ORIGINAL RESEARCH ARTICLE

Isolation, Screening, Characterization and Antagonism Assay of PGPR Isolates from Rhizosphere of Rice Plants in Cuddalore District

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ABSTRACT

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, screened and characterized the PGPR from the rhizosphere soil of rice field. Rhizosphere soils were collected from different areas of Cuddalore district in Tamil Nadu, India. 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 PGPR isolates for their antagonistic activity against phytopathogenic fungi such as *Fusarium oxysporum*, *Rhizoctonia solani* and *Sclerotium rolfsii*. In extend, the growth of PGPR isolates was optimized under different temperature conditions such as 10°C, 20°C, 28°C, 37°C and 45°C. 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. In case of Siderophore production, the isolates PGB4, PGT1, PGT2, PGT3 and PGG2 were found to be positive. Most of the isolates grown best under the temperature of 20°C & 28°C when compared to 10°C & 37°C and very few can grown at 45°C. Furthermore, most of the PGPR isolates shown antifungal activity against *Fusarium oxysporum*, and *Rhizoctonia solani*, and only one against *Sclerotium rolfsii*. The present study, therefore, suggests that the use of PGPR isolates PGB4, PGG2 and PGT3 as inoculants/ biofertilizers might be beneficial for rice cultivation as they enhanced growth of rice due to the production of IAA, Phosphate solubilization, Siderophore production and also having antifungal activity against phytopathogenic fungi.

Key words: PGPR, IAA, phosphorus solubilization, siderophore production, antagonism assay.

1. 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[1,2,3]. 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 [3]. Under salt stress, PGPR have shown positive effects in plants on such parameters as germination rate, tolerance to

drought, weight of shoots & roots, yield, and plant growth [4,5]. Another major benefit of PGPR is to produce antibacterial compounds that are effective against certain plant pathogens and pests [2]. Moreover, PGPR mediate biological control indirectly by eliciting induced systemic resistance against a number of plant diseases [6]. 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 [7]. PGPR have also been reported in cereal crops including rice [8,9]. 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 [10]. It has also

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been reported that PGPR is able to solubilize inorganic and organic phosphates in soil [11]

Inoculant development has been most successful to deliver biological control agents of plant disease i.e. organisms capable of killing other organisms pathogenic or disease causing to crops. Various bacteria which are predominantly studied and increasingly marketed as the biological control agents includes the genera *Bacillus*, *Streptomyces*, *Pseudomonas*, *Burkholderia* and *Agrobacterium*. They suppress plant disease through at least one mechanism; induction of systemic resistance and production of siderophores or antibiotics. Exposure to the PGPR triggers a defence response by the crop as if attacked by pathogenic organisms [12].

The discovery of IAA as a plant growth regulator coincided with the first indication of the molecular mechanisms involved in tumorigenesis induced by *Agrobacterium*. *Agrobacterium*-induced tumors were shown to be sources of IAA [13] and capable of growth in the absence of plant growth regulators, which are normally, required to incite growth of callus from sterile plant tissues [14]. It was later found that not only plants but also microorganisms including bacteria and fungi are able to synthesize IAA [15]. In recent years, advancement in understanding the IAA signaling pathway in plants has been truly spectacular. The role of IAA in bacteria has not thus far been investigated in such detail. Undoubtedly, the advancement in plant IAA signaling has also intensified research on the various aspects of bacterial IAA synthesis, including its role in bacteria– plant interactions. As more bacterial species have been analyzed, the role of auxins in plant–microorganism interactions appears diverse. Molecular studies on the biochemical pathways of bacterial IAA synthesis and their regulation have provided some clues on the possible outcomes of the interactions between plants and IAA-producing bacteria, varying from pathogenesis to phytostimulation. IAA producing PGPR strains can able to enhance the growth and development of plants by interfering in the concentration of known phytohormones [16]. One of the most important ways that those bacteria affect growth and development is by producing Indole-3-acetic acid (IAA) that this hormone is led to plant root system development and subsequently nutritional uptake increase by plant. Many of rhizobial species enable to produce IAA [17]. In order to produce Indole-3-acetic acid (IAA), the bacteria use Tryptophan (L-Trp) as precursor [18]. This substance can be converted to IAA by soil beneficial bacterial activities. In addition to root growth, the synthesized IAA by bacteria in some case by promoting enzyme 1-aminocyclopropane-1-carboxylate (ACC) synthase and increasing the synthesis of ACC, precursor of ethylene, can be caused a reverse result. The excess of produced ethylene, so-called named stress ethylene, causes to decrease vegetative period and finally, yield [19].

2. MATERIAL AND METHODS

2.1. Isolation of PGPR from rice rhizosphere

Soil samples were collected from the rhizosphere of 2 month old rice plants in different areas of Cuddalore district in India. The rhizosphere was dugout with intact root system. The samples were placed in plastic bags and stored at 4°C in refrigerator. 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-Bertany (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 perpetuated thrice and cultures were made single colony type.

2.2. 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 the shape was recorded. 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 side 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 seconds 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.

2.3. 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.

2.4. IAA production

Plant hormones can be natural or synthetic. There are several phytohormone groups and the best known is the auxin group. Diverse soil microorganisms including bacteria, fungi and algae are also capable of producing physiologically active quantities of auxins (IAA). The culture of 10 isolates was incubated in the peptone broth enriched with tryoptophan broth to check for the production of indole acetic acid, a precursor of auxin which is an important plant hormone. The quantitative estimation of IAA is performed by using Salkowski method by using the reagent, 1 ml of $FeCl_3$, 0.5 mM in 35% $HClO_4$. Mixtures were incubated at room temperature for 25 min and observed for pink colour production and read colorimetrically.

2.5. Phosphate solubilization

Phosphorus is only second to nitrogen in mineral nutrients which is most commonly limiting in the growth of plants. Many soil microorganisms are able to solubilize unavailable forms of bound P [20]. 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 [21]. Visual detection and semi quantitative estimation of phosphate solubilizing ability of microorganisms is possible by plate screening methods that show clear zone around the microbial colonies in media containing insoluble mineral phosphates (tricalcium phosphate or hydroxyapatite) as sole P source [22, 23].

2.6. Siderophore production

Siderophore production was tested qualitatively using chrome azurol S medium (CAS-medium) [24]. The culture of 10 isolates were streaked on the surface of CAS agar medium and incubated at room temperature for 1 to 3 days. Siderophore production was indicated by orange halos around the colonies after the incubation, and this test was done in two replications.

2.7. Antagonism assay against phytopathogenic fungi

All the 10 isolates were assayed for antifungal activities against *Fusarium oxysporum*, *Rhizoctonia solani* and *Sclerotium rolfsii* by using Potato Dextrose Agar (PDA) medium. The isolates were streaked on PDA medium 3 cm in distance opposite to pathogenic fungi inoculated at the center of the medium. The barrier between isolates and fungi indicated antagonist interaction between them. Antagonist activity was investigated for 4 to 7 days after incubation at room temperature. The value of inhibition was measured using the formula described by Kumar *et al.* [25] which is 1-(a/b) x 100% (a: distance between fungi in the center of Petri dish to test isolate, b: distance between fungi in the center of Petri dish to blank are without *Bacillus* isolate).

3. RESULTS

3.1. Isolation of PGPR

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

Table 1: Description of the PGPR isolates

S. No	Isolates	Location of rhizosphere soil	Variety of rice in the field
1	PGB1	Adoor	ADT 36
2	PGB2	Kattumannarkoil	ADT 36
3	PGB3	Orathur	ADT 36
4	PGB4	Aalappakkam	ADT 43
5	PGB5	Maruthur	ADT 36
6	PGT1	Mutlur	ADT 43
7	PGT2	Sethiyathope	ADT 36
8	PGT3	Srimusnam	ADT 43
9	PGG1	Bhuvanagiri	ADT 43
10	PGG2	Keerapalayam	ADT 43

3.2. 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 colour but all were odourless. No pigmentation was observed in the colonies of LB agar plates. Diameter of the colonies of isolates varied from 0.2 to 2 mm.

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

Isolates	Shape	Size (mm)	Elevation	Surface	Margin	Color	Odour	Pigmentation
PGB1	Round	0.9-1.1	Raised	Smooth Shiny	Smooth	Off whitish	Odourless	None
PGB2	Round	0.9-1.1	Raised	Smooth Shiny	Smooth	Off whitish	Odourless	None
PGB3	Round	1.0-1.5	Raised	Smooth Shiny	Smooth	Brownish	Odourless	None
PGB4	Round	1.0-1.5	Raised	Smooth Shiny	Smooth	Yolk brown	Odourless	None
PGB5	Round	1.8-2.0	Raised	Smooth Shiny	Smooth	Brownish	Odourless	None
PGT1	Round	1.0-1.5	Raised	Smooth Shiny	Smooth	Yellowish	Odourless	None
PGT2	Round	1.5-2.0	Raised	Smooth Shiny	Smooth	Yolk yellowish	Odourless	None
PGT3	Round	0.2-0.5	Raised	Smooth Shiny	Smooth	Whitish	Odourless	None
PGG1	Round	0.9-1.1	Raised	Smooth Shiny	Smooth	Yellowish	Odourless	None
PGG2	Round	0.5-1.0	Raised	Smooth Shiny	Smooth	Off white	Odourless	None

3.3. 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.

Table 3: Cell shape, Motility and Gram reaction of PGPR isolates

Isolates	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 condition

Isolates	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; ++ = good growth.

3.4. 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**).

Table 5: Production of IAA and Phosphorous solubilization by PGPR isolates

Isolates	IAA production	Phosphorous solubilization	Siderophore production
PGB1	-	Not solubilizing	Negative
PGB2	-	Not solubilizing	Negative
PGB3	-	Not solubilizing	Negative
PGB4	+++	Not solubilizing	Positive
PGB5	-	Not solubilizing	Negative
PGT1	+	Not solubilizing	Positive
PGT2	+	Not solubilizing	Positive
PGT3	++	Solubilizing	Positive
PGG1	+	Not solubilizing	Negative
PGG2	+++	Not solubilizing	Positive

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

3.5. Siderophore production

Out of 10 PGPR isolates, 5 isolates (PGG2, PGB4, PGT3, PGT2 and PGT1) were able to produce siderophore and it is confirmed by the development of orange halos surrounding those colonies (**Table 5**).

3.6. Antagonism assay against phytopathogenic fungi

Opposition assay was used to determine the isolates that inhibit the growth of *R. solani*, *F. oxysporum* and *S. rolfsii*. One isolate showed inhibition activity against *S. rolfsii* (PGG2), meanwhile numerous isolates were able to inhibit *F. oxysporum* and *R. solani* in various level of inhibition percentage. In this study, among 10 PGPR isolates that significantly promoted plant growth of rice seedling, there were only 3 isolates (PGG2, PGB4 and PGT3) that were able to inhibit *F. oxysporum*, 6 isolates (PGG2, PGB4, PGT3, PGT2, PGT1 and PGG1) inhibited *R. solani*, and only 1 isolate of PGPR (PGG2) inhibited *S. rolfsii* (**Table 6**).

Table 6: Antagonism assay of PGPR isolates against phytopathogenic fungi

Isolates	Antagonism assay against phytopathogenic fungi		
	R. solani	*F. oxysporum*	*S. rolfsii*
PGB1	-	-	-
PGB2	-	-	-
PGB3	-	-	-
PGB4	+++	++	-
PGB5	-	-	-
PGT1	++	-	-
PGT2	+	-	-
PGT3	++	++	-
PGG1	+++	-	-
PGG2	++	++	+

+ = low inhibition percentage (< 30 %); ++ = moderate inhibition percentage (30% to 40%);

+++ = strong inhibition percentage (>40%)

4. 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 [10,11].

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 [26]. Moreover, isolates from the rhizosphere are more efficient auxin producers than isolates from the bulk soil [27].

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 [28]. The ability of bacteria to solubilize mineral phosphates 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 [29]. In comparison to non-rhizospheric soil, a considerably higher concentration of phosphate-solubilizing bacteria is commonly found in the rhizosphere [30]. 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 [31] but their numbers are not usually high enough to compete with other bacteria commonly established in the rhizosphere [32].

Siderophore is one of the biocontrol mechanisms belonging to PGPR groups under iron limiting condition. PGPR produces a range of siderophore which have a very high affinity for iron. Therefore, the low availability of iron in the environment would suppress the growth of pathogenic organisms including plant pathogenic fungi [33]. In addition to siderophore, there are other mechanisms of biocontrol including antibiotics compounds, elicitation of induced systemic resistance (ISR) of plant, and lytic enzyme secretion [34]. This study has demonstrated that, the 10 PGPR isolates classified as plant growth promoter and produced siderophores such as PGB4, PGT1, PGT2, PGT3 and PGG2, and these isolates already found to be IAA producer.

Based on the siderophore produced by those isolates, it has been determined that, all the isolates which produced the bioactive compound siderophore were able to inhibit the phytopathogenic fungi (Table 6). The results of this study suggest that, siderophore produced by those bacteria functions as suppressor to the growth of phytopathogenic fungi such as *F. oxysporum*, *R. solani* and *S. rolfsii*.

REFERENCES

1. 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.

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

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

4. Kloepper JW, Ryu CM, Zhang S (2004). Induced systemic resistance and promotion

of plant growth by *Bacillus* spp. *Phytopathology*, 94: 1259-1266.

5. 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.

6. 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.

7. 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.

8. 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.

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

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

11. 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.

12. Joseph B, Patra RR, Lawrence R (2007). Characterization of plant growth promoting Rhizobacteria associated with chickpea (*Cicer arietinum* L). *International Journal of Plant Production*, 1 (Suppl 2): 141-152.

13. Link GKK and Eggers V (1941). Hyperauxiny in crown gall of tomato. *Bot Gaz.,* 103: 87–106.

14. White PR and Braun AC (1941). Crown gall productions by bacteria free tumor tissues. *Science* 93: 239–241.

15. Costacurta A, Keijers V and Vanderleyden J (1994). Molecular cloning and sequence analysis of an *Azospirillum brasilense* indole-3-pyruvate decarboxylase gene. *Mol Gen Genet*., 243: 463–472.

16. Khalid A, Arshad M, Zahir ZA and Khaliq A (1997). Potential of plant growth promoting rhizobacteria for enhancing wheat (*Triticum aestivum* L.) yield. *J. Anim. Plant Sci*., 7: 53-56.

17. Leinhos V (1994). Effects of pH and glucose on auxine production by phosphate-solubilizing rhizobacteria *in vitro*. *Microbiological Research*, 194: 135-138.

18. Sarwar M and Frankenberger WT (1994). Influence of L-Tryptophan and auxins applied to the rhizosphere on the vegetative growth of *Zea mays* L. *Plant and Soil*, 160: 97-104.

19. Neeru N, Vivek K, Rishi K and Wolfgancy M (2000). Effect of P-solubilizing *Azotobacter chroococcum* on N, P, K uptake in p-responsive genotypes grown under greenhouse condition. *J. Plant Nutr. Soil Sci*., 163: 393-398.

20. Daniel PS, Robert JR, Ayling SM (1998). Phosphorus uptake by plants, from soil to cell, pp. 447-453

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

22. Ames BN (1964). Assay of inorganic phosphate, total phosphate and phosphatases.

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

24. Husen E (2003). Screening of soil bacteria for plant growth promoting activities *in vitro*. Indones. *J. Agric. Sci*., 4: 27-31.

25. Kumar NR, Arasu VT, Gunasekaran P (2002). Genotyping of antifungal compounds producing plant promoting rhizobacteria, *Pseudomonas fluorescens*. Curr. Sci., 82: 1463-466.

26. Mirza MS, Ahmad W, Latif F, Haurat J, Bally R, Normand P, Malik KA (2001) Isolation, partial characterization, and

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the effect of plant growth-promoting bacteria (PGPB) on micro propagated sugarcane *in vitro*. Plant Soil. 237: 47-54.

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

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

29. 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.

30. 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.

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

32. 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.

33. Whipps JM (2001). Microbial interactions and biocontrol in the rhizosphere. J. Experiment. Bot., 52: 487-511.

34. Haas D, Defago G (2005). Biological control of soil-borne pathogens by fluorescent pseudomonads. Nature. Rev. Microbiol., 1038: 1-13.