhizobial inoculation on rice but the mechanisms still remain unclear and are far more dynamic than previously thought. A series of studies involving six experiments were conducted to observe the effects of locally isolated PGPR and rhizobial strains in combined and single inoculations on growth, nitrogen fixation and yield of rice plants under laboratory and glasshouse conditions. In the preliminary study, isolation of PGPR and rhizobial strains from four locations of rice growing areas around Peninsular Malaysia with different cultural practices were undertaken. A total of 205 strains were obtained with the highest number of bacterial isolates from Besut, Terengganu (45%). This rice farming site practises minimal chemical fertilizer input system which is believed to promote plant-microbe interaction and increase beneficial activities to the host plant. It was also observed that higher numbers of endophytes compared to rhizospheric PGPR were isolated at sampling locations which have been cultivated with rice for a much longer period namely at Tunjung, Kelantan (61%) and Besut, Terengganu (56%). The microbial populations were believed to have developed mechanisms for survival by becoming root endophytes and consequently protected from adverse soil environmental conditions and competition with other indigenous soil microbes. The isolated PGPR and rhizobial strains were characterized through several

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biochemical assays namely biological nitrogen fixation, phosphate and potassium solubilization, and phytohormone, iron siderophore and hydrolyzing enzyme production. Two superior PGPR and two rhizobial strains were selected for subsequent experiments namely UPMB19, UPMB20, UPMR30 and UPMR31 and identified as *Lysinibacillus xylanilyticus, Alcaligenes faecalis, Bradyrhizobium japonicum and Rhizobium etli* bv. *mimosa*, respectively, along with the reference strain, UPMB10, identified as *Bacillus subtilis*. These strains have high nitrogenase activities which ranged between 15.60 – 19.95 nmol C_2H_4 mL^{-1} h^{-1}. It was also observed that bacterial abilities to solubilize phosphate were positively correlated to the incubation period and negatively correlated to the media pH. UPMB10 produced highest soluble P at 6 days after incubation (51.14 µg mL^{-1} P), while UPMR31 produced 61.87 µg mL^{-1} soluble P at 12 days after incubation. These strains were also able to produce phytohormone (indole-3 acetic acid, IAA) which ranged between 8.932 – 23.681 µg mL^{-1} and solubilize potassium at the rates between 10.70 – 14.15 mg L^{-1}. An *in vitro* study demonstrated that these PGPR and rhizobial strains could effectively maintain a high population and produce phytohormones in the residual nutrient solution, with a significant enhancement of rice seedling heights and total dry weights. This initial study has demonstrated some positive effects of the locally isolated PGPR and indigenous rhizobial strains on the rice plant. In the glasshouse study with rice plants in 15 kg plastic pots, 33% of fertilizer-N supply from the conventional recommended rate was found to be optimum for bacterial inoculation treatment. In some cases, the growth and yield enhancements were even greater than the control plant with full fertilizer-N, suggesting the possibility that these bacterial inocula could be applied to minimize chemical fertilizer inputs while still producing higher yields compared to the conventional practice. It was also observed that tiller productions were positively correlated to the yield. Inoculation with UPMB19 resulted in a significantly more tillers (28) at 43 DAP and subsequently produced significantly higher spikelet weight (107 g) at terminal harvest (115 days after planting). The rhizobial strain, UPMR30, also performed better than other inoculants by producing significantly higher spikelet weights, directly correlated to rice grain yield. The final experiment was conducted to determine the effects of combined inocula which consisted of a selected PGPR (UPMB19) and rhizobia (UPMR30) on growth and N_2 fixation of rice plant using ^{15}N isotope dilution technique under glasshouse conditions for a 65 day-period. At the terminal harvest, the combined inocula successfully promoted rice plant top and root growth, tiller numbers, plant dry weights, nutrient accumulations in soil and plant tissues, and consequently increased the N_2 fixation rate of the rice plant ecosystem. The rhizobia supplied nitrogen through BNF while the PGPR increased root proliferation and overall plant growth through the production of phytohormone (IAA). This was supported by the significantly higher values of chlorophyll and nitrogen content in plant tissues and dilutions of ^{15}N a.e. % of the combined inocula and single inoculation with UPMR30 as compared to the single inoculation with UPMB19. The accumulation of N in the soil and plant tissues were also significantly increased in the inoculated plants and similar trends were observed with the reduction in ^{15}N a.e. % as compared to the uninoculated control. This indicated that the BNF processes successfully increased the N availability around the roots for greater plant uptake. It was observed that the BNF activities of the rhizobial strain (UPMR30) was higher than the PGPR strain (UPMB19) with Ndfa values of 19

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and 12%, respectively. Meanwhile, the combined inocula produced the highest BNF activities at 22% Ndfa, strongly suggesting a synergistic interaction and cumulative beneficial effects between the rhizobial and PGPR strains. Based on the BNF estimations, the combined inocula treatment could save up to 45% of fertilizer-N from the recommended rate for rice, equivalent to 63 kg N fixed ha^{-1} in the period of 65 days. Several co-inoculation studies have been undertaken by other researchers using rhizobial and PGPR strains. However, there has been no report regarding the multi-strain beneficial effects of BNF on rice involving combined applications of PGPR and rhizobia; thus, indicating the significance of this study.

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

PENGGUNAAN RHIZOBIA DAN RHIZOBAKTERIA PENGGALAK TUMBESARAN POKOK UNTUK MENINGKATKAN PERTUMBUHAN, PENGIKATAN N_2 DAN HASIL PADI

Oleh

TAN KEE ZUAN

Mac 2015

Pengerusi : Profesor Zulkifli Hj. Shamsuddin, PhD

Fakulti : Pertanian

Padi merupakan salah satu daripada makanan utama di kebanyakan negara terutamanya negara Asia dan ini termasuklah Malaysia. Disebabkan oleh peningkatan permintaan yang berterusan, petani terdorong untuk menggunakan baja kimia secara berlebihan sebagai kaedah untuk meningkatkan hasil padi, tetapi kaedah berisiko ini menjurus kepada pencemaran alam sekitar contohnya eutrofikasi yang berlaku akibat daripada kehilangan baja melalui larut resapan, pengewapan dan larian permukaan. Baja bio telah terkenal dalam sektor pertanian sebagai sumber alternatif baja nitrogen dan penggunaannya boleh melengkapkan keperluan N selain meningkatkan pengambilan air dan nutrien oleh tumbuhan. Rhizobakteria penggalak tumbesaran pokok (RPTP) telah dilaporkan berkesan kepada pelbagai jenis tumbuhan termasuk padi, manakala rhizobia telah sedia dikenali dengan perhubungan simbiotik bersama pokok kekacang. Terdapat laporan berkenaan faedah inokulasi rhizobia terhadap pokok padi tetapi mekanismanya masih tidak jelas dan lebih dinamik daripada yang disangkakan. Beberapa siri kajian melibatkan enam ekperimen telah dijalankan untuk melihat kesan inokulasi RPTP dan rhizobia yang diasingkan di kawasan sawah padi tempatan secara campuran dan berasingan ke atas pertumbuhan, pengikatan nitrogen dan hasil padi dalam keadaan makmal dan rumah kaca. Dalam kajian awalan, pengasingan RPTP dan rhizobia dari empat kawasan sawah padi sekitar Semenanjung Malaysia yang mengamalkan sistem penanaman yang berbeza telah dijalankan. Sejumlah 205 bakteria telah didapati dan pengasingan bakteria di Besut, Terengganu telah menghasilkan bakteria paling banyak iaitu 45% daripada jumlah keseluruhan tersebut. Petani di kawasan sawah padi ini mengamalkan penggunaan baja kimia secara minimal yang dipercayai telah menggalakkan interaksi antara pokok padi dan mikrob dalam tanah dan meningkatkan aktiviti yang berfaedah kepada pokok tersebut. Keputusan pengasingan ini juga telah menunjukkan bahawa terdapat lebih banyak RPTP endofitik berbanding rizosferik di kawasan yang telah ditanam dengan padi secara berterusan selama bertahun-tahun iaitu di Tunjung, Kelantan (61%) dan Besut, Terengganu (56%). Populasi

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mikrob di kawasan ini dipercayai telah menghasilkan mekanisma untuk hidup dengan menjadi endofitik yang membolehkannya terlindung dari keadaan sekitar tanah yang kurang sesuai dan juga daripada persaingan dengan mikrob tanah sedia ada yang lain. Ciri-ciri RPTP dan rhizobia yang diasingkan ini kemudiannya dikenalpasti menggunakan beberapa kaedah biokimia iaitu pengikatan nitrogen, pelarutan fosforus dan kalium, dan penghasilan fitohormon, siderofor zat besi dan enzim hidrolisasi. Dua jenis RPTP dan dua rhizobia yang paling berfaedah telah dipilih untuk ekperimen seterusnya iaitu UPMB19, UPMB20, UPMR30 dan UPMR31, yang dikenalpasti sebagai *Lysinibacillus xylanilyticus*, *Alcaligenes faecalis*, *Bradyrhizobium japonicum* dan *Rhizobium etli* bv. *mimosa*, termasuk bakteria rujukan iaitu UPMB10 yang dikenalpasti sebagai *Bacillus subtilis.* Bakteria-bakteria ini mempunyai kadar pengikatan nitrogen yang tinggi dalam lingkungan 15.60 – 19.95 nmol C_2H_4 mL^{-1} jam^{-1}. Hasil kajian juga menunjukkan bahawa kebolehan bakteria untuk melarutkan fosforus adalah berkait terus dengan tempoh inkubasi dan berkait secara negatif dengan pH media. UPMB10 menghasilkan fosforus larut yang tertinggi selepas inkubasi selama 6 hari (51.14 µg mL^{-1} P), manakala UPMR31 menghasilkan 61.87 µg mL^{-1} fosforus larut selepas 12 hari inkubasi. Bakteria-bakteria ini juga berupaya menghasilkan fitohormon (indole-3 acetic acid, IAA) dalam lingkungan 8.932 – 23.681 µg mL^{-1} dan melarutkan kalium dengan kadar lingkungan 10.70 – 14.15 mg L^{-1}. Kajian dalam makmal secara *in vitro* telah menunjukkan bahawa RPTP dan rhizobia ini boleh secara efektifnya mengekalkan populasi yang tinggi dan menghasilkan fitohormon di dalam media nutrien, dengan peningkatan yang signifikan pada ketinggian dan berat kering anak pokok padi. Kajian awalan ini telah menunjukkan kesan yang positif oleh RPTP dan rhizobia yang diasingkan di kawasan sawah padi tempatan terhadap pokok padi. Dalam kajian rumah kaca yang menggunakan pasu plastik seberat 15 kg, sebanyak 33% bekalan baja kimia nitrogen daripada jumlah yang disyorkan terbukti optimum untuk kesan inokulasi bakteria yang maksimum. Dalam beberapa kes, pertumbuhan dan hasil padi adalah lebih tinggi daripada pokok kawalan yang dibekalkan dengan 100% baja kimia nitrogen, dan ini menunjukkan bahawa inokulasi bakteria-bakteria ini boleh mengurangkan input baja kimia dan meningkatkan hasil padi pada masa yang sama, berbanding sistem yang sedia ada. Kajian ini juga menunjukkan penghasilan anak padi (tiller) berkait terus dengan hasil padi ketika menuai. Inokulasi dengan UPMB19 telah meningkatkan penghasilan anak padi secara signifikan iaitu sebanyak 28 batang selepas 43 hari menanam dan seterusnya juga meningkatkan berat spikelet secara signifikan iaitu 107 g ketika menuai (115 hari selepas menanam). Rhizobia UPMR30 juga menghasilkan berat spikelet yang lebih tinggi berbanding bakteria-bakteria lain, kesan tumbesaran pokok yang berkait rapat dengan hasil padi. Eksperimen terakhir dijalankan dalam rumah kaca untuk mengenalpasti kesan inokulasi campuran melibatkan satu RPTP (UPMB19) dan satu rhizobia (UPMR30) terpilih ke atas pertumbuhan dan pengikatan nitrogen oleh pokok padi, menggunakan kaedah pencairan isotop ^{15}N selama 65 hari. Keputusan kajian menunjukkan inokulasi campuran ini berjaya meningkatkan pertumbuhan akar dan pokok, bilangan anak padi, berat kering pokok, akumulasi nutrien dalam tanah dan tisu tumbuhan dan seterusnya meningkatkan kadar pengikatan nitrogen dalam ekosistem pokok padi. Rhizobia telah mengikat nitrogen manakala RPTP meningkatkan pertumbuhan akar dan pokok secara amnya melalui penghasilan fitohormon (IAA). Ini telah terbukti melalui peningkatan secara signifikan

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kandungan klorofil dan nitrogen dalam tisu tumbuhan dan pencairan ^{15}N oleh inokulasi campuran dan inokulasi berasingan UPMR30, jika dibandingkan dengan UPMB19. Akumulasi nitrogen di dalam tanah dan tisu tumbuhan juga telah meningkat secara signifikan dalam pokok yang telah diinokulasi dengan bakteria-bakteria ini dan senario yang sama berlaku terhadap pencairan ^{15}N, berbanding pokok kawalan tanpa inokulasi. Ini menunjukkan bahawa proses pengikatan nitrogen telah berjaya meningkatkan kandungan nitrogen di sekitar akar untuk diambil oleh pokok. Hasil kajian juga menunjukkan bahawa kadar pengikatan nitrogen oleh rhizobia (UPMR30) adalah lebih tinggi berbanding RPTP (UPMB19) dengan nilai masing-masing sebanyak 19 dan 12%. Inokulasi campuran pula telah menghasilkan kadar pengikatan nitrogen yang tertinggi iaitu pada nilai 22% dan ini telah membuktikan dengan nyata berlakunya interaksi sinergi dan faedah terkumpul oleh rhizobia dan RPTP tersebut. Berdasarkan kepada kadar pengikatan nitrogen ini, dianggarkan inokulasi campuran tersebut mampu menjimatkan sebanyak 45% baja kimia nitrogen daripada kadar yang disyorkan, dan ini adalah bersamaan dengan 63 kg N ha^{-1} selama 65 hari. Beberapa kajian terdahulu berkenaan inokulasi campuran rhizobia dan RPTP telah dijalankan. Namun demikian, tiada laporan berkenaan kesan berfaedah inokulasi campuran melibatkan RPTP dan rhizobia terhadap pertumbuhan dan pengikatan nitrogen oleh pokok padi; justeru mencerminkan signifikan kajian ini.

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I certify that a Thesis Examination Committee has met on 10 Mac 2015 to conduct the final examination of Tan Kee Zuan on his thesis entitled "Application of Rhizobia and Plant Growth Promoting Rhizobacteria for Increased Growth, N_2 Fixation and Yield of Rice" 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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Faculty of Agriculture
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Faculty of Agriculture
Universiti Putra Malaysia
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Felix Dapare Dakora, PhD
Professor
Department of Chemistry
Tshwane University of Technology
South Africa
(External Examiner)

ZULKARNAIN ZAINAL, PhD
Professor and Deputy Dean
School of Graduate Studies
Universiti Putra Malaysia

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Zulkifli Haji Shamsuddin, PhD
Professor
Faculty of Agriculture
Universiti Putra Malaysia
(Chairman)

Radziah Othman, PhD
Associate Professor
Faculty of Agriculture
Universiti Putra Malaysia
(Member)

Halimi Mohd Saud, PhD
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Faculty of Agriculture
Universiti Putra Malaysia
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LIST OF ABBREVIATIONS

a.e.	atom excess
ARA	Acetylene Reduction Assay
BNF	Biological Nitrogen Fixation
CRD	Completely Randomized Design
cv	cultivar
DAI	Days after Inoculation
DAT	Days after Transplanting
dia	diameter
g	gram
h	hour
ht	height
IAA	Indole-3 Acetic acid
kg	kilogram
mL	milliliter
mg	milligram
min	minute (s)
Ndfa	Nitrogen Derived from Atmosphere
OD	Optical Density
PGPR	Plant Growth-Promoting Rhizobacteria
TDM	Total Dry Matter
TDW	Total Dry Weight
TSA	Tryptic Soy Agar
µ	micro

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CHAPTER 1

INTRODUCTION

Recently, the world population had reached 7 billion and it is expected to double by 2040 (Masciarelli *et al*., 2013). This phenomena has resulted in increased concerns throughout the world regarding food security. The global food crisis, as in 2007 and 2008, has once again thrust hunger into the international spotlight (Shattuck and Holt-Gimenez, 2010). The food security problem has also put more and more people at risk of hunger and malnutrition, with the added threat caused by the rise of agricultural products being used for biofuel (Gleissman, 2014). Malaysia is among the countries which seems to be affected, with a current population of 28.4 million and rising at the rate of 1.9% from year 2000 to 2010 (FAO UN, 2012). Due to the increased human population, the Food and Agriculture Organization (FAO) of the United Nations has set the goal to double the food production by 2050 in order to meet the ever-increasing demand (FAO UN, 2009). Rice is one of the major foods taken up by people in most countries especially from the Asian region including Malaysia, with the national's self-sufficiency level about 75% and an average yield of 4.5 t ha^{-1} $season^{-1}$. It has therefore been proposed that the average yield needs to be increased to approximately 7 t ha^{-1} $season^{-1}$ in order to be fully self-sufficient.

Conventionally, rice farmers tend to over-apply chemical fertilizers as the easy and speedy solution to increase their yield but this indiscriminate practice has a toll on the environment due to the fertilizer loss through leaching, volatilization and surface runoff. Leaching and runoff will lead to the deposition of these inorganic fertilizers into the river and causes heavy euthrophication while volatilization through denitrification causes the production of nitrous oxide (NO_2) which is a potent greenhouse gas (GHG) that depletes the ozone layer (Gruber and Galloway, 2008). This method has also increased the input cost as the efficiency of chemical inorganic fertilizer is very low, generally around 30 - 40% for urea as the main source of fertilizer-N (Choudhury and Khanif, 2001; Choudhury and Kennedy, 2005). Another problem that arises from it is related to the high phosphate fixation rate, especially in tropical soils mainly due to the large quantities of Fe and/or Al oxides in acidic condition (Sanchez and Uehara, 1980; Harter, 2007). Nitrogen remains the highest applied fertilizer in agriculture in 2011 with a global demand of 105.3 million t, followed by phosphate with a demand of 41.7 million t, and both are expected to increase at the annual rate of 1.7% and 1.9%, respectively (FAO UN, 2011).

Therefore, a greener approache is to minimize the use of chemical fertilizer while increasing the crop yield by applying environmentally-friendly biofertilizer. Biofertilizer is a form of microbial inoculants which have been reported to be able to enhance the growth and yield of various crops. Numerous type of beneficial microbes have been used as a biofertilizer including fungi; *Glomus* (Duponnois 2005; Chen *et al*., 2007), bacteria; *Bacillus*, *Azospirillum*, *Acetobacter*, *Herbaspirillum* (Boddey *et al*., 1995; Mia *et al*., 2010a) and rhizobia;

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Bradyrhizobium and Rhizobium (Garcia-Fraile *et al*., 2012; Javaid and Mahmood, 2012). A compilation of research done in the past has shown that biofertilizer application benefits the crop by increasing growth and yield with a minimal use of inorganic fertilizer.

Plant growth-promoting rhizobacteria (PGPR) are biofertilizer derived from the root rhizosphere of crops. It has been reported that PGPR inoculation to sweet potato with 33% N of the total fertilizer-N requirement resulted in a similar plant biomass and yield as the fully fertilized plants, consequently presenting a 67% saving in fertilizer-N (Saad *et al*., 1999). A study by Malik *et al.* (1997) also showed that approximately 70% of nitrogen in the rice variety BAS-370 was nitrogen derived from atmosphere (Ndfa) upon inoculation with *Azospirillum* N-4. The PGPR are believed to promote crops mainly through biological nitrogen fixation (BNF), increases of growth, water, mineral and nutrient uptake, production of plant growth regulator/phytohormone and also as biological control for pest and diseases (Ramamoorthy *et al.*, 2001; Vessey, 2003; Garcia *et al.*, 2004; Mahmood *et al*., 2010; Kryuchkova *et al*., 2014). However, the exact modes by which PGPR promote plant growth at a specific step in the life cycle are not fully understood yet (Masciarelli *et al*., 2013). Research with PGPR on cereal crops such as rice have produced several beneficial effects mostly through biological N_2 fixation (Malik *et al*., 1997), increased root growth (Mia *et al*., 2012), enhanced nutrient uptake (Yanni *et al*., 1997), phytohormone production (Chabot *et al*., 1996), and disease control (Ramamoorthy *et al*., 2001). Despite the vast positive outcomes, there are inconsistent issues when it comes to the application of PGPR on rice plant, possibly due to the flooded conditions in wetland rice cultivation. The majority (85%) of the total rice-cropped area is under wetland culture (Choudhury and Kennedy, 2004) which includes deepwater rice cultivation whereby rice are grown in flooded conditions for almost the entire planting season and this creates a microaerophilic to anaerobic environment, that may change the rhizosphere microbial community (Tilak *et al*., 2005).

A good example to illustrate the benefit of microorganisms is the legume-rhizobial symbiosis, in which the bacteria fix nitrogen as endosymbionts inside root nodules in a nutrient-rich, oxygen-controlled microenvironment (Reinhold-Hurek and Hurek, 1998). Recently, researchers started to look into the possibility of inoculating non-legume crops such as rice with rhizobial strains with the idea to benefit from their high nitrogen-fixing abilities. There were reports on the benefits of rhizobial inoculation on rice in various countries but the mechanisms still remain unclear and far more dynamic than previously thought (Yanni *et al*., 1997; Biswas *et al*., 2000a; Peng *et al.*, 2002; Chi *et al*., 2005; Yanni and Dazzo, 2010; Bhattacharjee *et al*., 2012; Mia *et al*., 2012). Some studies have reported that the beneficial effects of rhizobial inoculation on non-legumes are similar to PGPR abilities such as BNF, phosphate solubilization, phytohormone production and many more (Chabot *et al*., 1996). Naher *et al*. (2009) found that rice seedlings inoculated with rhizobial strains contained higher nitrogen (4.47%) with an increased biomass of 36%, while Mia *et al*. (2012) showed that rhizobial inoculation increased the seedling germination, emergence, vigour and root growth, which in turn meant the production of seedlings with better establishment and consequently benefitting the crop growth and yield.

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Despite these reports, few studies have been conducted on the use of locally isolated rhizobia and PGPR on rice plants especially in Malaysia. The new approach is to apply the PGPR and rhizobial strains as multi-strain biofertilizer with the idea to benefit from the various beneficial characteristics derived from the synergistic effects between the strains. There were numerous positive reports on the application of combined inocula of PGPR to various crops with a significant improvement of nutrient accumulations (P and K), shoot, root, grain and dry matter yield of sorghum, barley and eggplant (Alagawadi and Gaur, 1988; Belimov *et al*., 1995; Han and Lee, 2005). However, very minimal work has been found to elucidate the mechanism(s) and determine the effects of combined inocula consisting of rhizobia and PGPR in any crops including rice. It is hypothesized that this multi-strain biofertilizer will enhance the rice plant growth and grain yield possibly through BNF along with several other known beneficial effects of PGPR and rhizobia such as solubilization of phosphate and potassium and production of plant growth regulators, siderophore and hydrolyzing enzymes. Biofertilizer application, which consists of locally isolated PGPR and rhizobial strains can help to reduce environmental pollution by minimizing inorganic fertilizer usage, while improving the growth and yield of rice. These can be achieved along with a reduction of input costs which will result in a substantive impact to the Malaysian rice farming sector. With this aim in view, the present study was undertaken with the following objectives:

1. To select and identify the superior rhizobacterial and rhizobial strains with multiple plant growth-promoting traits from rice fields in Peninsular Malaysia
2. To determine the effects of selected PGPR and rhizobial inoculations with different fertilizer-N rates on the growth and yield of rice.
3. To evaluate the performance of selected PGPR and rhizobia as a multi-strain biofertilizer on the growth, nutrient accumulations and tillering of rice.
4. To elucidate the BNF activities of single or combined inoculation of PGPR and rhizobia in a rice ecosystem.

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BIBLIOGRAPHY

Abd-Alla M.H. 1994. Solubilization of rock phosphates by *Rhizobium* and *Bradyrhizobium*. *Folia Microbiol*. 39(1):53-56

Abd-Alla M.H. El-Enany A.E., Nafady N.A., Khalaf D.M. and Morsy F.M. 2014. Synergistic interaction of *Rhizobium leguminosarum* bv. *viciae* and arbuscular mycorrhizal fungi as a plant growth promoting biofertilizers for faba bean (*Vicia faba* L.) in alkaline soil. *Microbiol. Res*. 169:49-58

Adesemoye A.O. and Kloepper J.W. 2009. Plant-microbes interactions in enhanced fertilizer-use efficiency. *Appl. Microbiol. Biotechnol*. 85:1-12

Ahemad M. and Khan M.S. 2011. Functional aspects of plant growth promoting rhizobacteria: recent advancements. *Insight Microbiol*. 1(3):39-54

Ahmad R., Arshad M., Khalid A. and Zahir Z.A. 2008. Effectiveness of organic-/bio-fertilizer supplemented with chemical fertilizers for improving soil water retention, aggregate stability, growth and nutrient uptake of maize (*Zea mays* L.). *J. Sustainable Agric*. 31(4):57-77

Ahmed I., Yokota A., Yamazoe A. and Fujiwara T. 2007. Proposal of *Lysinibacillus boronitolerans* gen. nov. sp. nov., and transfer of *Bacillus fusiformis* to *Lysinibacillus fusiformis* comb. nov. and Bacillus sphaericus to *Lysinibacillus sphaericus* comb. nov. *Int. J. Systematic and Evol. Microbiol*. 57:1117-1125

Ai'shah O.N., Amir H.G., Keng C.L. and Othman A.R. 2009. Influence of various combinations of diazotrophs and chemical N fertilizer on plant growth and N_2 fixation capacity of oil palm seedlings (*Elaeis guineensis* Jacq.). *Thai J. Agric. Sci*. 42(3):139-149

Akhtar M.S. and Siddiqui Z.A. 2010. Role of plant growth promoting rhizobacteria in biocontrol of plant diseases and sustainable agriculture. In *Plant Growth and Health Promoting Bacteria* eds: D.K. Maheshwari. pp 157-182. Springer-Verlag, Berlin Heidelberg

Alagawadi A.R. and Gaur A.C. 1988. Interaction between *Azospirillum brasilense* and 'phosphate solubilizing bacteria' and their influence on yield and nutrient uptake of sorghum (*Sorghum bicolor* L.). *Zentralbl. Mikrobiol*. 143:637-643

Alexander D.B. and Zuberer D.A. 1991. Use of chrome azurol S reagents to evaluate siderophore production by rhizosphere bacteria. *Biol. Fertil. Soils*. 12:39-45

Alias, H. and Manaf, A.M.R. 1993. Fertilizer research priorities for maximum yield of rice in Malaysia. In: Habibuddin H. and Suhaimi O. (Eds.).

© COPYRIGHT UPM

Proceedings of Malaysian National Rice Conference, 21-23 September, 1993. Pulau Pinang: MARDI/MACA/FIAM. Pp 125-140.

Amir, H.G., Z.H. Shamsuddin, M.S. Halimi, M.F. Ramlan and M. Marziah, 1999. Potential use of rhizobacteria for sustainable oil palm seedling production. *Malaysian J. Soil. Sci*. 3: 39-50

Amir, H.G., Z.H. Shamsuddin, M.S. Halimi, M.F. Ramlan and M. Marziah, 2003. Nitrogen fixation, nutrient accumulation and plant growth promotion by rhizobacteria in association with oil palm seedlings. *Pak. J. Biol. Sci.* 6:1269-1272

Amir H.G., Shamsuddin Z.H., Halimi M.S., Marziah M. and Ramlan M.F. 2005. Enhancement in nutrient accumulation and growth of oil palm seedlings caused by PGPR under field nursery conditions. *Communications in Soil Sci. and Plant Anal*. 36:2059-2066

Anderson F.N. and Peterson G.A. 1978. Optimum starter fertilizer placement for sugarbeet seedlings as determined by uptake of radioactive ^{32}P isotope. *J. American Society of Sugar Beet Technol.* 20(1):19-24

Andrews M., James E.K., Cummings S.P., Zavalin A.A., Vinogradova L.V. and McKenzie B.A. 2003. Use of nitrogen fixing bacteria inoculants as a substitute for nitrogen fertilizer for dryland graminaceous crops: progress made, mechanisms of action and future potential. *Symbiosis*. 35(1):209-229

Antoun H., Beauchamp C.J., Goussard N., Chabot R. and Lalande R. 1998. Potential of *Rhizobium* and *Bradyrhizobium* species as plant growth promoting rhizobacteria on non-legumes: effects on radishes (*Raphanus sativus* L.). *Plant Soil*. 204:57-67

Asea P.E.A., Kucey R.M.N. and Stewart J.W.B. 1988. Inorganic phosphate solubilization by two *Penicillium* species in solution culture and soil. *Soil Biol. Biochem*. 20(4):459-464

Ashrafuzzaman M., Hossen F.A., Ismail M.R., Hoque M.A., Islam M.Z., Shahidullah S.M. and Meon S. 2009. Efficiency of plant growth-promoting rhizobacteria (PGPR) for the enhancement of rice growth. *African J. Biotechnol*. 8(7):1247-1252

Azcon R. 1993. Growth and nutrition of nodulated mycorrhizal and non-mycorrhizal *Hedysarum coronarium* as a result of treatment with fractions from a plant growth-promoting rhizobacteria. *Soil Biol. Biochem*. 25(8):1037-1042

Bagyalakshmi B., Ponmurugan P. and Marimuthu S. 2012. Influence of potassium solubilizing bacteria on crop productivity and quality of tea (*Camellia sinensis*). *African J. Agric. Res*. 7(30):4250-4259

© COPYRIGHT UPM

Bai Y., Pan B., Charles T.C. and Smith D.L. 2002. Co-inoculation dose and root zone temperature for plant growth promoting rhizobacteria on soybean [*Glycine max* (L.) Merr] grown in soil-less media. *Soil Biol. Biochem*. 34:1953-1957

Barazani O.Z. and Friedmand J. 1999. Is IAA the major root growth factor secreted from plant-growth-mediating bacteria? *J. Chem. Ecol*. 25(10):2397-2406

Bartel B. 1997. Auxin biosynthesis. *Annu. Rev. Plant Physiol. Plant Mol. Biol*. 48:51-66

Barton C.J. 1948. Photometric analysis of phosphate rock. *Anal. Chem*. 20(11):1068-1073

Bashan Y. 1986. Significance of timing and level of inoculation with rhizosphere bacteria on wheat plants. *Soil Biol. Biochem*. 18(3):297-301

Bashan, Y. and Holguin G. 1997. *Azospirillum*/plant relationship: environmental and physiological advances (1990-1996). *Canadian J. Microbiol.* 43:103-121

Bashan Y., Holguin G. and Lifshitz R. 1993. Isolation and characterization of plant growth-promoting rhizobacteria. *Methods in Plant Mol. Biol. and Biotechnol*. 331-345

Bashan Y., Moreno M. and Troyo E. 2000. Growth promotion of the seawater-irrigated oilseed halophyte *Salicornia bigelovii* inoculated with mangrove rhizosphere bacteria and halotolerant *Azospirillum* spp. *Biol. Fertil. Soils*. 32:265-272

Bayano N.P.M., Cruz P.C.S., Aguilar E.A., Badayos R.B. and Haefele S.M. 2012. Evaluation of biofertilizers in irrigated rice: effects on grain yield at different fertilizer rates. *Agriculture*. 2:73-86

Becker M., Diekmann K.H., Ladha J.K., De Datta S.K. and Ottow J.C.G. 1991. Effect of NPK on growth and nitrogen fixation of *Sesbania rostrata* as a green manure for lowland rice (*Oryza sativa* L.). *Plant Soil*. 132:149-158

Belimov A.A., Kojemiakov A.P. and Chuvarliyeva C.V. 1995. Interaction between barley and mixed cultures of nitrogen fixing and phosphate-solubilizing bacteria. *Plant Soil*. 173:29-37

Bellone C.H., De Bellone S.D.V.C., Pedraza R.O. and Monzon M.A. 1997. Cell colonization and infection thread formation in sugar cane roots by *Acetobacter diazotrophicus. Soil Biol. Biochem*. 29(5):965-967

Beneduzi A., Peres D., Vargas L.K., Bodanese-Zanettini M.H. and Passaglia L.M.P. 2008. Evaluation of genetic diversity and plant growth promoting

© COPYRIGHT UPM

activities of nitrogen-fixing Bacilli isolated from rice fields in South Brazil. *Appl. Soil Ecol*. 39:311-320

Bergersen, F. J. and Turner, G. L. 1983. An evaluation of ^{15}N method for estimating nitrogen fixation in a subterranean clover-perennial ryegrass sward. *Aus. J. Agric. Res.* 34:391-401

Bhattacharjee R.B., Jourand P., Chaintreuil C., Dreyfus B., Singh A. and Mukhopadhyay S.N. 2012. Indole acetic acid and ACC deaminase-producing *Rhizobium leguminosarum* bv. *trifolii* SN10 promote rice growth, and in the process undergo zolonization and chemotaxis. *Biol. Fertil. Soils*. 48:173-182

Biswas J.C., Ladha J.K. and Dazzo F.B. 2000a. Rhizobia inoculation improves nutrient uptake and growth of lowland rice. *Soil Sci. Soc. Am. J.* 64:1644-1650

Biswas J.C., Ladha J.K., Dazzo F.B., Yanni Y.G. and Rolfe B.G. 2000b. Rhizobial inoculation influences seedling vigour and yield of rice. *Agron. J*. 92:880-886

Boddey R.M. 1987. Methods for quantification of nitrogen fixation associated with gramineae. *Crit. Rev. Plant Sci.* 6:209–266

Boddey, R. M., Alves, B. J. R. and Urquiaga, S. 1996. Evaluation of biological nitrogen fixation associated with non-legumes. In *Nitrogen Fixation with Non-Legumes* eds: K.A. Malik et al., pp 287-306. Kluwer Academic Publishers, London

Boddey R.M., Baldani V.L.D., Baldani J.I. and Dobereiner J. 1986. Effect of inoculation of *Azospirillum* spp. on nitrogen accumulation by field-grown wheat. *Plant Soil*. 95:109-121

Boddey R.M., de Oliveira O.C., Urquiaga S., Reis V.M., de Olivares F.L., Baldani V.L.D. and Dobereiner J. 1995. Biological nitrogen fixation associated with sugar cane and rice: contributions and prospects for improvement. *Plant Soil.* 174:195-209

Bottini R., Cassan F. and Piccoli P. 2004. Gibberellin production by bacteria and its improvement in plant growth promotion and yield increase. *Appl. Microbiol. Biotechnol*. 65:497-503

Brar S.K., Sarma S.J. and Chaabouni E. 2012. Shelf-life of biofertilizers: an accord between formulations and genetics. *J. Biofertil. Biopestici*. 3(5):1-2

Busse H. and Stolz A. 2006. *Achromobacter*, *Alcaligenes* and related genera. *Prokaryotes*. 5:675-700

© COPYRIGHT UPM

Chabot R., Antoun H. and Cescas M.P. 1996. Growth promotion of maize and lettuce by phosphate-solubilizing *Rhizobium leguminosarum* biovar *phaseoli*. *Plant Soil*. 184:311-321

Chaintreuil C., Giraud E., Prin Y., Lorquin J., Ba A., Gillis M., De Lajudie P. and Dreyfus B. 2000. Photosynthetic *Bradyrhizobia* are natural endophytes of the African wild rice *Oryza breviligulata*. *Appl. Environ. Microbiol*. 66(12):5437-5447

Chandra S., Choure K., Dubey R.C. and Maheshwari D.K. 2007. Rhizosphere competent *Mesorhizobium loti* MP6 induces root hair curling, inhibits *Sclerotinia sclerotiorum* and enhances growth of Indian mustard (*Brassica campestris*). *Brazilian J. Microbiol*. 38:124-130

Chen B.D., Zhu Y.G., Duan J., Xiao X.Y. and Smith S.E. 2007. Effects of the arbuscular mycorrhizal fungus *Glomus mosseae* on growth and metal uptake by four plant species in copper mine tailings. *Environ. Pollution.* 147:374-380

Chen, Y.P., Rekha, P.D., Arun, A.B., Shen, F.T., Lai, W.A. and Young, C.C. 2006. Phosphate solubilizing bacteria from subtropical soil and their tricalcium phosphate solubilizing abilities. *Appl. Soil Ecol.* 34:33-41

Chi F. Shen S. Cheng H. Jing Y. Yanni Y.G. and Dazzo F.B. 2005. Ascending migration of endophytic rhizobia, from roots to leaves, inside rice plants and assessment of benefits to rice growth physiology. *Appl. and Environmental Microbiol.* 71(11):7271-7278

Choudhury A.T.M.A. and Kennedy I.R. 2004. Prospects and potentials for systems of biological nitrogen fixation in sustainable rice production. *Biol. Fertil. Soils*. 39:219-227

Choudhury A.T.M.A. and Kennedy I.R. 2005. Nitrogen fertilizer losses from rice soils and control of environmental pollution problems. *Communications in Soil Sci. and Plant Anal.* 36:1625-1639

Choudhury A.T.M.A. and Khanif Y.M. 2001. Evaluation of the effects of nitrogen and magnesium fertilization on rice yield and fertilizer nitrogen efficiency using ^{15}N tracer technique. *J. Plant Nutr.* 24(6):855–871

Choudhury A.T.M.A., Kennedy I.R., Ahmed M.F. and Kecskes M.L. 2007. Phosphorus fertilization for rice and control of environmental pollution problems. *Pak. J. Biol. Sci*. 10(13):2098-2105

Cong P.T., Dung T.D., Hien N.T., Choudhury A., Rose M.T., Kecskes M.L., Deaker R. and Kennedy I.R. 2011. Effects of a multi strain biofertilizer and phosphorus rates on nutrition and grain yield of paddy rice on a sandy soil in southern Vietnam. *J. Plant Nutrition*. 34:1058-1069

© COPYRIGHT UPM

Cong P.T., Dung T.D., Hien T.M., Hien N.T., Choudhury A.T.M.A., Kecskes M.L. and Kennedy I.R. 2009. Inoculant plant-growth promoting microorganisms enhance utilization of urea-N and grain yield of paddy in southern Vietnam. *European J. Soil Biol*. 45:52-61

Coughlan, M. P. and Hazlewood, G. P. 1993. b-1,4-D-Xylan-degrading enzyme systems: biochemistry, molecular biology and applications. *Biotechnol. Appl. Biochem*. 17:259–289

Cummings S.P. 2009. The application of plant growth promoting rhizobacteria (PGPR) in low input and organic cultivation of graminaceous crops; potential and problems. *Environ. Biotechnol*. 5(2):43-50

Danso S.K.A. 1985. Methods of estimating biological nitrogen fixation. In *Biological Nitrogen Fixation in Africa.* ed: Ssali, H. and Keya, SO. pp 213-244. MIRCEN, Nirobi

Danso S.K.A. 1995. Assessment of biological nitrogen fixation. *Fertilizer Res*. 42:33-41

Datta C. and Basu P.S. 2000. Indole acetic acid production by a *Rhizobium* species from root nodules of a leguminous shrub, *Cajanus cajan*. *Microbiol*. *Res*. 155:123-127

Deaker R., Mijajlovic G. and Casteriano A. 2007. Estimating the most probable number of bacteria in multistrain biofertiliser inoculants using a multiple-tube fermentation test. *Proceedings of the Efficient nutrient use in rice production in Vietnam achieved using inoculant biofertilisers*. Hanoi, Vietnam. 12-13/10/2007

Department of Agriculture, Malaysia. 2009. A manual of rice planting through direct seeding

Department of Agriculture, Malaysia. 2011. Retrieved January 1st 2013, from http://www.doa.gov.my/c/document_library/get_file?uuid=8b3bb7ed-4363-4471-b760-6f528e6273dc&groupId=38371

Department of Statistics, Malaysia. 2011. Retrieved January 1st 2013, from http://www.statistics.gov.my/portal/download_Economics/download.php?file=DATA_SERIES/2011/pdf/08Padi.pdf

Dobbelaere S., Croonenborghs A., Thys A., Broek A.V. and Vanderleyden J. 1999. Phytostimulatory effect of *Azospirillum brasilense* wild type and mutant strains altered in IAA production on wheat. *Plant Soil*. 212:155-164

© COPYRIGHT UPM

Dobereiner J. and Day J. M. 1975. Associative symbiosis in tropical grasses : characterization of microorganisms and dinitrogen fixing sites. In *Proceedings of the 1st International Symposium on Nitrogen Fixation*, pp. 518-538. Edited by W.E. Newton and C.J. Nyman. Pullman : Washington State University Press

Doberman A. and Fairhurst T. 2000. Rice: nutrient disorders and nutrient management. Handbook series. Potash and Phosphate Institute, Potash and Phosphate Institute of Canada and International Rice Research Institute

Duijff B.J., De Kogel W.J., Bakker P.A.H.M. and Schippers B. 1994. Influence of pseudobactin 358 on the iron nutrition of barley. *Soil Biol. Biochem.* 26(12);1681-1688

Duponnois R., Colombet A., Hien V. and Thioulouse J. 2005. The mycorrhizal fungus *Glomus intraradices* and rock phosphate amendment influence plant growth and microbial activity in the rhizosphere of *Acacia holosericea*. *Soil Biol. Biochem*. 37:1460-1468

Eckert B., Weber O.B., Kirchhof G., Halbritter A., Stoffels M. and Hartmann A. 2001. *Azospirillum doebereinerae* sp. nov., a nitrogen-fixing bacterium associated with the C_4-grass *Miscanthus*. *Int. J. Systematic and Evolutionary Microbiol*. 51:17-26

Egamberdieva D., Kamilova F., Validov S., Gafurova L., Kucharova Z. and Lugtenberg B. 2008. High incidence of plant growth-stimulating bacteria associated with the rhizosphere of wheat grown on salinated soil in Uzbekistan. *Environ. Microbiol*. 10(1):1-9

Egener T., Hurek T. and Reinhold-Hurek B. 1999. Endophytic expression of nif genes of *Azoarcus* sp. strain BH72 in rice roots. *Mol. Plant-Microbe Interactions*. 12(9):813-819

Epstein E., Cohen J.D. and Bandurski R.S. 1980. Concentration and metabolic turnover of indoles in germinating kernels of *Zea mays* L. *Plant Physiol*. 65:415-421

Erturk Y., Ercisli S., Haznedar A. and Cakmakci R. 2010. Effects of plant growth promoting rhizobacteria (PGPR) on rooting and growth of kiwifruit (*Actinidia deliciosa*) stem cuttings. *Biol. Res*. 43:91-98

Farzana, Y., Radziah O., Kamaruzaman S. and Mohd S.S., 2007. Effect of PGPR inoculation on growth and yield of sweet potato. *J. Biol. Sci.* 7:421-424

Farzana, Y., Radziah O., Mohd S.S. and Kamaruzaman S. 2009. Growth and storage root development of sweetpotato inoculated with rhizobacteria under glasshouse conditions. *Australian J. Basic Appl. Sci*. 3:1461-1466

© COPYRIGHT UPM

Food and Agriculture Organization (FAO) of the United Nations. 2000a. Retrieved January 1st 2013, from http://www.fao.org/docrep/003/x6905e/x6905e0f.htm

Food and Agriculture Organization (FAO) of the United Nations. 2000b. Retrieved January 1st 2013, from ftp://ftp.fao.org/agl/agll/docs/barfinal.pdf

Food and Agriculture Organization (FAO) of the United Nations. 2004. Rice is Life. Italy: FAO. Retrieved January 1st 2013, from http://www.fao.org/newsroom/en/focus/2004/36887 (Accessed on January 3rd, 2013)

Food and Agriculture Organization (FAO) of the United Nations. 2009. Sixty-fourth General Assembly, Second Committee, Panel Discussion. In *New Cooperation for Global Food Security*. Retrieved January 1st 2012, from http://www.un.org/News/Press/docs/2009/gaef3242.doc.htm

Food and Agriculture Organization (FAO) of the United Nations. 2011. Retrieved January 1st 2013, from http://faostat3.fao.org/faostat-gateway/go/to/search/*/E

Food and Agriculture Organization (FAO) of the United Nations. 2012. Retrieved January 1st 2013, from http://faostat3.fao.org/home/index.html#VISUALIZE_BY_DOMAIN

Food and Agriculture Organization (FAO) of the United Nations. 2014. Retrieved January 1st 2013, from http://faostat.fao.org/site/567/DesktopDefault.aspx?PageID=567

Frommel M.I., Nowak J. and Lazarovits G. 1991. Growth enhancement and developmental modifications of *in* vitro grown potato (*Solanum tuberosum* ssp. *Tuberosum*) as affected by a nonflourescent *Pseudomonas* sp. *Plant Physiol*. 96:928-936

Garcia J.A.L., Probanza A., Ramos B., Flores J.J.C. and Manero F.J.G. 2004. Effects of plant growth promoting rhizobacteria (PGPRs) on the biological nitrogen fixation, nodulation and growth of *Lupinus albus* l. *cv*. Multolupa. *Eng. Life Sci*. 4(1):71-77

Garcia-Fraile P., Carro L., Robledo M., Ramirez-Bahena M., Flores-Felix J., Fernandez M.T., Mateos P.F., Rivas R., Igual J.M., Martinez-Molina E., Peix A. and Velazquez E. 2012. *Rhizobium* promotes non-legumes growth and quality in several production steps: towards a biofertilization of edible raw vegetables healthy for humans. PLoS ONE 7(5): 1-7

Gholami A., Shahsavani S. and Nezarat S. 2009. The effect of plant growth promoting rhizobacteria (PGPR) on germination, seedling growth and yield of maize. *World Academy Sci., Engineering and Technol.* 25:19-24

© COPYRIGHT UPM

Goh K.M. and Edmeades D.C. 1978. Field measurements of symbiotic nitrogen fixation in an established pasture using acetylene reduction and a ^{15}N method. *Soil Biol. Biochem*. 10:13-20

Gordon S.A. and Weber R.P. 1951. Colorimetric estimation of indoleacetic acid. *Plant Physiol*. 26:192-195

Goswami D., Dhandhukia P., Patel P. and Thakker J.N. 2014. Screening of PGPR from saline desert of Kutch: growth promotion in *Arachis hypogea* by *Bacillus licheniformis* A2. *Microbiol. Res*. 169:66-75

Gregersen T. 1978. Rapid method for distinction of gram-negative from gram-positive bacteria. *European J. Appl. Microbiol. Biotechnol*. 5:123-127

Gruber, N. and Galloway, J. N. 2008. An Earth-system perspective of the global nitrogen cycle. *Nature*. 451:293–296

Guerinot M.L., Meidl E.J. and Plessner O. 1990. Citrate as a siderophore in *Bradyrhizobium japonicum*. *J. Bacteriology*. 172(6):3298-3303

Halder A.K. and Chakrabartty P.K. 1993. Solubilization of inorganic phosphate by *Rhizobium*. *Folia Microbiol*. 38(4):325-330

Halder A.K., Mishra A.K., BhattaCharyya P. and Chakrabartty P.K. 1990. Solubilization of rock phosphate by *Rhizobium* and *Bradyrhizobium*. *J. Gen. Appl. Microbiol*. 36:81-92

Hall B.G. 2013. Bulding phylogenetic trees from molecular data with MEGA. *Mol. Biol. Evol.* 30(5):1229-1235

Han H.S. and Lee K.D. 2005a. Phosphate and potassium solubilizing bacteria effect on mineral uptake, soil availability and growth of eggplant. *Res. J. Agric. Biol. Sci*. 1(2):176-180

Han H.S. and Lee K.D. 2005b. Plant growth promoting rhizobacteria effect on antioxidant status, photosynthesis, mineral uptake and growth of lettuce under soil salinity. *Res. J. Agric. Biol. Sci*. 1(3):210-215

Han H.S., Supanji and Lee K.D. 2006. Effect of co-inoculation with phosphate and potassium solubilizing bacteria on mineral uptake and growth of pepper and cucumber. *Plant Soil Environ*. 52(3):130-136

Hardy R.W.F., Holsten R.D., Jackson E.K. and Burns R.C. 1968. The acetylene-ethylene assay for N_2 fixation: laboratory and field evaluation. *Plant Physiol*. 43:1185-1207

Harter R.D. 2007. Acid soils of the tropics. Technical note no. 48. 1-7 https://echocommunity.site-ym.com/resource/collection/E66CDFDB-0A0D-4DDE-8AB1 74D9D8C3EDD4/Acid_Soils_of_the_Tropics_[Web_Format].pdf

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Hayat R., Sheirdil R.A., Iftikar-ul-Hassan M. and Ahmed I. 2012. Characterization and identification of compost bacteria based on 16S rRNA gene sequencing. *Ann. Microbiol*. 63(3):905-912

Hegazi N.A. and Fayez M. 2001. Biological nitrogen fixation to maximize productivity of intercropped legumes and non-legumes: ten years of field experimentations in semi-arid deserts of Egypt. *Archives of Agron. and Soil Sci.* 47(1-2):103-131

Holguin G. and Glick Z.B.R. 2001. Expression of the ACC deaminase gene from *Enterobacter cloacae* UW4 in *Azospirillum brasilense*. *Microb. Ecol*. 41:281-288

Hossain M. and Fischer K.S. 1995. Rice research for food security and sustainable agricultural development in Asia: achievements and future challenges. *GeoJournal*. 35(3):286-298

Hu X., Chen J. and Guo J. 2006. Two phosphate- and potassium-solubilizing bacteria isolated from Tianmu Mountain, Zhejiang, China. *World J. Microbiol. and Biotechnol*. 22:983-990

Hun T.G. 2001. Isolation and identification of rhizobacteria from paddy land and their benefits as a biofertilizer. MSc. Thesis Universiti Putra Malaysia

Illmer P. and Schinner F. 1992. Solubilization of inorganic phosphates by microorganisms isolated from forest soils. *Soil Biol. Biochem*. 24(4):389-395

International Institute of Tropical Agriculture (IITA). 1982. Automated and Semi-automated methods for soil and plant analysis manual series No 7. Ibadan, Nigeria. IITA. pp. 33

International Rice Research Institute. 2007. Retrieved March 14 2014, from http://www.knowledgebank.irri.org/ericeproduction/0.2._Growth_stages_of_the_rice_plant.htm

International Rice Research Institute. 2011. Retrieved January 1st 2013, from http://ricestat.irri.org:8080/wrs2/entrypoint.htm

International Rice Research Institute. 2014. Retrieved January 1st 2013, from http://ricestat.irri.org:8080/wrs2/entrypoint.htm

James E.K. 2000. Nitrogen fixation in endophytic and associative symbiosis. *Field Crops Res.* 65:197-209

James E.K., Gyaneshwar P., Mathan N., Barraquio W.L., Reddy P.M., Iannetta P.P.M., Olivares F.L. and Ladha J.K. 2002. Infection and colonization of rice seedlings by the plant growth-promoting bacterium *Herbaspirillum seropedicae* Z67. *Mol. Plant-Microbe Interactions*. 15(9):894-906

© COPYRIGHT UPM

Javaid A. and Mahmood N. 2010. Growth, nodulation and yield response of soybean to biofertilizers and organic manures. *Pakistan J. Bot*. 42(2):863-871

Ji S.H., Gururani M.A. and Chun S. 2014. Isolation and characterization of plant growth promoting endophytic diazotroph bacteria from Korean rice cultivars. *Microbiol. Res*. 169:83-98

Kapulnik, Y., Gafny, R. and Okon, Y. 1985. Effect of *Azospirillum* spp. inoculation on root development and NO_3^- uptake in wheat (*Triticum aestivum* cv. Miriam) in hydroponics systems. *Canadian J. Botany*. 63: 627-631

Kasana R.C., Salwan R., Dhar H., Dutt S. and Gulati A. 2008. A rapid and easy method for the detection of microbial cellulases on agar plates using Gram's iodine. *Curr. Microbiol*. 57:503-507

Katupitiya S., Millet J., Vesk M., Viccars L., Zeman A., Lidong Z., Elmerich C. and Kennedy I.R. 1995. A mutant *Azospirillum brasilense* sp7 impaired in flocculation with a modified colonization pattern and superior nitrogen fixation in association with wheat. *Appl. Environ. Microbiol*. 61(5):1987-1995

Kennedy I.R., Choudhury A.T.M.A. and Kecskes M.L. 2004. Non-symbiotic bacterial diazotrophsin crop-farming systems: can their potential for plant growth promotion be better exploited? *Soil Biol. Biochem*. 36:1229-1244

Keyeo F., Ai'shah O.N. and Amir H.G. 2011. The effects of nitrogen fixation activity and phytohormone production of diazotroph in promoting growth of rice seedlings. *Biotechnol*. 10(3):263-273

Keyser H.H., Bohlool B.B., Hu T.S. and Weber D.F. 1982. Fast-growing rhizobia isolated from root nodules of soybean. *Science*. 215:1631-1632

Khan M.S., Zaidi A. and Wani P.A. 2007. Role of phosphate solubilizing microorganisms in sustainable agriculture – a review. *Agron. Sustain. Dev*. 27:29-43

Khorshidi Y.R., Ardakani M.R., Ramezanpour M.R., Khavazi K. and Zargari K. 2011. Response of yield and yield components of rice (*Oryza sativa* L.) to *Pseudomonas fluorescence* and *Azospirillum lipoferum* under different nitrogen levels. *American-Eurasian J. Agric. and Environ. Sci*. 10(3):387-395

Khush G.S. 2005. What it will take to feed 5.0 billion rice consumers in 2030. *Plant Mol. Biol*. 59:1-6

Kloepper J. W, Leong J., Teintze M. and Schroth M.N. 1980. Enhanced plant growth by siderophore produced by plant growth-promoting rhizobacteria. *Nature*. 286:885-886

© COPYRIGHT UPM

Kloepper J. W., Ryu C.M., and Zhang S. 2004. Induced systemic resistance and promotion of plant growth by *Bacillus* spp. *Phytopathology*. 94:1259-1266

Klonowska A., Chaintreuil C., Tisseyre P., Miche L., Melkonian R., Ducousso M., Laguerre G., Brunel B. and Moulin L. 2012. Biodiversity of *Mimosa pudica* rhizobial symbionts (*Cupriavidus taiwanensis*, *Rhizobium mesoamericanum*) in New Caledonia and their adaptation to heavy metal-rich soils. *FEMS Microbiol. Ecol*. 81:618-635

Kryuchkova Y.V., Burygin G.L., Gogoleva N.E., Gogolev Y.V., Chernyshova M.P., Makarov O.E., Fedorov E.E. and Turkovskaya O.V. 2014. Isolation and characterization of a glyphosate-degrading rhizosphere strain, *Enterobacter cloacea* K7. *Microbiol. Res.* 169:99-105

Kucey, R. M. N. 1988. Alteration of size of wheat root system and nitrogen fixation by associative nitrogen fixing bacteria measured under field conditions. *Canadian J. Microbiol.* 34:735-739

Kumar K.V., Srivastava S., Singh N. and Behl H.M. 2009. Role of metal resistant plant growth promoting bacteria in ameliorating fly ash to the growth of *Brassica juncea*. *J. Hazardous Materials*. 170:51-57

Kundu B.S. and Gaur A.C. 1984. Rice response to inculation with N_2-fixing and P-solubilizing microorganisms. *Plant Soil*. 79:227-234

Laranjo M., Alexandre A. and Oliveira S. 2014. Legume growth-promoting rhizobia: an overview on the *Mesorhizobium* genus. *Microbiol. Res*. 169:2-17

Lethbridge G., Davidson M.S. and Sparling G.P. 1982. Critical evaluation of the acetylene reduction test for estimating the activity of nitrogen-fixing bacteria associated with the roots of wheat and barley. *Soil Biol. Biochem*. 14:27-35

Lindau C.W., Bollich P.K., Delaune R.D., Patrick W.H.J. and Law V.J. 1991. Effect of urea fertilizer and environmental factors on CH_4 emissions from a Louisiana, USA rice field. *Plant Soil*. 136:195-203

Liu H., He Y., Jiang H., Peng H., Huang X., Zhang X., Thomashow L.S. and Xu Y. 2007. Characterization of a phenazine-producing strain *Pseudomonas chlororaphis* GP72 with broad-spectrum antifungal activity from green pepper rhizosphere. *Current Microbiol*. 54(4):302-306

Liu Y., Feng L., Hu H., Jiang G., Cai Z. and Deng Y. 2012. Phosphorus release from low-grade rock phosphates by low molecular weight organic acids. *J. Food, Agric. and Environ*. 10(1):1001-1007

© COPYRIGHT UPM

Lucy M., Reed E. and Glick B.R. 2004. Applications of free living plant growth-promoting rhizobacteria. *Antonie van Leeuwenhoek*. 86:1-25

Lugtenberg B. and Kamilova F. 2009. Plant-growth-promoting rhizobacteria. *Annu. Rev. Microbiol.* 63:541-556

Mahmood M., Rahman Z.A., Saud H.M., Shamsuddin Z.H., Subramaniam S. 2010. Influence of rhizobacterial and agrobacterial inoculation on selected physiological and biochemical changes on banana cultivar, *Berangan* (AAA) plantlets. *J. Agric. Sci*. 2(1):115-137

Malaysian Agricultural Research and Development Institute (MARDI). 2010. Retrieved January 1st 2013, from http://www.mardi.gov.my/c/document_library/get_file?uuid=29d5002c-afd5-4892-b118-838f828e0006&groupId=10138

Malboobi M.A., Owlia P., Behbahani M., Sarokhani E., Moradi S., Yakhchali B., Deljou A. and Heravi K.M. 2009. Solubilization of organic and inorganic phosphates by three highly efficient soil bacterial isolates. *World J. Microbiol. Biotechnol*. 25:1471-1477

Malik K.A., Bilal R., Azam F. and Sajjad M.I. 1988. Quantification of N_2-fixation and survival of inoculated diazotrophs associated with roots of Kallar grass. *Plant Soil*. 108:43-51

Malik K.A., Bilal R., Mehnaz S., Rasul G., Mirza M.S. and Ali S. 1997. Association of nitrogen-fixing, plant-growth-promoting rhizobacteria (PGPR) with kallar grass and rice. *Plant Soil* 194:37-44

Manjunath M., Prasanna R., Sharma P., Nain L. and Singh R. 2011. Developing PGPR consortia using novel genera *Providencia* and *Alcaligenes* along with cyanobacteria for wheat. *Archives Agron. Soil Sci.* 57(8):873-887

Martinez-Viveros O., Jorquera M.A., Crowley D.E., Gajardo G. and Mora M.L. 2010. Mechanisms and practical considerations involved in plant growth promotion by rhizobacteria. *J. Soil Sci. Plant Nutr.* 10(3):293-319

Masciarelli O., Llanes A. and Luna V. 2014. A new PGPR co-inoculated with *Bradyrhizobium japonicum* enhances soybean nodulation. *Microbiol. Res*. 169(7-8):609-615

McAuliffe, C., Chamblee, D. S., Uribe-Arang, H., Woodhouse, Jr., W. W. 1958. Influence of inorganic nitrogen on N_2 fixation by legumes as revealed by ^{15}N. *Agron. J.* 50:334-337

Mia M.A.B. 2002. Beneficial effects of rhizobacterial inoculation on nutrient uptake, growth and yield of banana (*Musa* Spp. Cv. 'Berangan'). Ph.D. Thesis Universiti Putra Malaysia

© COPYRIGHT UPM

Mia M.A.B., Shamsuddin Z.H. and Mahmood M. 2012. Effects of rhizobia and plant growth promoting bacteria inoculation on germination and seedling vigor of lowland rice. *African J. Biotech*. 11(16):3758-3765

Mia M.A.B., Shamsuddin Z.H., Wahab Z. and Marziah M. 2009. The effect of rhizobacterial inoculation on growth and nutrient accumulation of tissue-cultured banana plantlets under low N-fertilizer regime. *African J. Biotechnol*. 8(21):5855-5866

Mia M.A.B., Shamsuddin Z.H., Wahab Z. and Marziah M. 2010a. Rhizobacteria as bioenhancer and biofertilizer for growth and yield of banana (*Musa* spp. cv. 'Berangan'). *Scientia Horticulturae.* 126:80-87

Mia M.A.B., Shamsuddin Z.H., Wahab Z. and Marziah M. 2010b. Effect of plant growth promoting rhizobacterial (PGPR) inoculation on growth and nitrogen incorporation of tissue-cultured Musa- plantlets under nitrogen-free hydroponics condition. *Australian J. Crop Sci.* 4(2):85-90

Miche L. and Balandreau J. 2001. Effects of rice seed surface sterilization with hypochlorite on inoculated *Burkholderia vietnamiensis*. *Appl. Environ. Microbiol*. 67(7):3046-3052

Mirza M.S., Ahmad W., Latif F., Haurat J., Bally R., Normand P. and Malik K.A. 2001. Isolation, partial characterization, and the effect of plant groth-promoting bacteria (PGPB) on micro-propagated sugarcane *in vitro. Plant Soil*. 237:47-54

Miwa H., Ahmed I., Yokota A. and Fujiwara T. 2009. *Lysinibacillus parviboronicapiens* sp. nov., a low-boron-containing bacterium isolated from soil. *Int. J. Systematic and Evol. Microbiol.* 59:1427-1432

Morteza S., Alireza N. and Shankar L.L. 2011. Effect of organic fertilizer on growth and yield components in rice (*Oryza sativa* L.). *J. Agric. Sci*. 3(3):217-224

Naher U.A., Othman R., Shamsuddin Z.H., Saud H.M. and Ismail M.R. 2009. Growth enhancement and root colonization of rice seedlings by *Rhizobium* and *Corynebacterium* spp. *Int. J. Agric. Biol*. 11:586-590

Naher U.A., Othman R., Shamsuddin Z.H., Saud H.M., Ismail M.R. and Rahim K.A. 2011. Effect of root exuded specific sugars on biological nitrogen fixation and growth promotion in rice (*Oryza sativa*). *Australian J. Crop Sci.* 5(10):1210-1217

Nautiyal C.S. 1999. An efficient microbiological growth medium for screening phosphate solubilizing microorganisms. *FEMS Microbiol.Letters*. 170:265-270

© COPYRIGHT UPM

Nayak D.N., Ladha J.K. and Watanabe I. 1986. The fate of marker Azospirillum lipoferum inoculated into rice and its effect on growth, yield and N_2 fixation of plants studied by acetylene reduction, $^{15}N_2$ feeding and ^{15}N dilution techniques. *Biol. Fertil. Soils*. 2:7-14

Nehl D.B., Allen S.J. and Brown J.F. 1996. Deleterious rhizosphere bacteria: an integrating perspective. *Appl. Soil Ecol.* 5:1-20

Ng L.C., Sariah M., Sariam O., Radziah O. and Abidin M.A.Z. 2012. Rice seed bacterization for promoting germination and seedling growth under aerobic cultivation system. *Australian J. Crop Sci*. 6(1):170-1175

Nguyen H.T., Deaker R., Kennedy I.R. and Roughley R.J. 2003. The positive yield response of field-grown rice to inoculation with a multi-strain biofertiliser in the Hanoi area, Vietnam. *Symbiosis*. 35:231-245

Nguyen T.H.N., Brechenmacher L., Aldrich J.T., Clauss T.R., Gritsenko M.A., Hixson K.K., Libault M., Tanaka K., Yang F., Yao Q., Pasa-Tolic L., Xu D., Nguyen H.T. and Stacey G. 2012. Quantitative phosphoproteomic analysis of soybean root hairs inoculated with *Bradyrhizobium japonicum*. *Mol. and Cellular Proteomics.* 11(11):1140-1155

Nori H., Halim R.A. and Ramlan M.F. 2008. Effects of nitrogen fertilization management practice on the yield and straw nutritional quality of commercial rice varieties. *Malaysian J. Mathematics Sci*. 2(2):61-71

Ogbo F.C. 2010. Coversion of cassava wastes for biofertilizer production using phosphate solubilizing fungi. *Bioresources Technol.* 101:4120-4124

Okon Y. and Kapulnik Y. 1986. Development and function of Azospirillum-inoculated roots. *Plant Soil*. 90:3-16

Owens L. 1977. Use of ^{15}N enriched soil to study N_2 fixation in grasses. In *Genetic Engineering for Nitrogen Fixation*. ed: Hollaender, A. p.473. Plenum Press. New York

Panhwar Q.I., Othman R., Rahman Z.A., Meon S. and Ismail M.R. 2012. Isolation and characterization of phosphate-solubilizing bacteria from aerobic rice. *African J. Biotechnol.* 11(11):2711-2719

Pareek R.P., Ladha J.K. and Watanabe I. 1990. Estimating N_2 fixation by *Sesbania rostrata* and *S. cannabina* (syn. *S. aculeate*) in lowland rice soil by the ^{15}N dilution method. *Biol. Fertil. Soils*. 10:77-88

Patten C.L. and Glick B.R. 2002. Role of *Pseudomonas putida* indoleacetic acid in development of the host plant root system. *Appl. Environ. Microbiol.* 68(8):3795-3801

© COPYRIGHT UPM

Peng G., Yuan Q., Li H., Zhang W. and Tan Z. 2008. *Rhizobium oryzae* sp. nov., isolated form the wild rice *Oryza alta*. *Int. J. Systematic and Evol. Microbiol*. 58:2158-2163

Peng S., Biswas J.C., Ladha J.K., Gyaneshwar P. and Chen Y. 2002. Influence of rhizobial inoculation on photosynthesis and grain yield of rice. *Agron. J*. 94:925-929

Perez E., Sulbaran M., Ball M.M. and Yarzabal L.A. 2007. Isolation and characterization of mineral phosphate-solubilizing bacteria naturally colonizing a limonitic crust in the south-eastern Venezuelan region. *Soil Biol. Biochem*. 39:2905-2914

Peng S., Yang S., Xu J., Luo Y. and Hou H. 2011. Nitrogen and phosphorus leaching lossess from paddy fields with different water and nitrogen managements. *Paddy Water Environ.* 9:333-342

Perret X., Staehelin C. and Broughton W.J. 2000. Molecular basis of symbiotic promiscuity. *Microbiol. Mol. Biol. Rev*. 64(1):180-201

Phillips D.A. 1980. Efficiency of symbiotic nitrogen fixation in legumes. *Ann. Rev. Plant Physiol*. 31:29-49

Pikovskaya R. I. 1948. Mobilization of Phosphorus in soil in connection with vital activity by some microbial species. *Microbiologica* 17:362–370

Pilet P. and Saugy M. 1987. Effect on root growth of endogenous and applied IAA and ABA. *Plant Physiol*. 83:33-38

Poovaiah, B. and Reddy, A. S. N. 1991. Calcium and root development: importance of calcium in signal transduction. In: *Plant Roots the HiddenHalf* eds:Waise, Y., Eshel, A. and Kafkafi, U. pp.205-227. Marcel Dekker, New York

Prasanna R., Joshi M., Rana A., Shivay Y.S. and Nain L. 2011. Influence of co-inoculation of bacteria-cyanobacteria on crop yield and C-N sequestration in soil under rice crop. *World J. Microbiol. Biotechnol*. 28(3):1223-1235

Premalatha, P., 2006. Colonization of *Oryza sativa* roots by plant growth-promoting rhizobacterium, *Bacillus sphaericus* UPMB10 and subsequent inoculant formulation. PhD Thesis, Universiti Putra Malaysia.

Raja P., Uma S., Gopal H. and Govindarajan K. 2006. Impact of bio inoculants consortium on rice root exudates, biological nitrogen fixation and plant growth. *J. Biol. Sci*. 6(5):815-823

Rajkumar M., Nagendran R., Lee K.J., Lee W.H. and Kim S.Z. 2006. Influence of plant growth promoting bacteria and Cr^{6+} on the growth of Indian mustard. *Chemosphere*. 62:741-748

© COPYRIGHT UPM

Ramamoorthy V., Viswanathan R., Raguchander T., Prakasam V. and Samiyappan R. 2001. Induction of systemic resistance by plant growth promoting rhizobacteria in crop plants against pests and diseases. *Crop Protection*. 20:1-11

Rashid M., Khalil S., Ayub N., Alam S. and Latif F. 2004. Organic acids production and phosphate solubilization by phosphate-solubilizing microorganisms (PSM) under *in vitro* conditions. *Pak. J. Biol. Sci.* 7(2):187-196

Reinhold-Hurek B. and Hurek T. 1998. Life in grasses: diazotrophic endophytes. *Trend Microbiol.* 6:139-144

Rennie R. J. 1980. ^{15}N-isotope dilution as a measure of dinitrogen fixation by *Azospirillum brasilense* associated with maize. *Canadian J. Botany*. 58: 21-24

Rennie R. J., Rennie, D. A. and Fried, M. 1978. Concept of ^{15}N usage in dinitrogen fixation studies. In *Isotope in Biological Dinitrogen Fixation*. pp. 107-133. IAEA. Vienna

Requena N., Jimenez I., Toro M. and Barea J.M. 1997. Interactions between plant-growth-promoting rhizobacteria (PGPR), arbuscular mycorrhizal fungi and *rhizobium* spp. in the rhizosphere of *Anthyllis cytisoides*, a model legume for revegetation in Mediterranean semi-arid ecosystems. *New Phytologist*. 136(4):667-677

Ribeiro C.M. and Cardoso E.J.B.N. 2012. Isolation, selection and characterization of root-associated growth promoting bacteria in Brazil Pine (*Araucaria angustifolia*). *Microbiol. Res*. 167:69-78

Richardson A.E., Barea J., McNeill A.M. and Prigent-Combaret C. 2009. Acquisition of phosphorus and nitrogen in the rhizosphere and plant growth promotion by microorganisms. *Plant Soil*. 321:305-339

Roberts E.H. 1963. An investigation of inter-varietal differences in dormancy and viability of rice seed. *Annals of Botany*. 106(27):365-369

Rodriguez H. and Fraga R. 1999. Phosphate solubilizing bacteria and their role in plant growth promotion. *Biotechnol. Adv*. 17:319-339

Rodrigues E.P., Rodrigues L.S., Oliveira A.L.M.D., Baldani V.L.D., Teixeira K.R.D.S., Urquiaga S. and Reis V.M. 2008. *Azospirillum amazonense* inoculation: effects on growth, yield and N_2 fixation of rice (*Oryza sativa* L.). *Plant Soil*. 302:249-261

Roger P.A. and Ladha J.K. 1992. Biological N_2 fixation in wetland rice fields: estimation and contribution to nitrogen balance. *Plant Soil*. 141:41-55

© COPYRIGHT UPM

Saad M.S., Sabuddin A.S.A., Yunus A.G. Shamsuddin Z.H. 1999. Effects of *Azospirillum* inoculation on sweetpotato grown on sandy tin-tailing soil. *Communications in Soil Sci. and Plant Anal*. 30(11 and 12):1583-1592

Saharan B.S. and Nehra V. 2011. Plant growth promoting rhizobacteria: a critical review. *Life Sci. and Medicine Res*. 21:1-30

Sahrawat K.L. 2000. Macro and micronutrients removed by upland and lowland rice cultivars in West Africa. *Communications in Soil Sci. and Plant Anal.* 31:717–723

Sanchez P.A. and Uehara G. 1980. Management considerations for acid soils with high phosphorus fixation capacity. *In* The role of phosphorus in agriculture. Chapter 17. 471-514

Sangeeth K.P., Bhai R.S. and Srinivasan V. 2012. *Paenibacillus glucanolyticus*, a promising potassium solubilizing bacterium isolated from black pepper (*Piper nigrum* L.) rhizosphere. *J. Spices and Aromatic Crops*. 21(2):118-124

Saravanakumar D., Vijayakumar C., Kumar N. and Samiyappan R. 2007. PGPR-induced defence responses in the tea plant against blister blight disease. *Crop Protection.* 26:556-565

Sayyed R.Z. and Chincholkar S.B. 2010. Growth and siderophores production in *Alcaligenes faecalis* is regulated by metal ions. *Indian J. Microbiol*. 50(2):179-182

Schwyn B. and Neilands J.B. 1987. Universal chemical assay for the detection and determination of siderophores. *Anal. Biochem*. 160:47-56

Shattuck A. and Holt-Giménez E. 2010. Moving from Food Crisis to Food Sovereignty. *Yale Human Rights and Development J.* 2(13):421-434

Shen D. 1997. Microbial diversity and application of microbial products for agricultural purposes in China. *Agriculture, Ecosystems and Environment*. 62:237-245

Shrestha R.K. and Ladha J.K. 1996. Genotypic variation in promotion of rice dinitrogen fixation as determined by nitrogen-15 dilution. *Soil Sci. Soc. Am. J*. 60:1815-1821

Siddique Z.A. 2006. PGPR: Prospective biocontrol agents of plant pathogens. *In* PGPR: biocontrol and biofertilization. 111-142

Singh R.K., Kumar D.P., Solanki M.K., Singh P., Srivastva A.K., Kumar S., Kashyap P.L., Saxena A.K., Singhal P.K. and Arora D.K. 2012. Optimization of media components for chitinase production by chickpea rhizosphere associated *Lysinibacillus fusiformis* B-CM18. *J. Basic Microbiol*. 52:1-10

© COPYRIGHT UPM

Sitepu I.R., Hashidoko Y., Santoso E. and Tahara S. 2007. Potent phosphate-solubilizing bacteria isolated from dipterocarps grown in peat swamp forest in central kalimantan and their possible utilization for biorehabilitation of degraded peat swamp forest in Central Kalimantan. *Proceedings of the International symposium and workshop on tropical peatland. Yogyakarta,* Indonesia. 27-29/08/2007

Slininger P.J., Schisler D.A., Shea-Andersh M.A., Sloan J.M., Woodell L.K., Frazier M.J. and Olsen N.L. 2010. Multi-strain co-cultures surpass blends of broad spectrum biological control of maladies of potatoes in storage. *Biocontrol Sci. Technol.* 20(8):763-786

Somasegaran P. and Hoben H.J. 1985. Methods in legume-*rhizobium* technology. University of Hawaii NifTAL Project and MIRCEN

Spaepen S., Dobbelaere S., Croonenborghs A. and Vanderleyden J. 2008. Effects of *Azospirillum brasilense* indole-3-acetic acid production on inoculated wheat plants. *Plant Soil*. 312:15-23

Spaepen S., Vanderleyden J. and Remans R. 2007. Indole-3-acetic acid in microbial and microorganism-plant signaling. *FEMS Microbiol. Rev*. 1-24

Steenhoudt O. and Vanderleyden J. 2000. *Azospirillum*, a free-living nitrogen-fixing bacterium closely associated with grasses: genetic, biochemical and ecological aspects. *FEMS Microbiol. Rev*. 24:487-506

Sturz A.V., Christie B.R., Matheson B.G., Arsenault W.J. and Buchanan N.A. 1999. Endophytic bacterial communities in the periderm of potato tubers and their potential to improve resistance to soil-borne plant pathogens. *Plant Pathology*. 48:360-369

Subba Rao N. S. 1995. Nitrogen fixation in free-living and associative symbiotic bacteria. *Soil Microorganisms and Plant Growth*. Oxford and IBH Publishing Co. Pvt. Ltd.

Sugumaran P. and Janarthanam B. 2007. Solubilization of potassium containing minerals by bacteria and their effect on plant growth. *World. J. Agric. Sci*. 3(3):350-355

Suneja P., Dudeja S.S. and Narula N. 2007. Development of multiple co-inoculants of different biofertilizers and their interaction with plants. *Archives Agron. Soil Sci.* 53(2):221-230

Suzuki Y., Adhikari D., Itoh K. and Suyama K. 2014. Effects of temperature on competition and relative dominance of *Bradyrhizobium japonicum* and *Bradyrhizobium elkanii* in the process of soybean nodulation. *Plant Soil*. 374:915-924

© COPYRIGHT UPM

Tilak K.V.B.R., Ranganayaki N., Pal K.K., De R., Saxena A.K., Nautiyal C.S., Mittal S., Tripathi A.K. and Johri B.N. 2005. Diversity of plant growth and soil health supporting bacteria. *Current Sci*. 89(1):136-150

Timell T.E. 1967. Recent progress in the chemistry of wood hemicelluloses. *Wood Sci. Technol*. 1:45-70

Vargas L., Carvalho T.L.G.D., Ferreira P.C.G., Baldani V.L.D., Baldani J.I. and Hemerly A.S. 2012. Early responses of rice (*Oryza sativa* L.) seedlings to inoculation with beneficial diazotrophic bacteria are dependent on plant and bacterial genotypes. *Plant Soil*. 356(1-2):127-137

Verma S.C., Ladha J.K. and Tripathi A.K. 2001. Evaluation of plant growth promoting and colonization ability of endophytic diazotrophs from deep water rice. *J. Biotechnol.* 91:127-141

Vessey J.K. 2003. Plant growth promoting rhizobacteria as biofertilizers. *Plant Soil*. 255:571-586

Wani P.A., Khan M.S. and Zaidi A. 2007. Synergistic effects of the inoculation with nitrogen-fixing and phosphate-solubilizing rhizobacteria on the performance of field-grown chickpea. *J. Plant Nutr. Soil Sci.* 170:283-287

Wassmann R., Papen H. and Rennenberg H. 1993. Methane emission from rice paddies and possible mitigation strategies. *Chemosphere*. 26(1-4):201-217

Wasule D.L., Wadyalkar S.R. and Buldeo A.N. 2007. Effect of phosphate solubilizing bacteria on role of Rhizobium on nodulation by soybean. E. Velazquez and C. Rodriguez-Barrueco (eds.) In *First International Meeting on Microbial Phosphate Solubilization*, 139-142

Watanabe I., Barraquio W.L., De Guzman M.R. and Cabrera D.A. 1979. Nitrogen-fixing (acetylene reduction) activity and population of aerobic heterotrophic nitrogen-fixing bacteria associated with wetland rice. *Appl. Environ. Microbiol*. 37(5):813-819

Wei C. T. 1997. Development and application of biofertilizer in China. In *Proceedings Biological Nitrogen Fixation: The Global and Future Needs* pp 71-72. Italy

Wichaar Desk. 2012. BioNitrogen Corp.: Pakistan's urea fertilizer production issues may be a global precursor of the future. Available at: http://www.wichaar.com/news/296/ARTICLE/28093/2012-02-27.html (Accessed: 27 Dec 2012)

Witty J.F. 1979. Aceylene reduction assay can overestimate nitrogen-fixation in soil. *Soil Biol. Biochem*. 11:209-210

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Wu S.C., Cao Z.H., Li Z.G., Cheung K.C. and Wong M.H. 2005. Effects of biofertilizer containing N-fixer, P and K solubilizers and AM fungi on maize growth: a greenhouse trial. *Geoderma*. 125:155-166

Xu L.M., Ge C., Cui Z., Li J. and Fan H. 1995. *Bradyrhizobium liaoningense* sp. nov., isolated from the root nodules of soybeans. *Int. J. Systematic Bacteriology*. 45(4):706-711

Yanni Y.G. and Dazzo F.B. 2010. Enhancement of rice production using endophytic strains of *Rhizobium leguminosarum* bv. trifolii in extensive field inoculation trials within the Egypt Nile delta. *Plant Soil*. 336:129-142

Yanni Y.G., Rizk R.Y., Corich V., Squartini A., Ninke K., Philip-Hollingsworth S., Orgambide G., de Bruijin F., Stoltzfus J., Buckley D., Schmidt T.M., Mateos P.F., Ladha J.K., and Dazzo F.B. 1997. Natural endophytic association between *Rhizobium leguminosarum* bv. *trifolii* and rice roots and assessment of its potential to promote rice growth. *Plant Soil* 194:99-114

Yaser R.K., Mohammad R.A., Mahmoud R.R., Kazem K. and Kaveh Z. 2011. Response of yield and yield components of rice (*Oryza sativa* L.) to *Pseudomonas flouresence* and *Azospirillum lipoferum* under different nitrogen levels. *American-Eurasian J. Agric. and Environ. Sci.* 10(3):387-395.

Yasmin F., Othman R., Sijam K. and Saad M.S. 2007. Effect of PGPR inoculation on growth and yield of sweetpotato. *J. Biol. Sci*. 7(2):421-424

Yazdani M., Bahmanyar M.A., Pirdashti H. and Esmaili M.A. 2009. Effect of phosphate solubilization microorganisms (PSM) and plant growth promoting rhizobacteria (PGPR) on yield and yield components of corn (*Zea mays L.*). *World Academy Sci., Engineering and Technol*. 25:90-92

Yogesh K., Vamsi K.K., Amol B., Nikhil G., Soham T., Prasad P., Girish G., Mayank G., Amol J., Adarsh M., Joshi B. and Mishra D. 2009. Study of pectinase production in submerged fermentation using different isolates of *Aspergillus Niger. Int. J. Microbiol. Res.* 1(2):13-17

Yoshida S. 1981. Fundamentals of rice crop science. The International Rice Research Institute.

You C.B., Wong W., Wang H.X., Li J.P., Lin M. and Hai W.L. 1991. Association of *Alcaligenes faecalis* with wetland rice. *Plant Soil*. 137:81-85

Zaki N., Gomaa A.M., Galal A. and Farrag A.A. 2009. The associative impact of certain diazotrophs and farmyard manure on two rice varieties grown in a newly cultivated land. *Res. J. Agric. and Biol. Sci*. 5(2):185-190

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Zakry F.A.A., Shamsuddin Z.H., Rahim K.A., Zakaria Z.Z. and Rahim A.A. 2012. Inoculation of *Bacillus sphaerichus* UPMB-10 to young oil palm and measurement of its uptake of fixed nitrogen using the ^{15}N isotope dilution technique. *Microbes Environ*. 27(3):257-262

Zhao X., Xie Y., Xiong Z., Yan X., Xing G. and Zhu Z. 2009. Nitrogen fate and environmental consequence in paddy soil under rice-wheat rotation in the Taihu lake region, China. *Plant Soil*. 319(1):225-234

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