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PGPR Isolates from the Rhizosphere of Vegetable Crop *Momordica charantia*: Characterization and Application as Biofertilizer

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ABSTRACT

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Plant growth promoting Rhizobacteria (PGPR) enhance the plant growth and productivity through wide variety of mechanism. The strains isolated from rhizosphere of *Momordica charantia*, mainly belonged to *Bacillus*, *Azotobacter*, *Pseudomonas* and *Acinetobacter*. Nearly 50% isolates of *Pseudomonas* and *Acinetobacter* and all the isolates of *Bacillus* and *Azotobacter* were able to solubilise tricalcium phosphate. All the isolates were positive for Catalase activity and IAA production except *Acinetobacter* with 75% isolates were IAA producing. *Pseudomonas* (100%), *Bacillus* (80%), *Acinetobacter* (75%) and *Azotobacter* (40%) produced ammonia and all the isolates were siderophore producing except *Azotobacter*. The *Bacillus* (75%) and *Pseudomonas* (50%) also produce HCN. Best performers in above plant growth promotion (PGP) traits, isolate of *Bacillus* (R7), *Azotobacter* (RS7), *Pseudomonas* (RS1) and *Acinetobacter* (R12) evaluated for their effect on plant vigour. The antibacterial activity and plant growth promotion in *Pseudomonas* (RS1), salt tolerance in *Bacillus* (R7), and nitrogenase activity (C_2H_2 -reduction) in *Azotobacter* (RS7) was highest. *Pseudomonas* (RS1) was most efficient PGPR and *Bacillus* (R7) most dominant in rhizosphere of bitter melon. Nitrogenase (C_2H_2 -reduction), phosphate solubilisation and growth promotion activities in *Bacillus* (R7), *Azotobacter* (RS7) and *Pseudomonas* (RS1) suggested that these isolates may be used as a biofertilizer for bitter melon.

Introduction

Plant Growth Promoting Rhizobacteria (PGPR) are a group of naturally occurring soil bacteria that aggressively colonize plant roots and increase plant growth and yield (Wu *et al.*, 2005). They prevent the colonization of deleterious rhizospheric microorganisms (DRMOs) at micro-niches and can promote plant growth directly or indirectly. It is interesting to study the diverse bacterial taxa for their PGPR characteristics (Lucy *et al.*, 2004). The numbers of PGPR species has increased due to studies on a wide range of plant species, advancement in bacterial

taxonomy and progress in understanding the different mechanisms of action of PGPR. The increased number of PGPR for various crops and vegetables has been commercialize as a biofertilizer, recently includes *Pseudomonas* (Loper *et al.*, 2007), *Azospirillum* (Cassán and García Salamone, 2008), *Bacillus* (Jacobson *et al.*, 2004), *Stenotrophomonas* (Ryan *et al.*, 2009), *Rhizobium* (Long, 2001), *Serratia* (De Vleeschauwer and Höfte, 2007), *Azotobacter*, *Klebsiella*, *Enterobacter*, *Alcaligenes*, *Arthrobacter*, *Burkholderia* (Joseph *et al.*, 2007) and *Streptomyces* (Schrey and Tarkka.,

2008). *Pseudomonas* and *Bacillus* are the most commonly investigated PGPR and often dominate the rhizosphere (Morgan *et al.*, 2005).

The mode of action of PGPR involves complex mechanisms to promote plant growth, development and protection. As bioprotectant, they suppress the plant diseases through induction of systemic resistance and production of antibiotics and siderophore. PGPR promotes plant growth by improving nutrient acquisition, as a biofertilizer and by producing phytohormones such as indole acetic acid, gibberellic acid, cytokinin and ethylene (Glick, 1995) as a biostimulant. Some PGPR may promote plant growth indirectly by stimulating symbiotic N₂-fixation, nodulation and nodule occupancy (Joseph *et al.*, 2007) and by asymbiotic N₂-fixation (Boddey and Dobereiner, 1995). In addition to above traits, the plant growth promoting strain must be rhizospheric competent, able to survive and colonize in the rhizospheric soil (Cattelan *et al.*, 1999). Unfortunately, the interaction between associative PGPR and plants can be unstable and the good results obtained *in vitro* (Zhender *et al.*, 1999). The variability in the performance of PGPR may be due to various environmental factors that affect the plants growth, includes climate, weather conditions, soil characteristics or the composition and activity of the indigenous microflora of the soil (Joseph *et al.*, 2007). Inoculation of ornamentals, horticultural and forest trees nurseries, vegetable and agricultural crop seedlings, seeds or soil with PGPR may result in multiple effect on plant growth, such as enhancement of seed germination, seedling growth, seedling health, plant vigor, plant height, shoot weight, nutrient content of shoot tissues, early bloom, chlorophyll content and increased nodulation in legumes (Saharan, 2011).

Isolating native strains adapted to the local environment has always been successful to the formulation of PGPR inoculants to use in vegetables crops. Our interest is to isolate and screen some well known PGPR strains of *Bacillus*, *Pseudomonas*, *Azotobacter* and *Acinetobacter* from rhizosphere of nutritionally and pharmaceutically important vegetable crop, *Momordica charantia* (bitter melon) and also to evaluate plant growth promotion potentials of the above PGPR on bitter melon plants.

Materials and Methods

Site

The study conducted in earthen pot filled with amended natural soil kept in Botanical garden, Banaras Hindu University (20° 18' N and 83°36' E, elevation 80.71 m).

Plants and soil sampling

Momordica charantia Linn. (Bitter melon), seeds were sown in natural soil added with manure and sand (2:1:1) in earthen pots and seedlings appears within 10- 15 days after sowing (DAS). At flowering stage (45 DAS), rhizospheric soil was collected by gentle shaking the entire root system to remove loosely adhering soil. Physicochemical analysis of soil collected from bitter melon pots at the flowering stage was performed and brief description of methods was as given below:

Total soil organic carbon, Total N, Available N, P and K determined by method described by (Jackson, 1967).

Soil microbial biomass C and N were estimated by direct chloroform fumigation and extraction method as described by (Vance *et al.*, 1987).

Soil pH was measured in the soil solution (1:2; soil: water, w/v) using the combined glass electrode pH meter. 2.2.4 Soil Moisture Content was determined based on fresh weight and soil dried to constant weight at 80°C

Isolation of Plant Growth Promoting Bacteria

Rhizospheric soil (fresh weight) was suspended in 50 ml buffer solution (phosphate buffer 10mM, pH- 7.0), shaken vigorously 1h on a gravatory shaker and serially diluted up to 10^{-5} . Bacterial strains isolated by plating the soil dilution on agar plate; *Bacillus* and *Acinetobacter* were isolated on LB nutrient agar plates (Carlton and Brown, 1981), *Azotobacter* on Jensen's agar plates (Norris and Chapman, 1968) and *Pseudomonas* on King's B agar plates (King, 1954). The plates were incubated at 30°C for 2- 7 days. The bacterial colonies were chosen based on colony morphology and visual growth rates. Clones picked up and purified by streaking on the respective plates. Colonies were restreaked on fresh nutrient agar plates for isolation and maintenance of isolates.

Pure isolates of PGPR from rhizospheric soil was characterized by using the criteria described in Bergey's Manual of Systematic Bacteriology (Bergey's Manual of Determinative Bacteriology 9th, 1994) and Bergey's Manual of Determinative Bacteriology (Bergey's Manual of Determinative Bacteriology, 1984). The PGPR strain was identified on the basis of colony behaviour, morphological and biochemical characteristics.

Morphological Characterization of PGPR

Colony morphology of each isolate was examined on LB agar plates. After 3 day of incubation, different characteristics of

colonies such as shape, size, elevation, surface, margin, colour, optical characteristics, pigmentation, recorded.

A phenotypic characteristic of bacterial cell of each isolates were examined by preparing a smear of diluted a loopful of bacterial culture on slide and fixed by heating on a flame. The slide was flooded with crystal violet solution for 3min, washed gently in flow of tap water and air-dried. The slide was observed under microscope and recorded the shape.

The smear prepared as above flooded with crystal violet solution (1min), washed gently in flow of tap water and then flooded with iodine solution, for 1min. Iodine complex was drained out followed by washing with 95% ethanol. The smear incubated with safranin solution for 1 min. Then, the slide observed under microscope and Gram-reaction recorded.

Motility of bacteria was determined by hanging drop method. A drop of loopful 2-day-old bacteria in 1 ml of nigrosin solution took on a cover slip that hanged on a hollow slide with Vaseline. The slide was then observed under microscope to test motility of bacteria.

Biochemical characterization of PGPR

Isolates of *Bacillus* (15), *Pseudomonas* (2), *Azotobacter* (5) and *Acinetobacter* (4) were biochemically characterized by Oxidase test, H₂S production, Citrate test, Urease test, Carbohydrates production, Starch hydrolysis and Nitrate reduction by the following method.

Oxidase Test- The loop full culture from single colony was just touched on the Standard Oxidase disc and immediate development of blue colour considered as positive.

Hydrogen Sulphide Test- SIM medium with lead acetate as indicator used for testing the ability of strains to produce H₂S.

Citrate Test- Simmon's Citrate medium green in colour, if the inoculated bacteria utilize citrate the pH increases gradually and medium becomes blue in alkaline pH.

Urease Test- Few drop of indicator phenol red added to the medium, a colour change from yellow to bright pinkish-red is positive; lack of colour change is a negative result for urease.

Nitrate Reduction- Addition of three drops of each solution A (Sulphanilic acid) and solution B (dimethyl-alpha-naphthylamine) to two days old culture of an isolate in nitrate broth and appearance of pink colour considered as positive to nitrate reduction ability.

Carbohydrates fermentation Test- An isolate grown in Hugh- Leifson's test medium containing bromothymol blue at 37°C for 24 hours to 4 days. Development of yellow colour due to change in colour of indicator, shows acid production from carbohydrate fermentation.

Starch hydrolysis Test- Few drops of Gram's iodine solution were added to the two days old culture of an isolate grown on starch agar plate. The dark blue colour developed due to formation of starch iodine complex. Clear area around streaked culture indicates the degradation of starch due to production of amylase.

Characterization of Plant growth promoting bacteria for PGP traits

Phosphate solubilisation: A loopful (20 µl) of each bacterial isolate was placed on the centre of modified Pikovskaya agar plate

containing insoluble tricalcium phosphate (TCP) and incubated at 30 ± 0.1°C for 5 days. The formation of clear zone indicated the phosphate solubilising potential of bacteria (Gupta *et al.*, 1994).

Quantitative estimation of phosphate: The Phosphate solubilisation activity of the selected PGPR isolates was estimated as per methodology described by authors [Nautiyal and Mehta, 2001; Jackson, 1973]. Bacterial isolates grown in National Botanical Research Institute's Phosphate (NBRIP) broth containing 0.5% tricalcium phosphate. The flask containing 100 ml medium inoculated with 1 ml 48 h old bacterial culture in triplicate and incubated at 28°C for 7 days on a shaker at 180 rpm. Simultaneously, under similar conditions the uninoculated control also kept. The test cultures centrifuged at 10,000 rpm for 10 min. To a 0.5 ml aliquot of the supernatant, 2.5 ml Barton's reagent added and made the volume to 50 ml with de-ionized water. The absorbance of the resultant colour read after 10 min at 430 nm in UV/Visible Spectrophotometer. The total soluble phosphorus calculated from the regression equation of standard curve prepared from K₂HPO₄. The values of soluble phosphate liberated expressed as µg ml⁻¹ over control.

Ammonia production: Bacterial isolates were tested for the production of ammonia in peptone water. Freshly grown cultures were inoculated in 10 ml peptone water and incubated for 48 to 78 h at 36 ± 2°C. Nessler's reagent (0.5 ml) was added in each tube. Development of brown to yellow colour was a positive test for ammonia production (Cappuccino and Sherman, 1992)

Detection of indole acetic acid production: IAA production was detected by the modified method described by Brick *et al.*, 1991. Quantitative analysis of IAA was performed

using the method (Loper and Scroth, 1986) at 50 µg/ml concentrations of tryptophan. Bacterial cultures were grown for 48 h in LB Broth medium at 28±2 °C. Bacterial cultures were centrifuged at 3000 rpm for 30 min. The supernatant (2 ml) was mixed with two drops of orthophosphoric acid and 4 ml of the Salkowski reagent (50 ml, 35% of perchloric acid, 1 ml 0.5 M FeCl₃ solution). Development of pink colour indicates IAA production. Absorbance was read at 535 nm with the help of UV/Visible spectrophotometer. Concentration of IAA produced by cultures was measured with the help of standard graph of IAA (Hi-media) obtained in the range of 10–100 µg/ml.

Hydrogen cyanide production: Screening of bacterial isolates for hydrogen cyanide (HCN) production was done by adapted method (Castric, 1975). Bacterial cultures were streaked on nutrient agar medium containing 4.4 g per litre of glycine. A Whatman filter paper no.1 soaked in 0.5% picric acid solution (in 0.2% sodium carbonate) was placed inside the lid of a plate. Plates were sealed with paraffin and incubated at 30 ± 0.1°C for 4 days. Development of light brown to dark brown colour indicated HCN production.

Catalase activity: Immediate gas bubbles formation was considered Catalase positive when single colony was mixed 2-3 drops of 3% H₂O₂ on clean grease free glass slide.

Siderophore production: Bacterial strains grown on blue agar plates containing the dye chromazurol S (CAS), the formation of orange halos around the colonies were indicative for siderophore production (Schwyn and Neilands, 1987)

N₂ -fixation (C₂H₂ -reduction) assay: The Nitrogenase activity (C₂H₂- reduction assay) (Schollhorn and Burris, 1967) was performed in 10 ml vacuotainer tubes containing 2.5 ml of the medium. Isolates grown in Burk's N-

free medium (48 h at 30° C) were transferred (1 ml) to each vial sealed with airtight rubber stopper and the headspace was injected with 10% (v/v) acetylene after removing equal amount of air. Gas samples (50 µl) removed after 1h and assayed for ethylene production in a gas chromatograph equipped with Poropak-R column and a flame ionization detector (Varian, USA). Ethylene produced was determined using standard ethylene (Scott Specialty Gases, Deerfield IL 60015). Values expressed as nmol m⁻¹ h⁻¹.

Antimicrobial activity: The antimicrobial activity of selected isolates of PGPR strains was done by agar well diffusion method (Mehmood *et al.*, 1999). The PGPR isolates tested for their antibacterial activity were grown in LB broth medium. Test bacteria such as human pathogenic strains of *Escherichia coli* and *Pseudomonas aeruginosa* were grown in LB broth medium. LB broth was taken as negative control and 30µg/ml Chlorempenicol was used as positive control. After 5-6 days of incubation at 30°C, the antibacterial activity was evaluated by measuring inhibition zone against test organisms.

Salt stress

The selected bacterial strains were tested for their tolerance to salt stress by inoculating them in LB broth medium containing various concentration of NaCl (1% to12%) and 0.1 ml of culture (1.0 x 10⁷ cell ml⁻¹) was inoculated in 10 ml LB broth. The culture tubes incubated at 30°C for 48 hours and analyzed for influence of salt by taking OD at 600 nm by UV/Visible spectrophotometer just after inoculation and after incubation for 24 h.

Experimental Design

The effect of PGPR isolates *Bacillus* (R7), *Azotobacter* (RS7), *Pseudomonas* (RS10) and

Acinetobacter (R12) on bitter melon was determined by conducting a pot experiment. The experiment was designed in five sets with three earthen pots in each set and soil from botanical garden added with manure and sand in proportion 2:1:1 ratio. Set 1 served as control without treatment of bacterium and in Set. 2, 3, 4 and 5, (one set for each PGPR strain) sterile soil seeded with three bitter melon seeds. Seeds were inoculated with *Bacillus* (R7), *Azotobacter* (RS7), *Pseudomonas* (RS10) or *Acinetobacter* (R12) culture (10^9 cell ml^{-1}) and pelleted with CaCO_3 powder.

All five sets of pots kept under natural environment in botanical garden and watered on each alternate day. Different traits of plant were studied such as seed germination, seedling length, plant height, number of fruits per plant, fruits size and number of seeds per fruit.

Results and Discussion

The physicochemical characteristics of the rhizospheric soil such as soil organic carbon (0.85%), total nitrogen (0.085%), moisture content (17.10%), soil pH (7.1) and other parameters were at a level, which could be favorable for the growth of PGPR (data not presented).

The *Momordica charantia* rhizosphere was associated with a diversity of bacterial population. Among the selected bacterial strains population (CFU per gram dry soil) *Bacillus* spp (4.7×10^8) was dominant in the rhizosphere followed by, *Acinetobacter* (0.24×10^8), *Pseudomonas* spp (0.1×10^8), and *Azotobacter* spp (0.51×10^7) (Fig- 1). On the basis of cultural, morphological and biochemical characteristics out of total 42 soil isolates, 26 isolates grouped as *Bacillus*, *Azotobacter*, *Pseudomonas* and *Acinetobacter*. The cultural (3-4 days colony),

morphological and biochemical characteristics of all the test isolates are given in Table 1.

All the 26 isolates of four bacterial genera were characterized for their PGP traits *in vitro* are depicted in Table 2. Catalase activity was detected by all the bacterial strains that may be potentially very advantageous. Only *Bacillus* and *Pseudomonas* exhibited HCN production. The IAA production, ammonia production and phosphate solubilisation activity was present in all the isolates of *Bacillus*, *Azotobacter*, *Pseudomonas* and *Acinetobacter*. Except *Azotobacter*, all the bacterial isolates produced siderophore.

We had selected four isolates, one each of *Bacillus*, *Azotobacter*, *Pseudomonas* and *Acinetobacter* based on high PGP activity and coded as R7, RS7, RS1 and R12, respectively. These isolates formed high phosphate solubilisation zone (16- 24 mm) and liberated high quantity of soluble phosphate ($210-418 \mu\text{g ml}^{-1}$). All the selected isolates such as *Bacillus* (R7), *Azotobacter* (RS7), *Pseudomonas* (RS1) and *Acinetobacter* (R12) produced IAA (ranges from 0.2 - $3.1 \mu\text{g ml}^{-1}$) at $50 \mu\text{g}$ Tryptophan concentration. Except *Acinetobacter* (R12), *Bacillus* (R7), *Azotobacter* (RS7) and *Pseudomonas* (RS1) isolates exhibited high nitrogenase (C_2H_2 -reduction) activity which was maximum in *Azotobacter* (RS7) ($72 \text{ nmol. C}_2\text{H}_4 \text{ ml}^{-1} \text{ h}^{-1}$). All the three later strains also exhibited antibacterial activity against human pathogenic strains of *E. Coli* with inhibition zone (12 to 23 mm) whereas only *Bacillus* (R7) and *Pseudomonas* (RS1) exhibited antibacterial activity against *P. aeruginosa* with inhibition zone of 25 and 11 mm. *Pseudomonas* (RS1) has maximum P-solubilisation ($418 \mu\text{g ml}^{-1}$), IAA production ($3.1 \mu\text{g ml}^{-1}$) and antibacterial activities (23 mm in *E. coli* and 25 mm in *P. aeruginosa*) (Table 3). These strains further selected for

the salinity tolerance and traits of growth promotion on bitter melon.

Among all the four selected strains of plant growth promoting Rhizobacteria, *Bacillus* (R7) tolerated high concentration of salt (NaCl) and could survive at 12% salt conc. whereas complete death of *Azotobacter* (RS7), *Pseudomonas* (RS1) and *Acinetobacter* (R12) occurred at 11%, 8% and 10% respectively (Fig. 2).

The effect of *Bacillus* (R7), *Azotobacter* (RS7), *Pseudomonas* (RS1) and *Acinetobacter* (R12) on bitter melon plant has been presented in Table 4. *Azotobacter* (RS7) and *Pseudomonas* (RS1) supported 100% seed germination. *Pseudomonas* (RS1) treated seeds attained maximum seedling length (12.67 ± 0.9), plant height (158.83 ± 5.8), fruit number per plant (18 ± 1.7), fruit size (12 ± 0.6), and fruit has maximum number of seeds (16) followed by *Bacillus* (R7), *Azotobacter* (RS7) and least in *Acinetobacter* (R12).

PGPR are root colonising bacteria which exert beneficial effects on plant growth by production of phytohormones, HCN, ammonia and antibiotics, suppression of deleterious organisms, activation of phosphate solubilisation, promotion of mineral uptake and aggressive colonization of micro niches (Maier *et al.*, 2009). There are many papers related to the advantages and screening of PGP traits of PGPR from vegetable plants particularly tomato, potato, chilli, lettuce, cucumber but the studies performing isolation and characterization of selected genera of PGPR from bitter melon rhizosphere and its effect on plant growth and yield are extremely limited.

The bacterial rhizosphere community structure was soil-specific; bitter melon rhizosphere has all the physico-chemical

property, which was strongly affected plant growth promoting bacterial population. Rhizospheric soils have high soil organic carbon, total nitrogen and available nitrogen, essential for both PGPR population and plant growth.

The dominance of *Bacillus* as a PGPR in the rhizospheric soil of bitter melon further confirmed that *Bacillus* sp is a successful PGPR and its beneficial role has already been established (Probanza *et al.*, 2002). Phosphate solubilisation was most frequently detected in *Bacillus* and *Azotobacter* and to a lesser extent in *Pseudomonas* and *Acinetobacter* as evident by formation of phosphate solubilisation zone more than 11 cm. The results of bitter melon supported the view of Reyes and Valduz (2006) who reported the dominance of phosphate solubilising bacteria in the rhizosphere as compared to non-rhizospheric soil.

IAA production is an important trait of PGPR because it is most important phytohormones and function as signal molecule in the regulation of plant development. The production of IAA by PGPR can vary among different species and strains, as observed during the present study, also influenced by culture condition, growth stage and substrate availability (Mirza *et al.*, 2001). Other research workers (Xie *et al.*, 2006) also recorded IAA production in *Azotobacter* isolates are in agreement with other authors (Gonzalez- Lopez *et al.*, 1986) and high level of IAA production by *Pseudomonas*. Production of IAA and soluble phosphate are the most common mechanisms of actions implicated in PGPR and indeed microbes demonstrating the same are wide spread in rhizosphere (Vessey, 2003). Besides Phosphate solubilisation and IAA production, another important traits of PGPR that may indirectly influence the plant growth, is production of ammonia that is strain specific

characteristics among the PGPR isolates. Siderophore production was common in PGPR isolates but not a rule, as *Azotobacter* isolates did not produce siderophore. PGPR strains producing siderophore competitively ensures its unavailability to the phytopathogens and thus contribute to disease suppression. Siderophore may directly stimulate the biosynthesis of other anti microbial compound by increasing the availability of minerals to bacteria. HCN production also plays an important role in the biological control of pathogens and act as PGP traits. Production of HCN by isolates of *Bacillus* and *Pseudomonas* are positive further confirmed that HCN production is a strain specific PGP traits and acts as an inducer of plant resistance (Joseph *et al.*, 2007). All the bacterial isolates produce

Catalase and it has been postulated that strains showing Catalase activity must be highly resistant to environmental, mechanical and chemical stress (Joseph *et al.*, 2007; Kumar *et al.*, 2012). Nitrogen fixation is one of the major mechanisms utilized by PGPR for the plant growth promotion. The nitrogenase (C₂H₂- reduction) activity assay performed in all the four selected PGPR isolates of bitter melon detected the nitrogenase activity in *Bacillus* (R7), *Azotobacter* (RS7) and *Pseudomonas* (RS1) strains while *Acinetobacter* (R12) did not show the nitrogenase activity. A number of studies suggest that PGPR enhances the plant growth and contribute to the protection of plants against pathogens and pests (Dey *et al.*, 2004).

Table.1 Culture (3-4 days colony), morphological and biochemical characteristics of PGPR isolates

Characteristics	<i>Bacillus</i>	<i>Azotobacter</i>	<i>Pseudomonas</i>	<i>Acinetobacter</i>
Culture Colony				
Shape	Round	Round	Round	Round
Size (mm)	1.5- 2.0	1.0- 5.0	2.0- 3.0	Pin headed
Elevation	Raised	Raised	Raised	Raised
Surface	Rough	Slimy	Smooth	Smooth
Margin	Serrated	Smooth	Smooth	Smooth
Optical characteristics	Opaque	Opaque	Translucent	Opaque
Colour	White	Creamy beige white	Light green	Light cream
Morphology				
Gram's reaction	G +ve	G -ve	G -ve	G -ve
Shape	Rod	Rod	rod	Rod
Pigment	-	+	+/-	-
Motility	+	+	+	-
Biochemical				
Oxidase	+	+	+	-
Citrate	+	+	+	+
Indole	-	+	-	-
H ₂ S Production	-	-	+	-
Urease	-	+	+	-
Carbohydrate fermentation	+	+	-	-
Starch hydrolysis	+	+	+	+
Nitrate Reduction	+	+	+	-

Table.2 Plant growth promoting characteristics of bacterial isolates

Bacterial isolates	No. of isolates	P Solubilization	IAA production	Ammonia production	Siderophore production	HCN production	Catalase activity
<i>Bacillus</i> spp	15	+ (100)	+ (100)	+ (80)	+ (93.3)	+ (73.3)	+ (100)
<i>Azotobacter</i> spp	5	+ (100)	+ (100)	+ (40)	ND	ND	+ (100)
<i>Pseudomonas</i> spp	2	+ (50)	+ (100)	+ (100)	+ (50)	+ (50)	+ (100)
<i>Acinetobacter</i> spp	4	+ (50)	+ (75)	+ (75)	+ (50)	ND	+ (100)

Number in parenthesis shows percentage of isolates; += Positive; ND= Not detected

Table.3 Different Plant Growth Promoting activities of some selected bacterial isolates

Isolates name with code	Phosphate solubilisation		IAA Production ($\mu\text{g ml}^{-1}$) \pm SE at 50 μg Tryptophan conc.	N_2 - fixation (C_2H_2 -reduction; n mol $\text{C}_2\text{H}_4 \text{ ml}^{-1} \text{ h}^{-1}$)	Antibacterial Activity	
	Zone of clearance (diam. in mm) \pm SE	Concentration ($\mu\text{g ml}^{-1}$) \pm SE			Zone of Inhibition (mm \pm SE)	<i>Escherichia coli</i>
<i>Bacillus</i> sp. (R7)	24 \pm 1.7	372 \pm 1.2	0.21 \pm 0.03	47 \pm 2.3	21 \pm 1.0	11 \pm 0.4
<i>Azotobacter</i> sp. (RS7)	18 \pm 1.7	246 \pm 3.5	1.6 \pm 0.01	72 \pm 0.0	12 \pm 0.4	ND
<i>Pseudomonas</i> sp. (RS1)	29 \pm 2.5	418 \pm 2.9	3.1 \pm 0.3	42 \pm 1.2	23 \pm 0.9	25 \pm 0.74
<i>Acinetobacter</i> sp. (R12)	16 \pm 1.7	210 \pm 1.5	1.0 \pm 0.2	ND	ND	ND

ml⁻¹= Absorbance, 0.4 at 710 nm; ND= Not detected; *= 20 μ l inoculated on the plate

Fig.1 Population of bacterial species in rhizosphere soil by bitter melon

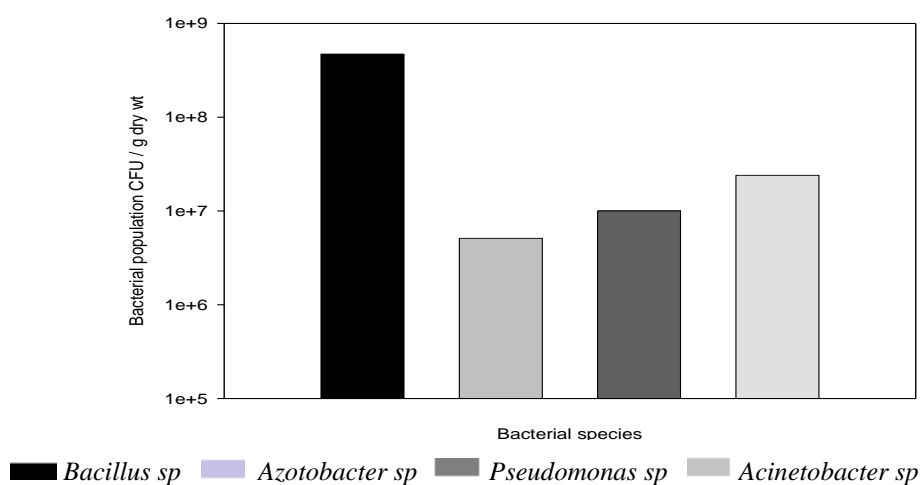


Fig.2 Effect of salinity (NaCl) on the growth of selected PGPR strains

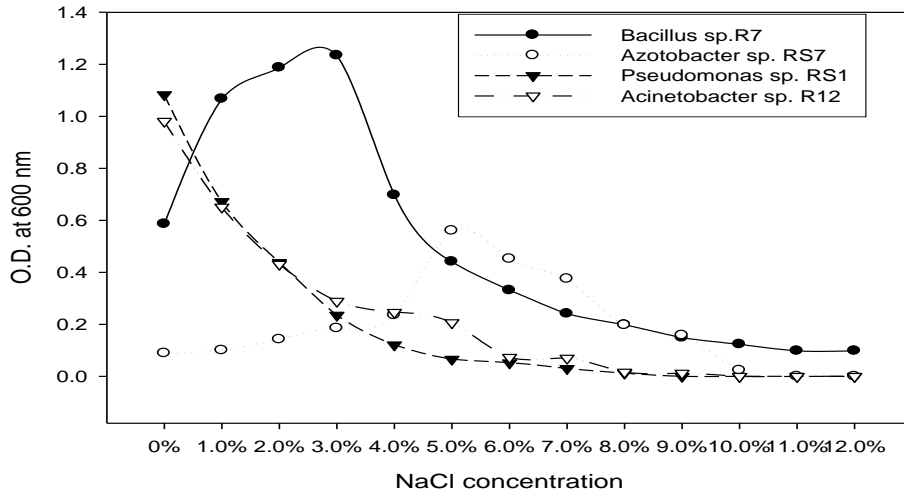


Fig.3 Cluster analysis (Bray Curtis) of selected. Data for analysis based on growth promotions activities of strains as compared to control (Table- 4)

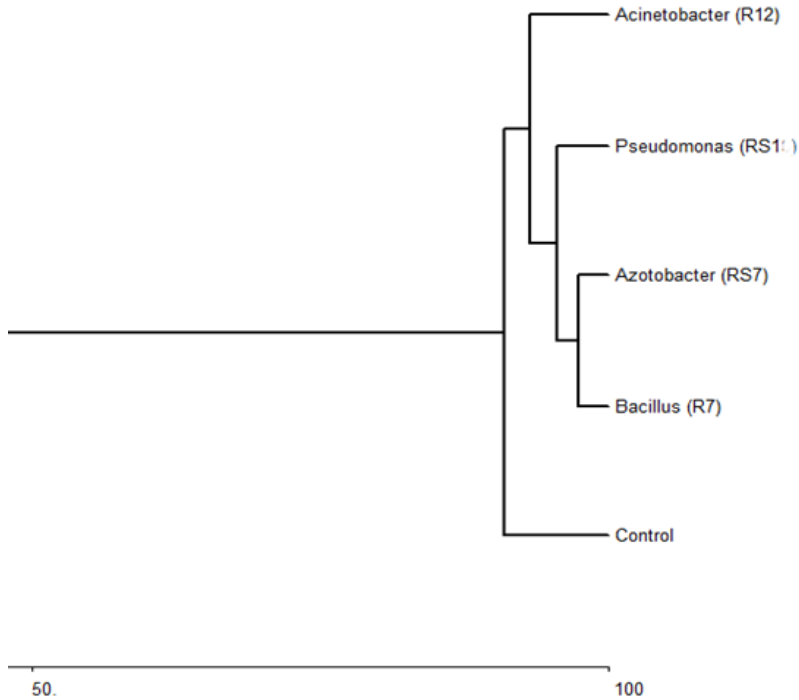


Table.4 Effect of PGPR strains on growth activity of Bitter melon

Plant with Bacteria inoculation	Percent of seed germination \pm SE	Seedling length at 15DAS (cm) \pm SE	Plant length at 60 DAS (cm) \pm SE	Fruit Number per plant 90 DAS \pm SE	Fruit size (cm) \pm SE	Seed Number per fruit \pm SE
Control	84.9 \pm 4.97	8.83 \pm 1.01	109.93 \pm 8.33	14 \pm 1.15	9.83 \pm 0.44	8 \pm 1.15
<i>Bacillus</i> sp. (R7)	89.9 \pm 0.00	8.84 \pm 1.09	143.4 \pm 10.36	16.33 \pm 0.88	10.00 \pm 0.29	10 \pm 0.58
<i>Azotobacter</i> sp. (RS7)	100 \pm 0.00	9.50 \pm 1.04 SE	142.20 \pm 2.35	15 \pm 1.20	10.33 \pm 0.33	11 \pm 0.00
<i>Pseudomonas</i> sp. (RS1)	100 \pm 0.00	12.67 \pm 0.88	158.83 \pm 5.8	18 \pm 1.73	12 \pm 0.58	16 \pm 0.58
<i>Acinetobacter</i> sp. (R12)	75 \pm 8.33	9.33 \pm 1.86	135.10 \pm 6.89	15.87 \pm 0.58	9.67 \pm 0.33	8 \pm 1.15

SE- Standard error of mean, DAS- Days after sowing

Antibiotic production is an important biocontrol trait of PGPR. *Pseudomonas* showed maximum antimicrobial activity in human pathogenic strain with *Escherichia coli*, followed by *Pseudomonas aeruginosa* and in decreasing order by *Bacillus*, *Azotobacter* and least by *Acinetobacter*. It is earlier reported trait for rhizobacteria with potential for biological control in pseudomonad (Kremmer and Kennedy, 1996). Although we have not characterized the exact chemical nature of active ingredient (s) but it is noteworthy that all the four bacteria possess antimicrobial activity similar to antibiotics.

PGPR strains are able to tolerate and survive the stress condition and alleviate the salinity stress when inoculated in salt stressed plant (Saharan and Nehra, 2011). The lower salt stress enhanced the *Bacillus* (R7) growth almost double and the strain tolerated maximum salt stress compared to other strains. The tolerance of salt stress in the various PGPR was strain specific property of bacteria. Our findings suggest that salt tolerance is associated with PGPR strains whether it can be taken as PGP trait for PGPR needs extensive investigations. Inoculation of bitter melon seed with selected PGPR isolates such as *Bacillus* (R7), *Azotobacter*(RS7),

Pseudomonas (SR10) and *Acinetobacter*(R12) enhanced plant growth by enhancing the percent of seed germination, seedling length, plant length, fruit per plant, fruit size and the seed number per fruit in comparison to control. *Pseudomonas* isolates had maximum PGP potential followed by *Azotobacter*, *Bacillus* and *Acinetobacter*. There were a huge number of evidences in support of the study that PGPR strains enhance the growth, seed emergence, crop yield (Kloepper *et al.*, 2004; Herman *et al.*, 2008 and Minorsky, 2008). In addition to high percent of seed germination, seedling and plant growth was also high which was contributed by Phosphate solubilisation and IAA production by the isolates. These results suggested that the increase growth of bitter melon plant by PGPR isolates such as *Bacillus* (R7), *Azotobacter* (RS7), *Pseudomonas* (Rs10) and *Acinetobacter* (R12) is probably due to multiple mechanisms and the best performance of *Pseudomonas* (Rs1) on growth and plant vigour of bitter melon is due to high activity of PGP traits as compared to other PGPR isolates. Cluster analysis based PGP traits of PGPR strains revealed that *Bacillus* (R7) and *Azotobacter* (RS7) is similar as they formed one cluster and *Pseudomonas* (Rs10) and *Acinetobacter* (R12) formed separate clusters as later have

maximum and minimum activities of PGP and their effect on bitter melon.

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Abbreviations- PGPR- Plant growth promoting Rhizobacteria; PGP- Plant Growth promotion; DRMOs- Deleterious rhizospheric microorganisms; NBRIP- National Botanical Research Institute's Phosphate; IAA- Indole Acetic acid.

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