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Full Length Research Paper

Screening of rhizobacteria containing plant growth promoting (PGPR) traits in rhizosphere soils and their role in enhancing growth of pigeon pea

M. Usha Rani[1]*, Arundhathi[2] and Gopal Reddy[1]

[1]Andhra University, Visakhapatnam, India.
[2]Department of Botany. Andhra University, India.

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Plant growth promoting rhizobacteria (PGPR) are beneficial bacteria that colonize plant roots and enhance plant growth with 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, enumerated and characterized the PGPR from the rhizosphere soil of pigeon pea for the enhancement of growth of pigeon pea. Rhizosphere soils were collected from different areas of Samalkot, Pithapuram, Peddapuram and Kakinada. Sixty five (65) isolates were identified and characterized for their morphological, cultural, staining and biochemical characteristics, of which 35 was selected for the screening of PGPR isolates. Sixteen isolates were successfully characterized for the PGPR traits like indole acetic acid (IAA) production, phosphorus solubilization, and production of enzymes like urease, chitinase, amylase, cellulase, protease and β-1,3 glucanase and were assayed. The antagonistic nature of these strains towards fungi and bacteria were estimated by siderophore estimation, 1-amino-cyclopropane-1-carboxylate (ACC) deaminase characterization, dual plate culture method and HCN production technique, and the best one was selected. These were further investigated to show the PGPR traits in pigeon pea seedling emergence, increase of shoot length, root length, dry matter production of shoot, nodule number and nodule mass. Furthermore, PGPR isolates remarkably increased seed germination of pigeon pea. Among the sixteen isolates, seven were found to be high IAA producing. Six were found to be efficient phosphate solubilizers, five isolates were found to be good antagonistic towards pathogen soil fungi and eight isolates were found to be better in enzyme productions, and thus, may enhance the mineralization efficiency of soils. Three isolates were shown to be promising in IAA production, phosphate solubilization, antagonism towards fungi, and mineralizing capacity. Thus, this study suggests the use of these isolates as inoculant biofertilizers which might be beneficial for pigeon pea cultivation as they enhanced the growth and other growth parameters.

Key words: Indole acetic acid (IAA), plant growth promoting rhizobacteria (PGPR), phosphorus solubilization, enzyme productions, seed germination.

INTRODUCTION

Bacteria that colonize the rhizosphere and plant roots, and enhance plant growth by any mechanisms 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 (Arnou et al., 1953). PGPR have been applied to various crops to enhance growth, seed emergence and crop yield, and some can be commercialized. A PGPR *Pseudomonas fluorescens* isolated

*Corresponding author. E-mail: mareedup@yahoo.com.

Abbreviations: PGPR, Plant growth promoting rhizobacteria; **ACC,** 1-amino-cyclopropane-1-carboxylate.

from roots of graminae plants has been shown to colonize the roots of various plants and increase the height, flower number, fruit number and total fruit weight of tomato plants (Andrews et al., 2003). 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 (Brown, 1974). Another major benefit of PGPR is that it produces antibacterial compounds that are effective against certain plant pathogens and pests. Moreover, PGPR mediate biological control indirectly by eliciting systemic induction against a number of plant diseases. 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. PGPR have also been reported in cereal crops including rice (Fauci and Dick, 1994).

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 (Gyaneshwar et al., 1998). It has also been reported that PGPR is able to solubilize inorganic and organic phosphates in soil. Pigeon pea, the most important staple food in several developing countries and as a fertilizer, is the most important input required for cultivation (Neeru et al., 2000). The high yielding variety has resulted in an increase in the production but requires large amounts of chemical fertilizers leading to health hazards and environmental pollution. In order to make 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 pigeon pea (Filip, 2001).

Recently, there has been growing interest in PGPR due to their efficacy as biological control and growth promoting agents in many crops. There is very little information regarding the use of PGPR as biofertlizer (Elliott et al., 1996). Therefore, this study was undertaken to screen the PGPR strains that are compatible with *Cajanus cajan and* use of PGPR as biofertilizer. We also investigated the effect of PGPR strains on seed germination and growth of pigeon pea seedlings cultivated in coastal areas of Andhra Pradesh. Under salt stress, PGPR have shown positive effects in plants on parameters such as germination rate, tolerance to drought, weight of shoots and roots, yield and plant growth. Another major advantage of PGPR is that it produces antibacterial compounds that are effective against certain plant pathogens and pests (Raju et al., 1999). The objective of this study was to screen out rhizobacteria with maximum PGPR traits like IAA production, phosphate solubilization, 1-amino-cyclopropane-1-carboxylate (ACC) deaminase producing ability, siderophore producing ability, antagonism to plant pathogens like *Fusarium udum* and *Macrophomina phaseolina*.

Plant growth promoting bacteria

In the era of sustainable agricultural production, the interactions in the rhizosphere by soil microorganisms play a pivotal role in transformation, mobilization and solubilization from a limited nutrient pool in the soil and ensure uptake of essential nutrients by the crop plants (Bolton et al., 1993; Mantelin and Touraine, 2004). Biocontrol PGPB has beneficial effects towards fungi that infect several crops like cereals, legumes, oil seeds, vegetables and fruits. James (1981) reported that soil borne diseases of economic crops alone cause 13 to 20% annual loss in production. As exact estimates are not found in India, it can be assumed that more than 50% of crop loss is due to soil inhabiting pathogenic microorganism (Rajash, 2005).

Thus, there is need to enhance the efficiency of scanty amount of external inputs by employing the best combinations of bacterial microbes for improved crop production (Chang et al., 2005). The IAA production amount of external inputs employs the best combinations of beneficial microbes for improved crop production (Goldstein, 1995). The use of PGPB inoculants as biofertilisers and antagonistis of plant pathogens provide a promising alternative to chemical fertilizers and pesticides (Goldstein, 1995).

MATERIALS AND METHODS

Isolation of PGPR from rhizosphere soil of pigeon pea

Soil samples were collected from the rhizosphere of 2 and 3 month old pea plants in different areas of Samalkot and Kakinada. To collect the rhizosphere soil in the field with a crop (pigeon pea) at actively growing vegetative phase, rhizosphere soil and cultivated field soil samples were dug out at a depth of 10 to 20 inch deep and collected in sterile cloth bags and polythene bags. The samples were collected in the fields of Samalkot, Pithapuram, Kakinada and Peddapuram regions of East Godavari. The rhizosphere was dug out with intact root system. The samples were placed in plastic bags and stored at 4°C in Biofertilizer and Microbiology laboratory. Samples of applied composts, rice straw compost and vermicompost were also taken for screening the PGPR traits of indigenous organisms. The soil samples were collected in Petri plates by fine brushing and used for dilution and plating.

Preparation of dilutions, inoculations and observations

Ten grams of rhizosphere soil were taken into a 250 ml of conical flasks, 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 the suspension was added to 10 ml vial and shaken for 2 min. Serial dilution technique was performed up to 10^{-7} dilution. An aliquot of this suspension was spread on the plates of Luria Bertani (LB) agar medium. Plates were incubated for 2 days at 28°C to observe the colonies of bacteria. Well isolated single colony was picked up and restreaked to fresh LB agar plate and incubated similarly. The

Table 1. Enumeration of rhizobacteria from five soil samples by SPC method.

S/N	Dilution	Amount of sample	Dilution factor (D)	Volume factor (V)	Number of colony	Cfu per ml. (nxDxV)	Mean cfu per g
1	10^{-2}	0.1ml	10^{2}	10	30	$30x10^{2}x10$	30000
2	10^{-3}	0.1ml	10^{3}	10	45	$45x10^{3}x10$	450000
3	10^{-4}	0.1ml	10^{4}	10	38	$38x10^{4}$ x10	3800000
4	10^{-5}	0.1ml	10^{5}	10	29	$29x10^{5}$ x10	29000000
5	10^{-5}	0.1ml	10^{5}	10	33	$33x10^{5}$ x10	33000000
6	10^{-5}	0.1ml	10^{5}	10	35	$33x10^{5}$ x10	33000000
7	10^{-4}	0.1ml	10^{4}	10	54	$54x10^{4}$ x10	5400000

technique was carried out thrice and cultures were made single colony type. Thus, 65 isolates were selected, from which 35 bacteria were sorted out in pure different colonies, exhibiting morphological and staining characteristics. Six fungal isolates were isolated from the soil sample and were used for further verification of growth promoting and antagonistic traits. They were characterized based on the colony morphological traits, staining characteristics and morphological features.

Standard plate count method: A viable and direct count

To enumerate the bacterial and fungal cultures, standard plate count method was used. The number of viable bacterial cells per unit volume of a sample using agar plate media was enumerated. The inoculum sample was spread across the plate and the colonies that were formed after incubation were counted. The colonies are referred to as colony forming units (CFU). Once the CFUs are counted on the plate, they are divided by the volume plated to determine the concentration of cells in the sample Table 1.

Fungal and bacterial identification

The fungi were identified by their mycelial nature, spores and spore bearing structures, together with colony characters. Different colony types were identified by the morphology of the colonies using characters such as color and texture pigmentation. Lactophenol cotton blue mounts were performed and observed for spores and spore bearing structures (Al-Raddad, 1995).

Bacteria were identified based on different morphological and staining characteristics. Based on the Gram staining property and cell morphology, the bacteria may be tentatively placed in 4 groups, that is, Gram +ve rods, Gram +ve cocci, Gram -ve cocci and Gram -ve rods. Usually, the predominant bacteria in rhizosphere of crop plants are Gram negative rods belonging to Gram -ve Pseudomonas and Gram +ve Bacillus. Further identification was done with specific biochemical tests.

Biochemical characterization of bacteria

Bacterial isolates were thus characterized based their morphological ability, staining characteristics and motility characteristics and were further investigated for their biochemical properties like indole production, catalase production, gelatin liquefaction, urease producing ability, saccharolytic activity, chitinase activity, acid production, citrate utilizing ability and nitrate producing abilities. The results are shown in Table 3. This helped in the bacterial identification up to the genus level (Dubey and Maheshwari, 2006). Morphological traits and biochemical results are shown in Tables 2 and 3.

Mobilization of soil phosphorus

Phosphorus is second only to nitrogen in mineral nutrients, which most commonly limit the growth of plants (Olsen et al., 1982) Soils have large reserves of total P, but the amount available to plants is a small proportion (Gyaneshwar et al., 1998). Many soil micro-organisms are able to solubilize unavailable forms of bound P. Visual detection and semi quantitative estimation of phosphate solubilizing ability of micro-organisms is possible by plate screening methods that show clearing zone around the microbial colonies in media containing insoluble mineral phosphates (tricalcium phosphate or hydro-xyapatite) as sole P source (Daniel et al., 1998). Quantitative estimation of the inorganic phosphorus produced by the screened rhizobacteria is performed by Fiske Subba Row method (Jayaraman, 1980). Cultures were incubated in PVK broth for 24, 48, 72 h, respectively, and lowering of pH and inorganic phos-phorus produced was estimated. The efficiency is shown as ranking in Table 4.

IAA production

Plant hormones can be natural or synthetic. There are several phytohormone groups; the best known group is the auxin group. Microorganisms inhabiting rhizosphere of various plants are likely to synthesize and release auxin as secondary metabolites. The ability to synthesize phytohormones is widely distributed among plant associated bacteria, 80% of the bacteria isolated from plant rhizosphere are able to produce IAA (Arshad and Frankenberger, 2002). IAA is a natural auxin and NAA, IBA, 2,4-D are synthetic auxins. It was in 1885 that Salkowski discovered IAA in fermentation media. IAA is the major auxin involved in many of the physiological processes in plants (Arshad and Frankenberger, 1998). The amount of IAA produced by rhizobacteria is estimated quantitatively by Salkowski method (Dubey and Maheshwari, 2006). The cultures were incubated in peptone broth together with tryptophan for 24 and 48 h, and IAA production by constructing standard curve was estimated. The results are reported as ranking in Table 4.

ACC deaminase

A number of PGPB contain the enzyme 1-amino-cyclopropane-1-carboxylate (ACC) deaminase and this enzyme could cleave ACC, and the immediate precursor to ethylene in the biosynthetic

Table 2. Morphological, cultural and microscopic characteristics of potential bacteria.

Name of test	RB 1	RB 3	RB 6	RB 7	RB 8	RB 9	RB 10	RB 11	RB 12	RB 13	RB 15	RB 16	RB 22	RB 23	RB 24	RB 27	RB 28	RB 29	RB 30	RB 31	RB 32
GS	+	-	+	+	-	+	+	-	-	-	-	-	-	+	+	-	+	-	-	-	-
SS	S	NS	S	NS	S	NS	NS	S	S	NS	S	NS	S	S	NS	NS	S	S	S	S	S
Cell Shape	SR	SR	R	SR	LR	R	R	SR	R	LR	LR	LR	LR	LR	R	SR	LR	LR	R	LR	LR
Density	O	O	Tl	Tl	Tl	O	Tl	Tl	O	O	Tl	Tl	O	O	O	Tl	Tl	O	Tl	O	O
Elevation	C	Ra	Ra	C	C	C	C	Ra	Ra	C	Ra	C	C	C	C	C	C	Ra	C	C	C
Texture	Sm	Sm	Sm	Sm	Sm	Sm	Sm	Sm	Sm	Sm	Sm	Sm	Sm	Sm	Sm	Sm	Sm	Sm	Sm	Sm	Sm
Margin	Ro	E	E	E	I	Ro	I	I	Ro	E	E	Ro	I	E	E	Ro	I	I	E	W	W
Pigments	NP	Y	Y	NP	NP	NP	P	P	G	Y	NP	NP	NP	P	Y	P	Y	P	G	NP	NP

GS = Gram stain, + = Gram positive rods, - = Gram negative rods, SS = spore stain, S = sporulating, NS = non sporulating, R= rods, LR = long rods, SR = short rods
O = opacity, Tl = translucent, C = convex, Ra = raised, Sm = smooth, W = wavy, Ro = round, E = entire, I = irregular, NP = no pigment, GY = greenish yellow on LB and NA plates, P = pink, G = green, Y = yellow.

Table 3. Identification of potential bacterial isolates based on biochemical tests.

Name of the test	RB 1	RB 3	RB 6	RB 7	RB 8	RB 9	RB 10	RB 11	RB 12	RB 13	RB 15	RB 16	RB 22	RB 23	RB 26	RB 27	RB 28	RB 29	RB 30	RB 31	RB 32
Motility	NM	M	NM	NM	NM	NM	NM	NM	NM	NM	NM	NM	NM	NM	NM	M	NM	NM	M	NM	NM
Indole	-	+	+	-	-	+	+	-	+	+	+	+	+	+	-	-	-	+	-	-	-
Methyl red	-	+	-	-	-	-	+	+	+	+	+	-	+	+	-	+	+	-	-	+	+
Voges Proskeur	+	-	+	+	+	+	+	-	-	-	-	+	-	-	+	-	-	-	-	-	-
Citrate utilization	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Urease	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	+	+	+	+	+	+
Starch hydrolysis	+	-	-	+	-	+	-	-	-	-	+	-	-	-	-	+	+	+	+	+	+
Casein hydrolysis	+	-	-	+	-	+	-	-	-	-	+	-	-	-	-	+	+	+	+	+	+
Gelatin hydrolysis	+	-	-	+	-	+	-	-	-	-	+	-	-	-	-	+	+	+	+	+	+
Nitrate reduction	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	+	+	+
Acid production from (A/G)																					
Glucose	A^+	A^+	A^+	A^-	A^+	A^+	A^+	A^+	A^+	A^+	A^+	A^+	A^-	A^+	A^+	A^+	A^-	A^-	A^-	A^-	A^-
Fructose	A^+	A^+	A^+	A^+	A^+	A^+	A^+	A^+	A^+	A^+	A^+	A^-	A^-	A^+	A^+	A^+	A^-	A^-	A^-	A^-	A^-
Mannitol	A^+	A^+	A^+	A^+	A^+	A^+	A^+	A^-	A^+	A^+	A^+	A^+	A^-	A^-	A^+	A^+	A^-	A^-	A^-	A^-	A^-
Sucrose	A^+	A^+	A^+	A^+	A^+	A^+	A^+	A^+	A^-	A^+	A^+	A^+	A^+	A^+	A^+	A^+	A^-	A^-	A^-	A^-	A^-
Identification of the isolate	*Z*	*Y*	*X*	*W*	*V*	*U*	*T*	*S*	*R*	*Q*	*P*	*O*	*N*	*M*	*L*	*K*	*J*	*I*	*H*	*G*	*F*

F= Bacillus subtilis, *G = Bacillus cereus*, *H = Pseudomonas* sp., *I = Serrratia marcescens*, *J = Azospirillum brasiliense, K = Klebsiella pneumoniae*, *L = Pseudomonas* sp., *M = Psuedomonas* sp., *N = Pseudomonas* sp., *O = Serratia* sp., *P = Bacillus circulans Q = Bacillus* sp., *R = Bacillus* sp., *S = Bacillus cereus T = Pseudomonas* sp., *U = Azotobacter* sp., *V = Rhizobium*, *W = Micrococcus* sp., X = *Klebsiella* sp., *Y = Clostridium* sp., *Z* = Azospirillum; A+ = acid producers, NM = non motile.

Table 4. Ranking of plant growth promoting bacteria for different traits used in the study.

Isolate	PSB	Phytase	Siderophore	Chitin	ACC deaminase	Indole	HCN	Root length	Antagonistic
RB1	2	2	0	0	2	1	0	2	++++
RB3	3	3	2	0	3	1	0	3	++
RB6	3	3	0	2	3	0	0	3	++
RB7	2	2	3	3	0	1	0	3	++
RB8	3	3	3	3	0	2	0	3	++
RB9	2	2	2	0	0	2	0	2	++
RB10	3	3	3	0	3	2	0	3	++
RB11	2	2	2	0	3	1	0	3	+++
RB12	3	3	0	0	2	3	0	3	+++
RB13	1	1	3	0	0	0	0	1	+++
RB15	3	3	2	0	1	3	0	3	++++
RB16	3	3	2	0	1	0	0	3	++++
RB22	2	2	0	0	0	0	0	2	++++
RB23	2	2	0	0	1	0	0	2	++++
RB24	3	3	0	0	0	0	0	3	++++
RB27	1	1	0	0	0	0	2	1	++++
RB28	1	1	0	0	1	0	1	1	++++
RB29	3	3	0	3	0	1	2	3	++++
RB30	2	2	0	3	3	0	1	2	++++
RB33	0	1	0	3	0	0	1	0	++++

pathway for ethylene in plants/ACC deaminase activity would decrease ethylene production in the roots (Jacobson et al., 1994) of host plants and result in root lengthening. For most of the plants, ethylene is required to break seed dormancy but after germination, high level ethylene would inhibit root elongation (Arshad and Frankenberger, 2002). PGPB that contain the enzyme ACC deaminase when bound to seed coat of a developing seedling act as a mechanism that ensures that the ethylene level does not become elevated to the point where initial root growth is impaired. By facilitating the formation of longer roots, these bacteria may enhance the survival of some seedling, especially during the first few days after the seeds are planted (Arshad and Frankenberger, 2002).

Siderophore and Fe nutrition

Plants prefer to absorb iron as the more reduced ferrous ion but the ferric ion (Fe^{3+}) is more common in well aerated soil although, it is easily precipitated in iron oxide forms (Duffy, 1994). Under iron deficiency conditions, many microorganisms produce siderophores for Fe acquisition. Most researches on microbial siderophores are associated with their biocontrol activities due to their competitive effects with plant pathogens. Bacteria can prevent the proliferation of phytopathogens (Kloepper et al., 1980) through the production of siderophores that bind iron that is available in the rhizosphere and as a result, effectively preventing any fungal pathogens in the vicinity from proliferating because of lack of iron. Siderophore producing bacteria are isolated using Chrome Azurol Agar media, a qualitative detection technique.

HCN production

Rhizabacteria were further screened for their HCN producing abilities so as to detect the antagonistic activity of bacteria towards the soil fungal phytopathogens (Bashan, and Holguin, 1997). Qualitative HCN production was detected by Lorck method. Isolates were cultured in glycine supplemented media and incubated for 48 h at 28°C. The filter paper was dipped in picrate and sodium carbonate was fixed under the lid of the Petri plate. A change from yellow to orange, red and brown is recorded as weak, moderate and strong cyanogenic bacteria.

Seed germination test

Cajanus cajan seeds collected from Agriculture research stations 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 (25) seeds were on agar 2% plates and incubated for 3 days in the dark. The germination of seeds was recorded. Sixteen bacterial isolates were designated for testing plant growth promotion. Seeds without treatment with any isolate were designated as control. Germination efficiency in terms of seedling emergence is tabulated in durations of one and two weeks. The germination efficiency of the seeds on treatment with the 16 isolates showing PGPR traits were analysed and are shown in Table 5. The data were analyzed statistically by F- test. The significance of differences between mean values was evaluated by DMRT (Duncan' new multiple range test).

RESULTS

Sixty five (65) bacterial isolates were selected for further characterization of biochemical and morphological traits. Thirty six (36) out of sixty five (65) were screened for the Gram nature, motility, colony characteristics, pigmentations and morphological characteristics (Table 2).

Table 5. Effect of seed treatment with bacteria from rhizosphere and with PGPR with cultivar in pots having unsterilized soil.

Isolate	Seed germination (%)	Seedling height (cm)	Root length (cm)	Dry weight (mg/plant)
control	82.10	10.30	4.10	5.60
RB1	84.10	12.30	4.50	6.40
RB3	86.63	12.50	4.30	6.60
RB6	84.31	11.80	4.40	6.40
RB7	84.01	13.80	5.30	6.80
RB10	86.11	10.90	4.60	6.50
RB16	90.26	12.60	4.50	6.20
RB22	92.59	13.10	4.80	6.60
RB23	92.07	12.70	4.40	6.08
RB24	90.15	13.20	4.50	6.50
RB28	90.26	11.20	5.10	7.10
RB29	92.59	10.30	5.59	6.20
RB32	86.36	9.59	5.60	6.30
RB33	82.15	6.80	4.98	5.80

Biochemical characteristics like indole production, catalase test, sugar fermentations, acid and gas productions were tested and shown in Table 3.

PGPR traits

Out of the thirty six (36) bacterial isolates tested for biochemical traits, sixteen were considered for the PGPR trait analysis, as these reported to be showing diverse characteristics. Sixteen isolates were named as RB1, RB2, RB3 and so on. Of the sixteen isolates, seven showed excellent IAA producing abilities, six were reported to show maximum phosphate solubilization, five showed HCN production and eight different isolates for enzyme productions like chitinase, β-1.3-glucanase. RB3, RB8, RB10, RB12, RB15, RB24 and RB29 showed IAA production. RB1, RB3, RB6, RB24, RB29 and RB33 showed phosphate solubilization; RB24, RB29 and RB33 were observed to be moderate HCN producers. Thus, three isolates, RB24, RB29 and RB15 were found with the PGPR traits and antagonistic traits. These three were found to be of the genus *Bacillus*, *Pseudomonas* and *Aeromonas* which are characterized to be up to the genus level.

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 known, although, several hypothesis 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.

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. Out of the sixteen isolates, twelve were found to be positive for IAA production and seven were found to be high IAA producing. Among them, it has been found that IAA production by PGPR can vary among different species and strains and it also influenced by culture condition, growth stage and substrate availability. Moreover, isolates from rhizosphere are more efficient auxin producers than isolates from the bulk soil (Glick and Bashan, 1997).

Phosphorus is one of the major nutrients, second only to nitrogen which is required by plants. Most of the phosphorus in the soil is present in the form of insoluble phosphates and cannot be utilized by the plants (Nautiyal, 1999). 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. In our experiment, six bacteria were found to be efficient phosphate solubilizers, and their phosphate solubilizing efficiency has been calculated and tested in plant growth. Seed germination was increased when seeds were pretreated with PGPR isolates

Further investigations, including efficiency test under greenhouse and field conditions are needed to clarify the role of PGPR as biofertilizers that exerts beneficial effects

on plant growth and development.

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