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Characterization of Phosphate Solubilizing Plant Growth Promoting Rhizobacteria Isolated From Pea Rhizosphere (*Pisum sativa*)

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A B S T R A C T

The rhizosphere soil is a source for a diversity of microorganisms which play a vital role in the enhancement of plant health through the mechanism of symbiotic interaction thereby influencing the plant growth. The present study aimed at isolating potential phosphate solubilizing rhizobacteria from pea (*pisum sativa*) crop for which, rhizosphere soil samples were collected from two pea growing districts of Kashmir valley *viz*. Srinagar and Baramulla. Isolates were cultured on nutrient agar medium followed by serial dilutions and different colonies with morphological variations were isolated from each dilution. A total of 80 bacteria were isolated and maintained as pure cultures. Out of the 80 isolates, 25 strains showed phosphate solubilizing ability and amongst them, 9 were highly potential which were subjected to morphological and biochemical characterization. Furthermore, the potential bacteria were analysed for Indole Acetic Acid (IAA) production, which was found to be directly proportional to the plant growth promotion. After screening the best nine isolates PGP1, PGP10, PGP28, PGP40, PGP49, PGP52, PGP60, PGP72 and PGP75 were selected for their effect on growth and yield in field conditions using pea as test crop.

Introduction

Phosphorus (P) is one of the major essential macronutrients for biological growth and development. Microorganisms offer a biological rescue system capable of solubilizing the insoluble inorganic P of soil and make it available to the plants. The ability of some microorganisms to convert insoluble phosphorus (P) to an accessible form, like orthophosphate, is an important trait in a PGPB for increasing plant yields (Rodriguez *et al.*, 2004). Over the years, several scientists have discovered the ability of certain microorganisms that aid in phosphate solubilization.

The microorganisms in the rhizosphere mainly bacteria, have the ability to compose complex substances based on the plant exudates (Glick, 2012;

Brahmaprakash *et al.*, 2017). The bacteria colonizing the soil and those that are associated with the plant rhizosphere are called rhizobacteria. Most bacterial species affect the chemical properties of the soil and also have a beneficial effect upon the plant growth, hence are termed as plant growth promoting rhizobacteria (PGPR). Their influence can also be neutral or deleterious (Schroth and Hancock, 1981, 1982; Beneduzi *et al.*, 2012).

The genera *Bacillus* and *Pseudomonas* are among the most abundant and phylogenetically diverse group of PGPR which show avid rhizosphere colonization and provide a considerable interest in enhancing crop production and yield (Podile and Kishore, 2007). Different bacterial strains such as *Bacillus, Rhizobium and Pseudomonas* are reported as the dominant solubilizers of phosphorus (Rodriguez and Fraga, 1999).

Diverse bacterial strains such as *Azospirillum, Enterobacter, Azotobacter, Acetobacter and Pseudomonas* increase the developmental reactions within the plant by either increasing the bioavailability of both micro and macro nutrients (e.g. iron, zinc and phosphorus), production of the phytohormones or by fixing atmospheric nitrogen (Ahmad *et al.*, 2008; Barea and Richardson, 2015; Arya *et al.*, 2020; Ummara *et al.*, 2021; Nawaz *et al.*, 2020).

Occurrence of PSB in the soil are ubiquitous in different forms and an elevated PSB population are seen in agricultural soils (Khan *et al.*, 2009; Kalayu, 2019). Studies have reported that bacterial strains isolated from pea fields have the remarkable ability to solubilize phosphorus from insoluble phosphorus through production of organic acids and phytohormones that increases phosphate uptake and improves the growth of pea plants (Othman and Panhwar, 2014). The present study was conducted with the objectives to isolate, identify, characterize the diversity of culturable bacteria from the pea rhizosphere. Also, to establish the ability of the bacteria for phosphate solubilization for its plant growth promoting capabilities on pea seeds.

Materials and Methods

Collection of samples

The rhizospheric soil of pea plants *(Pisum sativa*) was collected from two different pea grown areas in kashmir (Srinagar and Baramulla). Soil samples were collected in polyethene bags, transferred to the Division of Plant Pathology, Faculty of Horticulture, Shalimar, SKUAST. Where, these samples were processed for the separating rhizobacteria that possess phosphate solubilizing ability.

Isolation of phosphate solubilizing rhizobacteria

Plant growth-promoting rhizobacteria were isolated using serial dilution method and samples were serially diluted upto (10^{-5}). Pour plate and spread plate method were used for the enrichment, isolation, screening, and maintenance of PSRB using Pikovskaya's agar medium (Pikovskaya, 1948) containing tricalcium phosphate (TCP). In order to confirm the phosphate solubilizing activity, these plates were incubated at 37°C for 48 -72 h till the clear halo zones were formed around their colonies. The phosphate solubilization index (SI) was calculated for all the potential phosphate solubilizers and the pure cultures were analysed for their colony morphology and following evaluations were carried out.

Characterization of phosphate solubilizing bacteria

The PSB isolates showing maximum phosphate solubilization were subjected to morphological and biochemical characterization like indole production, catalase, urease, citrate utilization, oxidase which helped in identifying the bacteria up to genus level (Gupta *et al.*, 2000) by Bergey's manual of Determinative bacteriology (Holt *et al.*, 1994).

Indole acetic acid (IAA) Production

The production of IAA was evaluated by the method of Brick *et al.*, (1991). For the production of auxins,

bacterial cultures were grown in Luria Bertani broth, amended with 5 mM L-tryptophan, 0.065 per cent sodium dodecyl sulphate and 1 per cent glycerol for 72 hours at 37°C under shake conditions. Supernatant was collected by the centrifugation of cultures at 15,000 rpm for 20 minutes and stored at 4°C.

For measuring IAA equivalents, 3 ml supernatant was pipetted into test tube and 2 ml Salkowski's reagent (2 ml 0.5 M FeCl3+ 98 ml 35% HClO4) added to it. Tubes containing the mixture was left as such for 30 minutes (in dark) for the development of pink colour. The colour intensity was measured at 535 nm by UV-VIS spectrophotometer.

Effect of PSB on growth and yield characteristics of pea

On the basis of Phosphate solubilizing characteristics, nine isolates were selected and applied to the pea crop at the time of sowing to evaluate their efficiency to enhance plant growth and yield characteristics under field conditions. The parameters studied were number of nodules per plant, plant height, number of pods per plant, pod length and fresh plant weight.

Results and Discussion

Isolation of PSB and detection of phosphate solubilization index (SI)

A clear zone around the colony indicates phosphate solubilization after the incubation period at 37°C for 48–72 h. These clear zones were formed as the PVK medium acts as a specific isolation medium for PSB due to the presence of calcium triphosphate which is known to be the factor for the halo zone formation. A total number of 80 isolates were obtained from the two different rhizosphere samples. Upon screening these 80 isolates, 25 isolates were possessing phosphate solubilizing potency of which, 9 strains were revealing remarkable phosphate solubilizing activity with the high colony-halo zone ratio, the isolate PGP72 exhibited maximum SI (12.67) (Table 1) and was significantly superior to majority of isolates (Fig.1).

Morphological and Biochemical characterization of the phosphate solubilizing isolates

These nine phosphate solubilizing isolates exhibited great variations in their morphological characters. The shape of colonies varied from circular, filamentous, punctiform to irregular, with margins varying from entire, undulate, lobate, erose to curled. The colony elevation varied from flat, raised, convex to umbonate (Table 2). Amongst these nine isolates, seven were Gram positive and two isolates were Gram negative bacteria. All of the phosphate producing isolates were positive for maximum biochemical activities (Tables 3).

Screening for IAA

The potential nine isolates proved to have the ability to produce IAA. The concentration of IAA produced by the isolates ranged between 8.50 µg/ml – 25.00 µg/ml. It was found that isolate PGP72 produced the highest concentration of IAA (25.00 µg/ml), followed by PGP10(20.50 µg/ml) (Table 4).

Effect of PSB for growth and yield parameters of pea

The data presented in (Table 5) shows that the application of isolates increased the number of nodules, plant height, number of pods, fresh plant weight and pod length in all nine isolates as compared to control where no culture was applied. The isolate PGP52 showed increased number of nodules (60.44) as compared to control (40.70). Maximum plant height was recorded with respect to isolate PGP72 (47.53 cm) while minimum was recorded in control (44.12 cm). The maximum number of pods was observed in isolate PGP1 (32.66) while it was minimum in control (21.33). Highest plant fresh weight was also recorded with respect to isolate PGP72 (427 g) than control (229 g). Highest pod length was recorded in isolate PGP75 (8.52 cm) and lowest in control (6.55cm).

Table.1 Phosphate solubilizing efficacy of the nine isolates

Isolates	Zone of clearance (cm)	Colony diameter (cm)	Solubilization index (SI)
PGP1	0.41	1.50	4.66
PGP10	0.20	1.80	10.00
PGP28	0.20	2.30	12.50
PGP40	0.50	1.50	4.00
PGP49	0.30	1.30	5.33
PGP52	0.60	1.90	4.17
PGP60	0.30	1.50	6.00
PGP72	0.30	3.50	12.67
PGP75	0.20	1.30	7.50

Table.2 Morphological characterization of the isolates

Morphological Traits	PGP1	PGP10	PGP28	PGP40	PGP49	PGP52	PGP60	PGP72	PGP75
Colour	Creamy white	White	brownish	yellow	Light brown	White	creamy	brownish	Creamy white
Texture	Smooth	Rough	Shiny	glossy	smooth	Rough	shiny	Shiny	glossy
Margin	Erose	Entire	Lobate	Entire	undulate	Entire	curled	umbonate	Entire
Elevation	Convex	Umbonate	convex	Flat	Convex	Raised	Flat	convex	convex
Shape	Circular	filamentous	irregular	circular	punctiform	filamentous	irregular	circular	circular
Size in diameter	0.3cm	0.3cm	o.2cm	0.5cm	0.4cm	o.5cm	0.2cm	o.3cm	0.5cm

Table.3 Biochemical characterization of the isolates

Characterization test	PGP1	PGP10	PGP28	PGP40	PGP49	PGP52	PGP60	PGP72	PGP75
Gram Staining	+	+	+	-	+	+	+	-	+
Indole production	-	+	-	+	-	+	-	+	-
Methyl Red	+	-	+	-	-	+	+	+	+
Vogesproskauer	-	+	-	+	+	-	+	+	+
Catalase	+	+	+	+	-	+	+	+	-
Oxidase	+	-	+	+	+	-	+	+	+
Citrate utilization	-	-	-	-	-	-	-	-	-
Urease	+	+	+	+	+	+	+	+	+

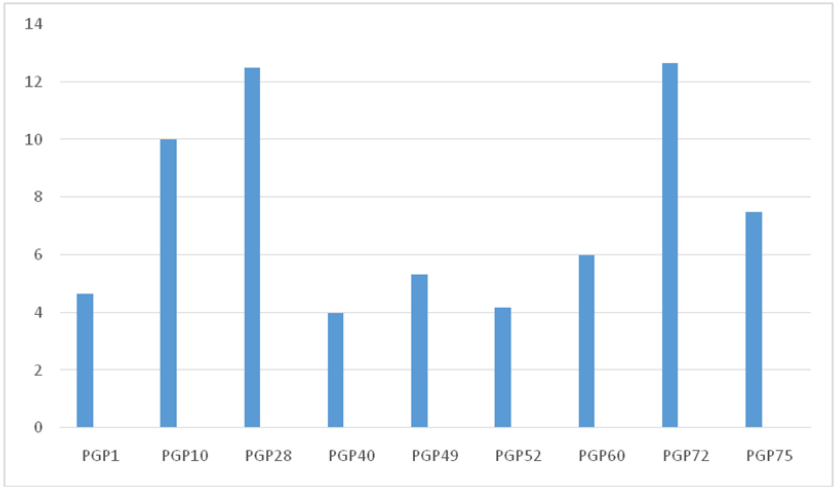
Table.4 IAA production of the isolates

Isolates	Indole acetic acid Production(µg/ml)
PGP1	12.00
PGP10	20.50
PGP28	22.50
PGP40	10.00
PGP49	19.00
PGP52	20.50
PGP60	8.50
PGP72	25.00
PGP75	11.00

Table.5 Effect of nine isolates of PSB for growth and yield characteristics on pea rhizosphere

Isolate	Number of nodules per plant	Plant height (cm)	Number of pods per plant	Plant fresh weight (g)	Pod length
Control	40.70	44.127	21.334	229	6.559
PGP1	54.20	46.712	32.667	397	8.342
PGP10	58.78	46.305	29.667	377	7.657
PGP28	58.69	44.727	30.000	282	8.270
PGP40	49.76	45.217	31.500	312	7.289
PGP49	52.85	45.679	29.667	364	7.375
PGP52	60.44	45.087	28.334	362	8.308
PGP60	57.90	45.112	29.167	360	6.785
PGP72	47.92	47.535	29.667	427	7.570
PGP75	54.94	44.130	29.000	357	8.522

Fig.1 Solubilization index (SI) of the nine isolates exhibiting remarkable phosphate solubilizing ability



Nature has provided the soil with ample faunal diversity that provides a significant function to maintain the fertility of soil and development of plant. Among the micro fauna, bacteria hold a promising significance as they actively invade the rhizosphere thereby enhancing the plant growth and development (Kumar *et al.*, 2017). The interaction of microbes with plant roots within a rhizosphere appears as detrimental factors for growth and yield of the crop species (Mumtaz *et al.*, 2017; Yin *et al.*, 2017). Strains from the genera *Pseudomonas, Bacillus and Rhizobium* are amongst the most powerful phosphate solubilizers (Rodríguez and Fraga, 1999). In the present study, nine potential pea rhizobacterial isolates exhibited phosphate solubilizing efficacy. There were variations in SI of the isolates and isolate PGP72 was revealing the highest phosphate solubilizing ability by showing maximum zone of clearance with a phosphate solubilization index of 12.67. In addition to phosphate solubilizing activity, all the nine isolates produced considerable amount of IAA, a plant growth inducing trait. Phosphate solubilizing bacteria when used as inoculants simultaneously increases P uptake by the plant and crop yield. All the nine isolates when studied for their growth promoting ability on pea seeds, all of them enhanced plant height and plant weight with remarkable increase in the size of the nodules when compared to the control. PGP72 was the most promising amongst the nine with significant enhancement of the plant growth promotion ability and IAA production.

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