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Full Length Research Paper

Efficiency of plant growth-promoting rhizobacteria (PGPR) for the enhancement of rice growth

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Plant growth-promoting rhizobacteria (PGPR) are beneficial bacteria that colonize plant roots and enhance plant growth by a wide variety of mechanisms. The use of PGPR is steadily increasing in agriculture and offers an attractive way to replace chemical fertilizers, pesticides, and supplements. Here, we have isolated and characterized the PGPR from the rhizosphere soil of rice field for the enhancement of growth of rice. Rhizosphere soils were collected from different areas of Mymensingh in Bangladesh. Ten isolates of bacteria, designated as PGB1, PGB2, PGB3, PGB4, PGB5, PGT1, PGT2, PGT3, PGG1 and PGG2, were successfully isolated and characterized. Subsequently, to investigate the effects of PGPR isolates on the growth of rice, a pot culture experiment was conducted. Prior to seeds grown in plastic pots, seeds were treated with PGPR isolates and seedlings were harvested after 21 days of inoculation. Isolates PGB4, PGT1, PGT2, PGT3, PGG1 and PGG2 induced the production of indole acetic acid (IAA), whereas only PGT3 isolate was able to solubilize phosphorus. Most of isolates resulted in a significant increase in plant height, root length, and dry matter production of shoot and root of rice seedlings. Furthermore, PGPR isolates remarkably increased seed germination of rice. Among the ten isolates, PGB4 and PGG2 were found almost equally better in all aspects such as dry matter production, plant height and root length of rice, and IAA production. Isolate PGT3 was also found to be promising in IAA production having an additional property of phosphate solubilization. The present study, therefore, suggests that the use of PGPR isolates PGB4, PGG2 and PGT3 as inoculant biofertilizers might be beneficial for rice cultivation as they enhanced growth of rice, and induced IAA production and phosphorus solubilization.

Key words: IAA, PGPR, phosphorus solubilization, rice growth, seed germination.

INTRODUCTION

Bacteria that colonize the rhizosphere and plant roots, and enhance plant growth by any mechanism are referred to as plant growth-promoting rhizobacteria (PGPR). In the context of increasing international concern for food and environmental quality, the use of PGPR for reducing chemical inputs in agriculture is a potentially important issue. PGPR have been applied to various crops to enhance growth, seed emergence and crop yield, and some have been commercialized (Dey et al., 2004; Herman et al., 2008; Minorsky, 2008). A PGPR *Pseudomonas fluorescens* B16 isolated from the roots of graminaceous plants has been shown to colonize the roots of various plants, and to increase the height, flower number, fruit number and total fruit weight of tomato plants (Minorsky, 2008). Under salt stress, PGPR have shown positive effects in plants on such parameters as germination rate, tolerance to drought, weight of shoots and roots, yield, and plant growth (Kloepper et al., 2004; Kokalis-Burelle et al., 2006). Another major benefit of PGPR is to produce antibacterial compounds that are effective against certain plant pathogens and pests (Dey et al., 2004; Herman et al., 2008; Minorsky, 2008). More-

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over, PGPR mediate biological control indirectly by eliciting induced systemic resistance against a number of plant diseases (Jetiyanon and Kloepper, 2002). Application of some PGPR strains to seeds or seedlings has also been found to lead to a state of induced systemic resistance in the treated plant (Kloepper et al., 1999). PGPR have also been reported in cereal crops including rice (Yanni et al., 1997; Biswas et al., 2000a, b).

In addition to improvement of plant growth, PGPR are directly involved in increased uptake of nitrogen, synthesis of phytohormones, solubilization of minerals such as phosphorus, and production of siderophores that chelate iron and make it available to the plant root (Lalande et al., 1989; Glick, 1995; Bowen and Rovira, 1999). It has also been reported that PGPR is able to solubilize inorganic and/or organic phosphates in soil (Liu et al., 1992).

Rice is the most important staple food in several developing countries, and chemical fertilizer is the most important input required for rice cultivation. In Bangladesh, 70% of the total cropped land and 82% of the irrigated land are used for rice cultivation (Bangladesh Bureau of Statistics, 2002). The high-yielding rice variety has resulted in an increase in rice production but requires large amounts of chemical fertilizers, leading to health hazards and environmental pollution. In order to make rice cultivation sustainable and less dependent on chemical fertilizers, it is important to know how to use PGPR that can biologically fix nitrogen, solubilize phosphorus and induce some substances like indole acetic acid (IAA) that can contribute to the improvement of rice growth.

Recently, there is a growing interest in PGPR due to their efficacy as biological control and growth promoting agents in many crops (Thakuria et al., 2004). There is very little information regarding the use of PGPR as biofertilizers in rice. Therefore, the present study was undertaken to screen the PGPR strains that are compatible with rice in Bangladesh. We also investigated the effect of PGPR strains on seed germination and growth of rice seedlings as well.

MATERIALS AND METHODS

Isolation of PGPR from rice rhizosphere

Soil samples were collected from the rhizosphere of 2-month-old rice plants in different areas of Mymensingh district in Bangladesh. The rhizosphere was dugout with intact root system. The samples were placed in plastic bags and stored at 4°C in the Biofertilizer and Microbiology Laboratory of Bangladesh Institute of Nuclear Agriculture.

Ten grams of rhizosphere soil were taken into a 250 mL of conical flask, and 90 mL of sterile distilled water was added to it. The flask was shaken for 10 min on a rotary shaker. One milliliter of suspension was added to 10 mL vial and shaken for 2 min. Serial dilution technique was performed upto 10^{-7} dilution. An aliquot (0.1 mL) of this suspension was spread on the plates of Luria Bartany (LB) agar medium. Plates were incubated for 3 days at 28°C to observe the colonies of bacteria. Bacterial colonies were streaked to other LB agar plates and the plates were incubated at 28°C for 3 days. Typical bacterial colonies were observed over the streak. Well isolated single colony was picked up and re-streaked to fresh LB agar plate and incubated similarly. The technique was perpetrated thrice and cultures were made single colony type.

Characterization of isolates

Morphological characteristics of the colony of each isolate were examined on LB agar plates. All the isolates were streaked on LB agar plates. After 3 days of incubation, different characteristics of colonies such as shape, size, elevation, surface, margin, color, odor, pigmentation, etc were recorded.

A loopful of bacterial culture from each isolates was diluted into a test tube containing 1 mL sterile distilled water and was vortexed. A loopful was then taken on a glass slide and smeared. The slide was air dried and fixed by heating on a Bunsen flame. The slide was flooded with crystal violet solution for 3 min. The slide was washed gently in flow of tap water and air dried. The slide was observed under microscope and recorded the shape.

A drop of sterile distilled water was placed in the center of glass slide. A loopful of growth from young culture was taken, mixed with water, and placed in the center of slide. The suspension was spread out on slide using the tip of inoculation needle to make a thin suspension. The smear was dried in air and fixed through mild heating by passing the lower site of the slide 3 to 4 times over the flame. The smear was then flooded with crystal violet solution for 1 min and washed gently in flow of tap water. Then the slide was flooded with iodine solution, immediately drained off, and flooded again with iodine solution. After incubation at room temperature for 1 min, iodine solution was drained out followed by washing with 95% ethanol. After that, it was washed with water within 15 to 30 s and blot dried carefully. The smear was incubated with safranin solution for 1 min. The slide was washed gently in flow of tap water and dried in air. The slide was observed under microscope and data were recorded.

Motility of bacteria was observed by hanging drop method. A loopful of 2-day-old bacterial culture was suspended in 1 mL of nigrosin solution. A drop of suspension was taken on a cover slip. The cover slip was hanged on a hollow slide with vaseline. The slide was then observed under microscope to test the motility of bacteria.

Growth under different temperature conditions

The culture of 10 isolates were streaked on LB agar plates and incubated at 10, 20, 28, 37 and 45°C. The change in growth and color was observed and recorded after 3 days of incubation.

IAA production

A single colony of bacterial culture was grown on LB liquid medium. A loopful of the respective culture was transferred to the 100 mL of conical flask containing LB liquid medium with the help of a sterile inoculation needle. The flask was then incubated for 7 days on a rotary shaker. The cultures in the flask showed dense milky white growth were tested for purity (Bric et al., 1991).

Phosphate solubilization

The plates were prepared with Pikovskya's medium. The culture of ten isolates were streaked on the plates and incubated in an incubator at 28°C for 7 days. The plates were then examined and data were recorded (Pikovaskya, 1948).

Seed germination test

Rice seeds (*Oryza sativa* L. cv. BINA Dhan5) collected from Agronomy Division of Bangladesh Institute of Nuclear Agriculture were soaked in H_2SO_4 for 5 min and washed with sterile water three times to remove the H_2SO_4. Then seeds were treated with bacterial strain for 30 min. Twenty five seeds were placed on agar (2%, w/v) plates and incubated for 3 days in the dark. Finally, germination of seeds was recorded.

Preparation of broth culture

The bacterial inoculants were prepared according to the method of Vincent (1970). A loopful of the respective rhizobacterial isolate was transferred to the liquid medium of 100 mL conical flask and incubated for 7 days on a rotary shaker. When the cultures in the flask showed dense milky white growth, the broth cultures (>1 x 10^7cells/mL) were tested for purity and growth.

PGPR isolates and pot experiments

Ten bacterial isolates were designated as PGB1, PGB2, PGB3, PGB4, PGB5, PGT1, PGT2, PGT3, PGG1 and PGG2. Rice seeds were soaked in H_2SO_4 for 3 min and washed with sterile water seven times. Seeds were then treated with bacterial isolates for 30 min. No treated seeds with any isolate were designated as control. Sands collected from Biofertilizer Laboratory of Bangladesh Institute of Nuclear Agriculture were sterilized by autoclaving. An amount of 0.3 kg sand was placed into a plastic pot. Ten seeds were sown at 4 to 5 cm depth of sand in each plastic pot.

Harvesting of the plants and analysis

Rice plants were harvested after 21 days of seed sowing through separating of plants from soil. The plants were washed through dipping into a vessel. Plant height (cm plant^{-1}) and root length (cm plant^{-1}) of each plant were recorded. Dry weights of shoot and root were recorded after drying in an oven for 1 day at 70°C. Data was analyzed statistically by *F*-test. The significance of differences between mean values was evaluated by DMRT (Duncan's New Multiple Range Test).

RESULTS

Isolation of PGPR

Ten bacterial isolates were successfully isolated from the rhizosphere soils of rice field (Table 1). They were designated as PGB1, PGB2, PGB3, PGB4, PGB5, PGT1, PGT2, PGT3, PGG1 and PGG2.

Morphological characteristics of PGPR isolates

As shown in Table 2, the morphological characteristics of PGPR isolates widely varied. The isolates were found to be first growers. All the isolates produced round shaped and raised colonies having smooth shiny surface with smooth margin. They differed in color but all were odor less. No pigmentation was observed in the colonies of LB agar plates. Diameter of the colonies of isolates varied from 0.2 to 2 mm.

Microscopic observation of PGPR isolates

Microscopic observations were performed to investigate the some characteristics of PGPR isolates such as shape, gram reaction and motility (Table 3). Eight isolates were rod shaped while PGB2 and PGB5 showed ellipsoidal shape. All the isolates were motile and gram negative in reaction. It was also noted that the growth of isolates on LB agar plates varied in temperature (Table 4). The growth of all isolates was good in the temperature ranges of 20 to 28°C. In addition, PGB3 and PGB4 isolates were found to grow at 45°C.

Production of IAA and solubilization of phosphorus

We investigated the IAA production and phosphorus solubilization of PGPR isolates. As shown in Table 5, isolates PGG2, PGB4, PGT3, PGT2, PGT1 and PGG1 induced the production of IAA. Isolates PGG2 and PGB4 were found to be good producers of IAA. On the contrary, PGT3 was found to be a medium producer of IAA in comparison to the weak producer isolates PGT1, PGT2 and PGG1. On the other hand, only PGT3 isolate had ability to solubilize the phosphorus (Table 5).

Seed germination

To see the effect of PGPR isolates on seed germination, rice seeds were pretreated with different isolates. The PGPR isolates remarkably affected the germination of rice seeds (Table 6). It is also noted that PGPR isolates increased seed germination by 2.3 to 14.7% over control. The highest seed germination was recorded when seeds were pretreated with PGB4 isolate. The isolates PGT2, PGT3, PGG1 and PGG2 also showed the better performances to increase the seed germination.

Plant height

The PGPR isolates significantly affected the height of rice seedlings (Table 6). Results reveal that plant height increased in PGPR treated plants over uninoculated control. The highest plant height (13.80 cm) was recorded in PGB4 isolate which was statistically similar to isolates PGT3 (13.10 cm) and PGG2 (13.20 cm).

Shoot dry weight

A significant increase in shoot dry matter of rice seedlings was observed in response to PGPR isolates (Table 6). The lowest shoot dry weight was noted in uninoculated control (7.20 mg plant^{-1}). The highest shoot dry matter was recorded in isolate PGG2 (9.60 mg plant^{-1}) followed by PGB4 (9.40 mg plant^{-1}) and PGT3 (9.20 mg plant^{-1}).

Table 1. Description of the PGPR isolates.

S/N	Isolates	Location of rhizosphere soil	Variety of rice in the field
1.	PGB1	Digharkanda, Sadar, Mymensingh	BRRI dhan 30
2.	PGB2	Digharkanda, Sadar, Mymensingh	BRRI dhan 30
3.	PGB3	Digharkanda, Sadar, Mymensingh	BRRI dhan 30
4.	PGB4	Digharkanda, Sadar, Mymensingh	BRRI dhan 30
5.	PGB5	Digharkanda, Sadar, Mymensingh	BRRI dhan 30
6.	PGT1	Darirampur, Trishal, Mymensingh	BRRI dhan 40
7.	PGT2	Darirampur, Trishal, Mymensingh	BRRI dhan 40
8.	PGT3	Darirampur, Trishal, Mymensingh	BRRI dhan 40
9.	PGG1	Koltapara, Gauripur, Mymensingh	BR11
10.	PGG2	Koltapara, Gauripur, Mymensingh	BR11

Table 2. Morphological characteristics of 3-day-old colony of PGPR isolates.

Isolate	Shape	Size (mm)	Elevation	Surface	Margin	Color	Odor	Pigmentation
PGB1	Round	0.9-1.1	Raised	Smooth shiny	Smooth	Off whitish	Odorless	None
PGB2	Round	0.9-1.1	Raised	Smooth shiny	Smooth	Off whitish	Odorless	None
PGB3	Round	1.0-1.5	Raised	Smooth shiny	Smooth	Brownish	Odorless	None
PGB4	Round	1.0-1.5	Raised	Smooth shiny	Smooth	Yolk brown	Odorless	None
PGB5	Round	1.9-2.0	Raised	Smooth shiny	Smooth	Brownish	Odorless	None
PGT1	Round	1.0-1.5	Raised	Smooth shiny	Smooth	Yellowish	Odorless	None
PGT2	Round	1.5-2.0	Raised	Smooth shiny	Smooth	Yolk yellowish	Odorless	None
PGT3	Round	0.2-0.5	Raised	Smooth shiny	Smooth	Whitish	Odorless	None
PGG1	Round	0.9-1.1	Raised	Smooth shiny	Smooth	Yellowish	Odorless	None
PGG2	Round	0.5-1.0	Raised	Smooth shiny	Smooth	Off white	Odorless	None

Table 3. Cell shape, motility and gram reaction of PGPR isolates.

Isolate	Cell shape	Motility	Gram reaction
PGB1	Rod	Motile	Gram negative
PGB2	Ellipsoidal	Motile	Gram negative
PGB3	Rod	Motile	Gram negative
PGB4	Rod	Motile	Gram negative
PGB5	Ellipsoidal	Motile	Gram negative
PGT1	Rod	Motile	Gram negative
PGT2	Rod	Motile	Gram negative
PGT3	Rod	Motile	Gram negative
PGG1	Rod	Motile	Gram negative
PGG2	Rod	Motile	Gram negative

Table 4. Growth of PGPR isolates at different temperature conditions.

Isolate	Temperature				
	10°C	20°C	28°C	37°C	45°C
PGB1	+	++	++	+	-
PGB2	+	++	++	+	-
PGB3	+	++	++	++	+
PGB4	++	++	++	++	++
PGB5	+	++	++	++	-
PGT1	+	++	++	+	-
PGT2	+	++	++	-	-
PGT3	-	++	++	+	-
PGG1	+	++	++	+	-
PGG2	-	++	++	++	-

- = No growth, + = weak growth; and ++ = good growth.

Root length

The PGPR isolates significantly increased the root length of rice seedlings (Table 6). Root length ranged from 4.10 to 5.30 cm. The isolate PGB4 produced the highest root length (5.30 cm), which was statistically similar to isolate PGG2 (5.10 cm). In comparison to other isolates, PGB5 and PGT3 also showed superior root length (4.60 and 4.80 cm, respectively).

Root dry weight

A significant variation in root dry weight was observed in response to different PGPR isolates (Table 6). Results show that all the isolates significantly increased dry weight of root. The highest root dry weight was recorded

Table 5. Production of IAA and solubilization of phosphorus by PGPR isolates.

Isolate	IAA production	Phosphorus solubilization
PGB1	-	Not solubilizing
PGB2	-	Not solubilizing
PGB3	-	Not solubilizing
PGB4	+++	Not solubilizing
PGB5	-	Not solubilizing
PGT1	+	Not solubilizing
PGT2	+	Not solubilizing
PGT3	++	Solubilizing
PGG1	+	Not solubilizing
PGG2	+++	Not solubilizing

- = No production; + = weak producer; ++ = medium producer; and +++ = good producer.

Table 6. Seed germination and growth of rice seedlings.

Isolate	Seed germination (%)	Seedling height (cm)	Shoot dry weight (mg/plant)	Root length (cm)	Root dry weight (mg/plant)
Control	82.10	10.30e	7.20e	4.10e	5.60e
PGB1	90.15	12.30cd	7.60de	4.50cde	6.40bcd
PGB2	90.26	12.50bcd	8.60b	4.30de	6.60bc
PGB3	84.31	11.80d	8.0cd	4.40cde	6.40bcd
PGB4	94.15	13.80a	9.40a	5.30a	6.80ab
PGB5	83.97	10.90e	7.80cd	4.60cd	6.50bc
PGT1	86.63	12.60bc	7.60de	4.50cde	6.20cd
PGT2	92.23	12.00cd	8.20bc	4.40cde	6.60bc
PGT3	92.00	13.10ab	9.20a	4.80bc	6.0d
PGG1	92.07	12.70bc	7.60de	4.50cde	6.50bc
PGG2	92.59	13.20ab	9.60a	5.10ab	7.10a
Significance	-	**	**	**	**
CV (%)	-	4.00	3.57	5.59	4.23

In a column values having similar letter(s) do not differ significantly as per DMRT.
** = Significant at 1% level.

in isolate PGG2 (7.10 mg plant^{-1}), which was followed by PGB4 (6.80 mg plant^{-1}).

DISCUSSION

PGPR colonize plant roots and exert beneficial effects on plant growth and development by a wide variety of mechanisms. To be an effective PGPR, bacteria must be able to colonize roots because bacteria need to establish itself in the rhizosphere at population densities sufficient to produce the beneficial effects. The exact mechanism by which PGPR stimulate plant growth is not clearly established, although several hypotheses such as production of phytohormones, suppression of deleterious organisms, activation of phosphate solubilization, and promotion of the mineral nutrient uptake are usually believed to be involved (Lalande et al., 1989; Liu et al., 1992; Glick, 1995; Bowen and Rovira, 1999).

IAA, a member of the group of phytohormones, is generally considered to be the most important native auxin. IAA may function as important signal molecule in the regulation of plant development. Of ten isolates, six isolates are positive for IAA production (Table 5). Among them, two isolates PGG2 and PGB4 are found to be good producers of IAA (Table 5). It has been reported that IAA production by PGPR can vary among different species and strains, and it is also influenced by culture condition, growth stage and substrate availability (Mirza et al., 2001). Moreover, isolates from the rhizosphere are more efficient auxin producers than isolates from the bulk soil (Sarwar and Kremer, 1992).

Phosphorus is one of the major nutrients, second only to nitrogen in requirement for plants. Most of phosphorus in soil is present in the form of insoluble phosphates and cannot be utilized by the plants (Pradhan and Sukla, 2006). The ability of bacteria to solubilize mineral phos-

phates has been of interest to agricultural microbiologists as it can enhance the availability of phosphorus and iron for plant growth. PGPR have been shown to solubilize precipitated phosphates and enhance phosphate availability to rice that represent a possible mechanism of plant growth promotion under field conditions (Verma et al., 2001). In comparison to non-rhizospheric soil, a considerably higher concentration of phosphate-solubilizing bacteria is commonly found in the rhizosphere (Raghu and MacRae, 1966). In our experiments, only PGT3 isolate was able to solubilize phosphate in the rhizosphere soil (Table 5). Furthermore, this isolate was found to be medium producer of IAA. It is important to note that several phosphate-solubilizing bacilli occur in soil (Skrary and Cameron, 1998) but their numbers are not usually high enough to compete with other bacteria commonly established in the rhizosphere (Lifshitz et al., 1987).

A large body of evidence suggests that PGPR enhance the growth, seed emergence and crop yield, and contribute to the protection of plants against certain pathogens and pests (Dey et al., 2004; Kloepper et al., 2004; Kokalis-Burelle et al., 2006; Herman et al., 2008; Minorsky, 2008). In this study, we investigated the effectiveness of PGPR isolates whether they could increase the seed germination rate as well as growth of seedlings. Most of isolates significantly increased plant height, root length, and dry matter production of shoot and root of rice seedlings (Table 6). Seed germination was also increased when seeds were pretreated with PGPR isolates. Of ten isolates, two isolates PGB4 and PGG2 showed better performances in aspects of seed germination and growth of seedlings with IAA production (Table 6). In addition to increment of seed germination and growth of seedlings by isolate PGT3, it was positive for both phosphorus solubilization and IAA production. These results suggest that the increased growth of rice seedlings by application of PGPR is probably due to induction of IAA production and phosphorus solubilization.

Taken together, results suggest that PGPR are able to induce the production of IAA, solubilization of phosphorus, and resistance to pathogens and pests, thereby improving growth of plants. The use of PGPR as inoculants biofertilizers is an efficient approach to replace chemical fertilizers and pesticides for sustainable rice cultivation in Bangladesh. Further investigations, including efficiency test under greenhouse and field conditions, are needed to clarify the role of PGPR as biofertilizers that exert beneficial effects on plant growth and development.

REFERENCES

Bangladesh Bureau of Statistics (2002). Statistical Yearbook of Bangladesh. Statistics Division, Ministry of Planning, Govt. of the People's Republic of Bangladesh, Dhaka.

Biswas JC, Ladha JK, Dazzo FB (2000a). Rhizobial inoculation improves nutrient uptake and growth of lowland rice. Soil Sci. Soc. Am. J. 64: 1644-1650.

Biswas JC, Ladha JK, Dazzo FB, Yanni YG, Rolfe BG (2000b). Rhizobial inoculation influences seedling vigor and yield of rice. Agron. J. 92: 880-886.

Bowen GD, Rovira AD (1999). The rhizosphere and its management to improve plant growth. Adv. Agron. 66:1-102.

Bric JM, Bustock RM, Silversone SE (1991). Rapid in situ assay for indole acetic acid production by bacterial immobilization on a nitrocellulose membrane. Appl. Environ. Microbiol. 57: 535-538.

Dey R, Pal KK, Bhatt DM, Chauhan SM (2004). Growth promotion and yield enhancement of peanut (*Arachis hypogaea* L.) by application of plant growth-promoting rhizobacteria. Microbiol. Res. 159: 371-394.

Glick BR (1995). The enhancement of plant growth by free-living bacteria. Can. J. Microbiol. 41: 109-117.

Herman MAB, Nault BA, Smart CD (2008). Effects of plant growth-promoting rhizobacteria on bell pepper production and green peach aphid infestations in New York. Crop Protect. 27: 996-1002.

Jetiyanon K, Kloepper JW (2002). Mixtures of plant growth promoting rhizobacteria for induction of systemic resistance against multiple plant diseases. Biol. Contr. 24: 285-291.

Kloepper JW, Rodriguez-Ubana R, Zehnder GW, Murphy JF, Sikora E, Fernandez C (1999). Plant root bacterial interactions in biological control of soil borne diseases and potential extension to systemic and foliar diseases. Austr. Plant Pathol. 28: 21-26.

Kloepper JW, Ryu CM, Zhang S (2004). Induced systemic resistance and promotion of plant growth by *Bacillus* spp. Phytopathology, 94: 1259-1266.

Kokalis-Burelle N, Kloepper JW, Reddy MS (2006). Plant growth-promoting rhizobacteria as transplant amendments and their effects on indigenous rhizosphere microorganisms. Appl. Soil Ecol. 31: 91-100.

Lalande R, Bissonnette N, Coutlée D, Antoun H (1989). Identification of rhizobacteria from maize and determination of their plant-growth promoting potential. Plant Soil. 115: 7-11.

Lifshitz R, Kloepper JW, Kozlowski M, Simonson C, Carlson J, Tipping EM, Zalesca I (1987). Growth promotion of canola (rapeseed) seedlings by a strain of *Pseudomonas putida* under gnotobiotic conditions. Can. J. Microbiol. 33: 390-395.

Liu ST, Lee LY, Tai CY, Hung CH, Chang YS, Wolfram JH, Rogers R, Goldstein AH (1992). Cloning of an *Erwinia herbicola* gene necessary for gluconic acid production and enhanced mineral phosphate solubilization in *Escherichia coli* HB101. J. Bacteriol. 174: 5814-5819.

Minorsky PV (2008). On the inside. Plant Physiol. 146: 323-324.

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

Pikovaskya RI (1948). Mobilization of phosphorus in soil in connection with the vital activity of some microbial species. Mikrobiologiya. 17: 362-370.

Pradhan N, Sukla LB (2006) Solubilization of inorganic phosphates by fungi isolated from agriculture soil. Afr. J. Biotechnol. 5: 850-854.

Raghu K, MacRae IC (1966). Occurrence of phosphate-dissolving microorganisms in the rhizosphere of rice plants and in submerged soils. J. Appl. Bacteriol. 29: 582-586.

Sarwar M, Kremer RJ (1992). Determination of bacterially derived auxins using a microplate method. Lett. Appl. Microbiol. 20: 282-285.

Skrary FA, Cameron DC (1998). Purification and characterization of a *Bacillus licheniformis* phosphatase specific for d-alpha-glycerphosphate. Arch. Biochem. Biophys. 349: 27-35.

Thakuria D, Talukdar NC, Goswami C, Hazarika S, Boro RC, Khan MR (2004). Characterization and screening of bacteria from the rhizosphere of rice grown in acidic soils of Assam. Curr. Sci. 86: 978-985.

Verma SC, Ladha JK, Tripathi AK (2001). Evaluation of plant growth promoting and colonization ability of endophytic diazotrophs from deep water rice. J. Biotechnol. 91: 127-141.

Vincent JM (1970). A manual for the practical study of root-nodule bacteria. Blackwell Scientific Publ., Oxford, UK.

Yanni YG, Rizk RY, Corich V, Squartini A, Ninke K, Philip-Hollingsworth S, Orgambide G, de Bruijn F, Stoltzfus J, Buckley D, Schmidt TM, Mateos PF, Ladha JK, Dazzo FB (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.