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Screening of PGPR from the Rhizosphere of Groundnut (*Arachis hypogea*): Characterization and Application

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A B S T R A C T

Rhizosphere of a groundnut plant (*Arachis hypogea*) from agricultural fields of Chittoor district of Andhra Pradesh (India) was explored for isolation of PGPR. A total of 6 isolates from the rhizosphere belonging to different species were isolated. All the isolates showed phosphate solubilization, out of these an isolate coded as Psm6 showed most prominent halo zone was tested in vivo for growth promotion of Groundnut (Arachis hypogaea) under field conditions. Root and Shoot length, Dry and Wet weights were found to be excellent in Psm6 treatment compare to control. Psm6 treated plants showed increase in fresh biomass, dry biomass, root length and shoot length by 19%, 11%, 10% and 12% respectively. Physicochemical properties of soil revealed that soil was slightly alkaline; Nitrogen and phosphorous contents were low in the soil. After treatment with PGPR they showed slight increase and excellent growth of Groundnut under field condition.

Introduction

The rhizosphere is the soil found around the root it is a site with complex interactions between the root and associated microorganisms. Rhizobacteria are the group of bacteria that colonize the rhizosphere naturally. These are soil bacteria that stimulate plant growth after inoculation of seeds or roots and beneficial interactions can be enhanced if these are effectively managed. The beneficial bacteria termed as plant growth promoting rhizobacteria (PGPR) stimulate growth of plants. The PGPR have been studied in various crop plants (Burr and Caesar 1983). Plant growth promoting rhizobacteria (PGPR) are functionally diverse group of bacteria having immense potential as biofertilizers and biopesticides. Depending upon their function, they may serve as partial replacements for chemical fertilizer or pesticides as an eco-friendly and cost-effective alternative as compared to their synthetic counterparts. Hence isolation, characterization and practical evaluation of PGPRs having multifaceted beneficial characteristics, are essentially required (Pradhan *et al.*, 2017). Therefore, the objectives of this study were to isolate native

bacterial strains from the groundnut (Arachis hypogaea) rhizosphere under in vitro conditions and to characterize these isolates for phosphate solubilization, to assess the PGPAs of these isolates in vivo and their eff ect on the nutrient contents (N and P) of groundnut plants at growth stage.

Materials and Methods

Sample collection

Soil samples were collected from rhizosphere of groundnut plants grown in fields of Chittoor district of Andhra Pradesh were uprooted carefully, shoot portion cut off and roots along with the rhizosphere soil aseptically in small plastic bags / bottles were brought to the laboratory and prior to their processing kept at 4°C.

Screening and qualitative analysis of isolates for plant growth promoting activity

10g of soil samples was suspended in 90ml of sterilized distilled water and 10^{-1}dilution was obtained. Serial dilutions were prepared by mixing 1ml of the suspension made into 9ml sterilized water blanks until the 10^{-7} dilution was obtained.

From these dilutions 100µl was spread plated on Pikovskaya's Agar plates (Pikovskayas, 1948). These plates were then incubated at 30^{o}C and were observed for 2-7 days. The total bacterial types were counted after 48 hours of incubation.

The Phosphate solubilizing bacteria (PSB) showing halo zones of clearance were streaked again on PVK agar plate to check for purity and Phosphate solubilizing ability. The pure strains forming zone of clearance were maintained by streaking on nutrient agar slants and stored at 4^{o}C.

Quantitative estimation of Phosphate solubilization in liquid medium

The phosphate in solution was determined by using Calorimetric Chlorostannous reduced molybdo phosphoric acid blue method (Jackson, 1973). The PSB were grown in 50 ml NB for 24 hours at 30^{o}C in incubator shaker. 1ml of each PSB was aseptically transferred to 50 ml of PVK broth contained in 150 ml conical flask. The flasks were incubated at 30^{o}C for 3-7 days in incubator shaker at 120 rpm. After 3 days of incubation, 5 ml culture was withdrawn from each flask and cultures were centrifuged at 10,000 rpm for 30min. The supernatant was diluted to 100 ml with autoclaved distilled water. Then 5ml aliquot of each dilution was transferred to 50ml volumetric flask. This was followed by addition of 10ml chloromolybdic acid, which was added along the sides of the flask. The contents of the flasks were diluted to 40 ml with distilled water. Then 5 drops of chlorostannous acid was added. After mixing, the volume was made up to 50ml with distilled water. The blue colour intensity of the solution was measured in a spectrophotometer at O.D. 660nm. The soluble Phosphate was estimated from standard curve of KH_2PO_4 (0-2 ppm) drawn against O.D. 600 nm.

Field study

The potential strain Psm6 showing good PGPR activity was tried with Groundnut for determination of effect on plant growth and crop productivity under field and natural environmental conditions. The isolate was grown in LB medium with agitation (125 rpm) for 48 h at 28°C to a final concentration of 108 $CFUml^{-1}$.

Groundnut seeds were then inoculated with bacterial suspension for 30 min at room temperature. Control seeds were treated in the same manner with uninoculated LB medium.

The parameters evaluated were dry weight, wet weight, root length and shoot length (Rocheli de Souza *et al.*, 2012).

Physiochemical analysis of soil samples

To validate the potency of the bacterial PGPR activity field experiment was conducted where various analysis were done to evaluate strength of the soil before and after field experiment. Analysis of physico-chemical properties such as pH, Electrical conductivity (EC), available phosphorus, organic carbon and organic matter (OC/OM), available nitrogen of Groundnut field soil was analysed by standard method (DIRD, Pune, 2009).

Results and Discussion

Plant growth promoting activity of the bacterial isolates

In search of efficient plant growth promoting activity, a total of 6 bacterial strains were isolated and checked their activity on phosphate solubilization. All the strains were positive to phosphate solubilization and showed halo zones on PVK agar plates (fig.1).

Screening of bacterial isolates for phosphate solubilization revealed variations among different groups of organisms. Fig.2 shows the selection of efficient Phosphate Solubilizing Bacterial isolates on qualitative basis. Similar criteria of selection of efficient PSB were followed by Oswal and Bhide, (1972).

Phosphate Solubilization in liquid medium

After confirming the Phosphate Solubilizing Activity on solid medium, the phosphorus solubilization was confirmed quantitatively in liquid medium using (PVK Broth). Different investigators have used various media for studying phosphate solubilization in liquid medium. Pradhan and Sukla, (2006) found a suitable medium formulation as an ideal one for new isolates. Considering amount of glucose used in medium and corresponding efficacy of Phosphate solubilization, PVK medium proved to be most effective without compromising the solubilization.

All bacteria tested were found to be solubilizers of Tri calcium phosphate in PVK broth. The Phosphate content released into the medium from Tri calcium phosphate were given in the Fig.3. Results revealed that an isolate coded as Psm-6 showed maximum Phosphate solubilization in liquid medium. It was evident that in the medium with Tri calcium phosphate, the values of dissolved phosphate obtained with the isolate was convincingly showing that the tested isolate have effectively converted the inorganic, insoluble phosphate into soluble form and was selected for further studies.

Incubation in PVK broth supplemented with tri calcium phosphate, Phosphorus solubilizing bacteria are reported to dissolve insoluble phosphates by production of inorganic or organic acids and/or by the decrease of the pH (Whitelaw, 2000). Most of the previous reports stated that calcium phosphates are dissolved by acidification. Therefore any microorganism that acidifies its external medium will show some level of Phosphate Solubilizing Activity (Pradhan and Sukla, 2006). It is well known that Phosphate Solubilizing Bacteria in soil solubilize insoluble phosphates mainly by secreting acids into the medium (Dave and Patel, 2003). Isolates showed maximum Phosphate solubilization activity might have used the same mechanism to solubilize the insoluble form of phosphate into soluble form. Similar method was also used by Achal *et al.*, (2007) to analyze the soluble content of phosphate in

culture filtrate of *Aspergillus tubingensis* and by Himani and Reddy, (2011) to analyze the soluble content of phosphate in culture supernatant of *Bacillus sp.*

Table.1 Physico chemical properties of soil before and after treatment of PGPR in Groundnut field

Physico chemical properties	Isolate code	
	Control	Psm6
pH	8.1	8.3
Electrical conductivity (μ s/cm)	0.17	0.25
Organic matter (kg/g of soil)	5.9	6.2
Total nitrogen (kg/h)	1.15	1.19
Phosphorus (kg/h)	119	123
Carbon (kg/h)	9.5	9.8

Fig.1 Screening of Bacteria for Phosphate solubilization on Pikovskayas Agar Medium

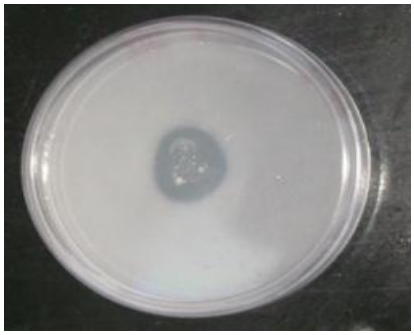


Fig.2 Phosphate solubilization by Bacterial isolates

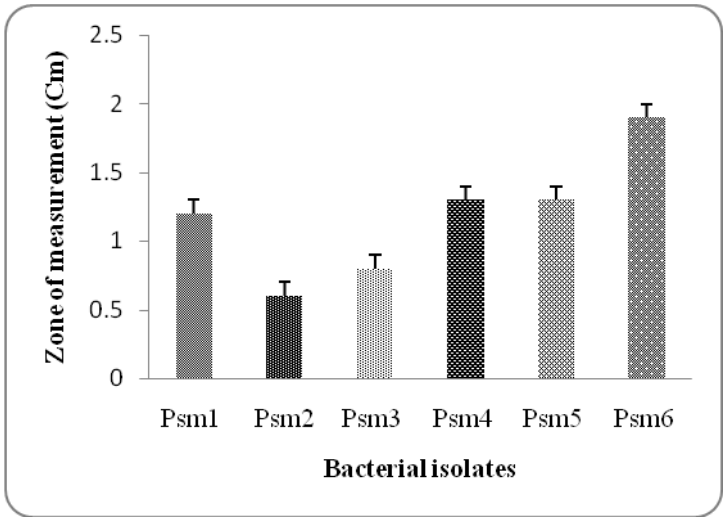


Fig.3 Phosphate solubilizing activity of Bacterial isolates

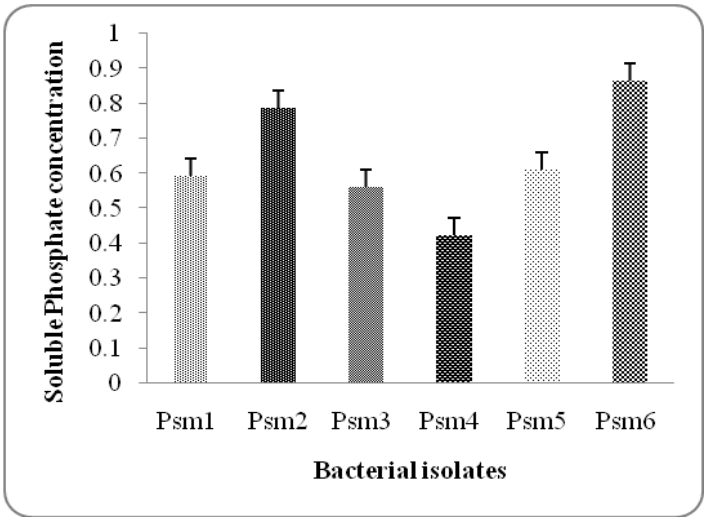


Fig.4 Growth of control and PGPR treated ground nut in field condition

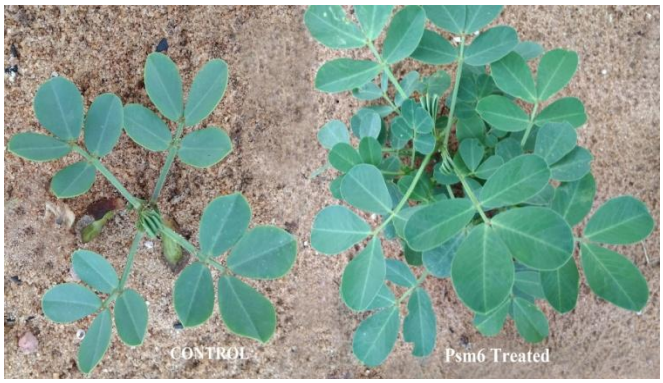
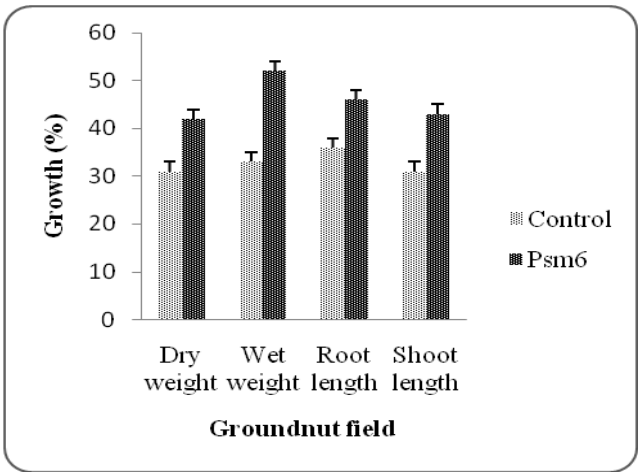


Fig.5 Dry weight, wet weight, root length and shoot length of control and PGPR treated Groundnut in field conditions



Field study

Biometric observation of Groundnut increases with treatment of Psm6 compare to control. Root and Shoot length, Dry and Wet weights were found to be excellent in Psm6 treatment compare to control (fig.4). Field study results revealed that there was significant increase in plant growth, Root length, Shoot length, Dry weight, Wet weight were of Groundnut with the inoculation of selected PGPR strains. When compared with PGPR treated groundnut, the control plant grown in field conditions showed that dry weight of the plant was reduced by 11% and fresh biomass was reduced by 19% and root length and shoot length of 10% and 12% (fig.5). This results of present study clearly showed the efficiency of Psm6 in plant growth enhancement, phosphorus uptake and soil fertility. Many studies in relation to crop improvement by PGPR were carried out either in pot cultures or field conditions [3, 16].

Physiochemical properties of soil

Physico chemical properties of soil used for field study was determined. After Psm6 inoculums treatment the physical-chemical properties such as pH, Electrical Conductivity, Organic Carbon and organic matter, Nitrogen and available Phosphorus were also increased significantly from control in Groundnut at field conditions (Table.1). From several studies and literature review it has been found that after treatment of PGPR as bio inoculants organic carbon, nitrogen and phosphorus also increases in the soil environment (Gunasekaran *et al.,* 2004) with slight reduction in pH (Shinde *et al.,* 2008). It has already been reviewed that production of organic acids by soil microorganisms and commensurate pH decrease is the major mechanism of phosphate solubilization (Whitelaw MA, 2000).

In conclusion, soil was slightly alkaline and belongs to low salinity class and are neutral in nature. Nitrogen and phosphorous content was low in the soil; organic matter was medium in the sample. After addition of PGPR isolate not only show in-vitro activity but they are showing excellent growth of plants in natural environmental condition. Among all isolates Psm6 exhibited better activities, which can be directed for farming. However, a lot of research is needed for evaluating the biotechnological properties of these bacterial species showing PGPR activity.

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