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Exploring the Potential of *Pseudomonas* Species as Phosphate Solubilizer, Plant Growth Promoter, Biocontrol Agent and Pesticide Degrader

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ABSTRACT

Nine Pseudomonas isolates from rhizosphere and seven from graveyard soil were isolated, out of which eleven showed phosphate solubilization. Isolates PSP IV, V, VI, VIII, IX, X and XI showed maximum phosphate solubilizing efficiency while isolates PSP V, VI, VIII, IX and X were also found to produce IAA ranging from 1.06 to 4.12 µg/ml. The culture supernatant of isolates V and X showed antimicrobial activity against Serratia, Xanthomonas (bacteria), Fusarium, Aspergillus and Colliotricum species (fungi). Both isolates PSP V and X were found to produce siderophore while neither of them was capable to produce chitinase. When adopted to use organochlorine pesticide endosulfan as sole source of carbon and nitrogen, PSP V isolate was able to use endosulfan as sole source of carbon and nitrogen; thus it was selected for the pot experiments. In the pot experiment, maximum growth was observed in both Wheat (Triticum vulgare) and Gram (Cicer arietinium) supplemented with phosphate fertilizer and phosphate solubilizer compared to the other treatments. So the PSP V isolate of Pseudomonas can serve as a promising candidate to be used as bioinoculant.

KEYWORDS: ***Pseudomonas, Phosphate solubilization, Antimicrobial, Endosulfan Bioinoculant***

INTRODUCTION

The economic stability of India is dependent on the agricultural yield. Great deal of research carried out in the last 50 years by agricultural scientists, has its major thrust on increasing crop productivity. The process has gathered momentum with the development of high yielding varieties of crop plant in response to the challenge of feeding the rapidly increasing human population in most developing countries. However; most of the developing countries do not have the industrial development, which helps to subsidize high input agriculture in developed countries. For this reason, and as a result of major scientific advances in the field of microbiology and molecular genetics in recent years, scientists are exploring new ways of meeting the nutrient needs of crop plant aiming at high productivity. Soil microorganisms like bacteria; cyanobacteria and fungi have a particularly important role in the exploration of these new approaches.

Interaction of soil microorganisms and plant in the rhizosphere can be beneficial, neutral, variable or deleterious for plant growth. The beneficial activities of these organisms include production or changes in the concentrations of plant hormones like IAA, Gibberelic acid, Cytokinine and ethylene; fixation of nitrogen, suppression of growth of deleterious organisms by production of siderophores, chitinase, antibiotics; and dissolution of phosphate and other nutrients[1].

The organisms belonging to *Pseudomonas* genera are gram negative, strictly aerobic, polarly flagellated rods. They are aggressive colonizers of rhizosphere of various crop plants and known to grow on simple media [2]. Pseudomonas are one of the well known solubilizer of phosphate [3], to produce plant growth hormones like IAA, they have broad spectrum antagonistic activity against plant pathogens such as antibiosis [4], siderophore production [5], and nutrition or site competition [6]. Some *Pseudomonas* species produce higher levels hydrogen cyanide that is toxic to certain pathogenic fungi [7]. These characteristics make *Pseudomonas* species good candidate for use as seed inoculants and root dips for biological control of soil borne plant pathogens.

In the present study, phosphate-solubilizing *Pseudomonas* were isolated and then evaluated for their ability to produce plant growth promoting hormones, to function as an antimicrobial agent and to degrade organochlorine

pesticide.

MATERIALS AND METHODS

Isolation, characterization and identification of *Pseudomonas*

Pseudomonas strains were isolated from rhizosphere and graveyard soil. After serial dilution of soil samples they were plated on King's B agar (Proteose peptone 20g, K_2HPO_4 1.5g, $MgSO_4.7H_2O$ 1.5g, Glycerol 15 ml, Agar 20.0g) and Cetrimide agar (pancreatic digest of gelatin 20.0g, K_2SO_4 10.0g, $MgCl_2$ 1.4g, Cetrimide 0.3g, Glycerol 10.0 ml, Agar 13.6g) for isolation of *Pseudomonas*. [2]. Distinct colonies observed on the plates were selected, purified by repeated culturing and maintained on Kings's B agar slants at 4ºC.

Enrichment and screening of phosphate solubilizing *Pseudomonas*

All isolates were inoculated in the Pikovskaya broth and incubated at 37 °C on rotary shaker at 100 rpm for enrichment. Preliminary screening for phosphate solubilization was done by a plate assay method using Pikovskaya (PVK) agar medium supplemented with tricalcium phosphate (TCP). The medium contained: glucose 10 g; $Ca_3(PO_4)_2$ 5 g; $(NH_4)_2SO_4$ 0.5 g; NaCl 0.2 g; $MgSO_4.7H_2O$ 0.1 g; KCl 0.2 g; Yeast extract 0.5 g; $MnSO_4.7H_2O$ 0.002 g; $FeSO_4.7H_2O$ 0.002g and agar 15 g. The initial pH of the medium was adjusted to 7 [8]. The plates were incubated at 37 °C for four days and the ability of isolates to solubilize the phosphate was assessed by observing halo zone around the growing colonies indicating phosphate solubilization. The results were expressed as solubilization efficiency (E):

$$E = \frac{\text{Solubilization diameter}}{\text{Growth diameter}} \times 100$$

The isolates showing maximum phosphate solubilizing efficiency were selected for further studies.

Capacity to solubilize tricalcium phosphate in liquid cultures

Solubilization activity was carried out in 100 ml PVK broth medium supplied with 0.5% tricalcium phosphate. Then, after sterilization 1.0 ml suspension of each of the isolates was added to the broth. A control without any inoculation was maintained. The cultures were incubated on a rotary shaker at 30ºC for 7 days. The phosphate solubilizing ability was qualitatively monitored by ammonium molybdate and nitric acid system. Available phosphorus in broth cultures was estimated by the paramolybdate blue method and was expressed in terms of µg/ml phosphorus released in culture medium [9]. In addition pH change of the medium was monitored for production of organic acids.

Screening of bacterial isolates for indole acetic acid (IAA) production

Pseudomonas strains showing maximum phosphate solubilizing efficiency were screened for IAA production by inoculating them in Jensen's nutrient broth supplemented with 2-mg/ml tryptophan followed by incubation at 30 °C for 5 days. Cultures were centrifuged at 10,000 rpm for 10 min. Two milliliters of the supernatant was mixed with 2 drops orthophosphoric acid and 4 ml of Solawaski's reagent (50 ml, 35% perchloric acid; 1 ml 0.5 $FeCl_3$) [10]. Development of a pink colour indicates IAA production. O.D. was read at 530 nm using UV 1800 spectrophotometer (Shimadzu). The level of IAA produced was estimated by a standard IAA graph.

Antimicrobial activity against various phytopathogens

Three fungal phytopathogens species *Fusarium* species, *Aspergillus* species and *Colliotricum* species were grown on potato dextrose agar and two bacterial phytopathogens *Serratia* species and *Xanthomonas* species were grown on nutrient agar. The investigation of antifungal activity was done by dual culture plate method [11]. The antibacterial activity of *Pseudomonas* was done by agar cup method; in which the culture supernatant from King's B medium was added to the agar well at the center of the nutrient agar plate inoculated with the test organisms. Control was kept for each phytopathogen without addition of supernatant. Growth inhibition around the well indicated the antimicrobial activity of supernatant. The isolates showing antimicrobial activity were evaluated for production of chitinase and siderophore.

Evaluation of pesticide degradation ability

The isolates showing maximum phosphate solubilization, IAA production and antimicrobial activity against phytopathogens were selected and adopted to degrade organochlorine pesticide endosulfan. Davis Mingiolis medium (DM) (KH_2PO_4 3.0g, K_2HPO_4 7.0g, $MgSO_4.7H_2O$ 0.1g, $(NH_4)_2.SO_4$ 1.0g) supplemented with 1g peptone and 10 ml endosulfan per 1000ml broth was used. The isolates were adopted in the above medium to use pesticide as sole source

of carbon and nitrogen by successively decreasing concentration of peptone and ammonium sulphate from 1g to 0.75g, 0.5g, 0.25g, 0.1g and 0.0g. Growth was observed on visual basis and staining. The isolates so adopted were inoculated in DM medium with endosulfan as sole source of carbon and nitrogen; incubated at 30 °C on rotary shaker for 5 days [12].

Pot experiment

Sterilized soil was mixed with sand in 1:1 ratio and filled in plastic pots. These pots were placed under natural conditions. Seeds of Wheat (*Triticum vulgare*) and Gram (*Cicer arietinium*) were surface sterilized, rinsed 6 times with sterile water and dried. The surface disinfected seeds were coated with the selected isolates of *Pseudomonas* in liquid culture medium for 2 h using 10% gum Arabic. Ten seeds were sown in each pot; treatments were: A1-Control (seeds), A2 (seeds + Phopshate solubilizer), A3 (seeds + phosphate fertilizer) and A4 (seeds +Phosphate solubilizer + Phosphate fertilizer). The growth promotional activities of *Pseudomonas* were observed on visual basis.

RESULTS

Nine isolates from rhizosphere soil and seven from graveyard soil were isolated on King's B medium and cetrimide medium. On the basis of morphological, cultural and biochemical characteristics isolates were tentatively identified as species of *Pseudomonas* according to Bergey's Manual of Determinative Bacteriology, 9th Edition.

Out of sixteen isolates eleven showed halo zone around the colonies indicating phosphate solubilization when inoculated on Pikovskaya (PVK) agar medium. The solubilization efficiency (E) and solubilization capacity for the eleven isolates were evaluated after seven days of incubation beyond which no further increase in phosphate solubilization was seen (Table 1). On an average reduction in pH by 2-3 units in all the flasks indicated the production of organic acids.

Out of seven isolates PSP IV, V, VI, VIII, IX, X and XI showing maximum phosphate solubilizing efficiency five (PSP V, VI, VIII, IX and X) were found to produce IAA in Jensen's nutrient broth supplemented with tryptophan after five days of incubation at 30 °C. The amount of IAA produced by the selected isolates PSP V, VI, VIII, IX and X were 4.12, 2.13, 1.06, 0.94 and 2.47 µg/ml respectively.

The culture supernatant of isolates V, VI and X showed antibacterial activity against *Serratia* species and *Xanthomonas* species while isolate V and X also showed antifungal activity against *Fusarium* species, *Aspergillus* species and *Colliotricum* species. Both isolates PSP V and X were found to produce siderophore while neither of them was capable to produce chitinase.

Finally these two isolates were adopted to use organochlorine pesticide endosulfan as sole source of carbon and nitrogen, but only PSP V isolate was able to use endosulfan as sole source of carbon and nitrogen.

Thus considering the phosphate solubilizing activity, ability to produce IAA, antimicrobial activity and ability to degrade the organochlorine pesticide isolate PSP V was selected for the pot experiments. In the pot experiment, maximum growth was observed in both Wheat (*Triticum vulgare*) and Gram (*Cicer arietinium*) supplemented with phosphate fertilizer and phosphate solubilizer compared to the other treatments.

DISCUSSION

Rhizosphere microorganisms like bacteria; cyanobacteria and fungi are important for plant growth. But extensive use of chemicals fertilizers and pesticide disturb ecological balance of soil microorganisms hence there is need to search for alternative strategies to improve soil health with out causing damage to environment as well as soil.

Because of chemical fixation, phosphate fertilizer are not available for plant growth hence use of efficient phosphate solubilizing microorganisms as seed inoculants or direct use in soil when crops are raised greatly help in phosphate solubilization and mobilization for crop use [13]. In the present study sixteen isolates of *Pseudomonas* were isolated out of which eleven showed potential of phosphate solubilization.

Many different traits of these bacteria are responsible for growth promotion activities it includes the ability to produce or change the concentration of the plant hormones, indole acetic acid, gibberlic acid, cytokinins and ethylene and they suppress the growth of deleterious microorganisms by production of siderophores, β-1,3 glucanase, chitinase, antibiotics and cyanides [14]. Thus seven of the efficient phosphate solubilizer isolates when analyzed for production

of IAA five were found to produce IAA. Two of them showed antifungal and antibacterial activity. When assessed for the production siderophore and chitinase as the probable means of antimicrobial activity both isolates were able to produce siderophore but none of them could produce chitinase indicating that in addition to the production of siderophore other mechanisms may be involved in their antifungal activity.

Biotransformation of pesticide have led to the isolation of some naturally occurring soil bacteria capable of using certain pesticides like endosulfan, monocrotophos and rogor as a sole source of carbon [15-17]. Out of the two isolates, PSP V isolate showed phosphate solubilizing activity, ability to produce IAA, antimicrobial activity and ability to degrade the organochlorine pesticide.

Our study indicates that *Pseudomonas* isolate PSP V has the potential to promote plant growth by making phosphorus available for plant, by releasing growth promoting phytohormone IAA. In addition this isolate showed an intrinsic capacity to produce and release antimicrobial compounds active against phytopathogens. Interestingly the isolate also showed the ability to degrade the organochlorine pesticide endosulfan. In summary, these attributes of *Pseudomonas* isolate will be of great advantages in agriculture field; and hence will play a vital role in plant growth promotion, disease suppression and subsequent enhancement of yield.

Table 1 Solubilization efficiency (E) and Solubilization capacity for the eleven isolates

Isolate	*Solubilization efficiency (E)*	*Solubilization capacity in µg/ml*
PSP I	122.77	638
PSP II	153.24	712
PSP III	144.36	670
PSP IV	172.51	725
PSP V	189.47	823
PSP VI	202.3	1107
PSP VII	153.18	637
PSP VIII	198.67	914
PSP IX	177.00	743
PSP X	200.40	977
PSP XI	168.73	784

PSP: Phosphate Solubilizing *Pseudomonas*

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