African Journal of Microbiology Research Vol. 7(2), pp. 147-151, 8 January, 2013
Available online at http://www.academicjournals.org/AJMR
DOI: 10.5897/AJMR12.1308
ISSN 1996-0808 ©2013 Academic Journals

Full Length Research Paper

Plant growth promoting abilities of phosphate solubilizers from the rhizosphere of *Parthenium hysterophorus*

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Accepted 19 December, 2012

Analysis of diversity of phosphate solubilizers in agricultural soil is essential to understand their ecological role and their utilization in sustainable agriculture. One of the factors contributing to the success of weeds, even in nutrient limiting conditions, is the microbial community they select in their vicinity. Phosphate solubilizers from the rhizosphere of a widely growing weed, *Parthenium hysterophorus*, were enumerated on Pikovskaya's medium, with an aim to screen for their plant growth promoting abilities for crops. The isolates were further assayed for multi-trait plant growth promoting properties. Two potential isolates, P1 and P2, were employed in seed germination and pot experiments with crop species. While bacterization led to an increase of 70 and 200% in shoot length in both seedling germination and pot experiments with *Cajanus cajan* (red gram), *Vigna radiata* (green gram) displayed an increase in shoot length by about 20% in pot assay using isolates P1 and P2, respectively. The present study reveals the presence of phosphate solubilizers in the rhizosphere of *P. hysterophorus* with plant growth promoting effect on other crop species. Due to their potential in exhibiting plant growth promoting properties, these phosphate solubilizing isolates provide a new dimension to the significance of weeds in agricultural ecosystems. The study opens up possibilities of utilization of this property of weeds in plant growth promotion, disease suppression and subsequent enhancement of yield in agriculture.

Key words: *Parthenium hysterophorus*, rhizosphere, phosphate solubilizers, plant growth promotion, *Bacillus subtilis*.

INTRODUCTION

Bacterial communities in the rhizosphere can greatly regulate the functioning of soil ecosystems including nutrient transformation and organic matter decomposition (Zhang et al., 2006). Nevertheless, there is much less knowledge about the role of the composition of microbial communities in weed rhizosphere which can flourish even under stress conditions. *Parthenium hysterophorus* L. (Asteraceae, congress grass) is an invasive weed competing strongly with crops, thereby suppressing yield (Kohli et al., 2006). The feature that contributes to replacement of dominant flora by *Parthenium* in various habitats, is its wide adaptability to varying physico-chemical conditions of soil. This strong ability of the weed to grow under stress conditions can be brought to use. Beneficial microorganisms, such as phosphate solubilizers, which are present in the plant's rhizosphere contributing to the competitiveness of this luxuriantly growing weed, can be harnessed. We hypothesize that rhizosphere of *P. hysterophorus* would harbour phosphate solubilizers possessing other plant growth promoting (PGP) properties. The aim of this study, therefore, was to evaluate the efficiency of multi trait phosphate solubilizers from the rhizosphere of *P. hysterophorus* in plant growth promotion of other crops.

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MATERIALS AND METHODS

Sampling

Rhizospheric soil (soil tightly adhering to roots, approximately 10 mm from root surface) samples of *P. hysterophorus* (L.) and bulk soil were collected from agricultural fields located in and around Delhi, India at a depth of approximately 30 cm. The alluvial soil had pH of 7.9, 0.09 g kg^{-1} nitrogen, 0.34 g kg^{-1} carbon, 0.24 g kg^{-1} hydrogen, and 24.4 µS cm^{-1} of electrical conductivity. Bioavailable P was also measured (Bray and Kurtz, 1945).

Enumeration of phosphate solubilizing bacteria (PSB)

PSB were isolated from samples by spread plating on Pikovskaya's (PVK) medium (Pikovskaya 1948) supplemented with tricalcium phosphate as insoluble inorganic phosphate source and incubated at 28°C for 3 days.

Quantification of phosphate solubilization and other PGP properties of isolates

Estimation of phosphate was carried out in flasks containing 10 ml PVK broth inoculated with the PSB incubated at 28°C at 180 rpm for 5 days. Phosphate in culture supernatant (cells harvested by centrifugation at 10,000 rpm for 10 min) was estimated by modified molybdenum blue method (Harold, 1988) in triplicates and expressed as equivalent phosphorus (µg ml^{-1}). Absorbance at 690 nm was measured after 7 min. Linearity of 0.9983 was obtained for the standard curve.

PGP properties were assayed in triplicates with the PSB: cellulase and chitinase production (Cattelan et al., 1999), NH_3 production (Cappuccino and Sherman, 1992), protease production (Vazquez et al., 1995), and indole acetic acid (IAA) production (Husen, 2003).

Seed germination and axenic pot experiment

Seeds of Green gram (*Vigna radiata*, var K851), Red Gram (*Cajanus cajan* var Upas 120), Okra (*Abelmoschus esculentus* var A4) and Chilli (*Capsicum annuum* var PJ) for each isolate were taken in sterile beakers (25 seeds per isolate). Seeds were surface sterilized (Abdul Baki, 1974) followed by incubation in bacterial cell suspension adjusted to 10^7 CFU ml^{-1} as described (Ashrafuzzaman et al., 2009). 25 bacterised seeds were placed on agar (2% w/v) plates and incubated for 3 days in the dark.

For pot experiments, soil (described earlier) was sterilized by autoclaving twice for axenic growth. The seeds treated with inoculum (10 per isolate) were sown in plastic pots and incubated in plant growth chamber at 25 ± 2°C at the photoperiod of 18/6 h. Growth parameters, in terms of shoot length, shoot biomass, root length and root biomass, were recorded after 3-week. The data were subjected to analysis of variance (ANOVA) using SPSS Statistical System (SPSS 16.0 for Windows) and comparison between treatments means was made using Duncan's multiple range test (DMRT) at $p < 0.05$ (Little and Hills, 1978).

Genomic DNA extraction and sequencing

Total genomic DNA of isolates with plant growth promoting effects on crops was extracted (Ausubel et al., 1995). Further sequencing was done using MicroSEQ® Full Gene 16S rDNA Bacterial Identification Kit (Applied Biosystem, USA) on outsource basis. Sequences of approximately 500 base pairs obtained were compared to known 16S rRNA sequences in the GenBank™ Database, using BLAST (Basic Local Alignment Search Tool).

RESULTS AND DISCUSSION

CFU count of 3.33 x 10^7 g^{-1} soil was obtained for rhizospheric samples as compared to 1.9 x 10^5 g^{-1} soil for bulk soil on PVK plates. However, no morphotypes obtained from bulk soil formed visible dissolution halo. Three halo forming morphotypes from rhizosphere soil of *P. hysterophorus* were confirmed for phosphate solubilization using bromophenol (100 mg ml^{-1}) as indicator.

Lower CFU counts of PSBs in rhizospheric soils was reported earlier ranging from 0 to 74 x 10^3 g^{-1} dry rhizosphere of weeds found in paddy, sugarcane, garden, riverbed and wasteland soils (Seshadri and Lakshminarasimhan, 2007), 32 to 95 x 10^3 g^{-1} soil (Vikram et al., 2007) to 9 to 67 x 10^5 CFU g^{-1} soil in chickpea (Kundu et al., 2009). The difference in population of PSB by 2 orders of magnitude is probably because most studies consider only colonies producing a distinct halo on medium used for enumeration of phosphate solubilizers. However, there have been reports wherein isolates showing no distinct halo in solid plates have also been shown to be efficient phosphate solubilizers in broth (Seshadri et al., 2000).

Phosphate solubilization in PVK broth ranged from 29 (isolate P4) to 432.46 (isolate P2) µg ml^{-1} after 4 days of inoculation. While the pH dropped to 5 for isolates P1 and P4, it went down to 4.2 for isolate P2. P1 was observed to solubilize the maximum amount of phosphate (137.46 µg ml^{-1}) in 24 h. However, P2 displayed a constant rise in the amount of phosphate solubilized starting from 201.47 to 432.46 µg ml^{-1} on the 4th day.

Similar large variations in isolates' abilities to solubilize phosphorus have been reported with values ranging from 22.7 µg ml^{-1} to 247 µg ml^{-1}, 12.6 µg ml^{-1} to 227.0 µg ml^{-1}, 2.2 µg ml^{-1} to 123 µg ml^{-1} in rhizospheres of chick pea, mustard and wheat, respectively (Kundu et al., 2009). Based on other studies on phosphate solubilizing efficiency of rhizospheric isolates (Chen et al., 2006; Naik et al., 2008; Minaxi et al., 2012), isolates P1 and P2 can be considered as highly efficient phosphate solubilizers. The other PGP properties exhibited by the isolates have been summarized in Table 1. P4 was positive only for ammonia and protease production. Tamilarsi et al. (2008) characterized the population of different components of microbial diversity in 50 medicinal plants including *P. hysterophorus* in which the predominant bacterial species was found to be *Bacillus*. In an attempt to enumerate phosphate solubilizing microorganisms from rhizosphere of *Piper betel*, Tallapragada and Seshachala (2012) observed dominant *Bacillus sp.* to exhibit the most phosphate solubilizing ability. IAA production value of 74.29 µg ml^{-1} by P1 was higher than what has been normally reported for other *Bacillus* isolates from rhizospheres (Gangwar and Kaur, 2009; Tsavkelova et al., 2007; Minaxi et al., 2012). Similar reports of certain strains exhibiting IAA production while others being more efficient in phosphate solubilization have earlier been

Table 1. Plant growth promoting assays of isolates P1 and P2 and identification.

Isolate	Phosphate solubilization (µg ml^{-1})	Protease production	Cellulase production	Ammonia production	Chitinase production	IAA production (µg ml^{-1})	Similarity with sequence in database (%)
P1	137.46* 74.96#	-	-	+	-	74.29	*Bacillus subtilis* (100)
P2	201.47* 432.46#	-	-	+	-	-	*Bacillus subtilis* (99.93)

*After 24 h; #after 96 h.

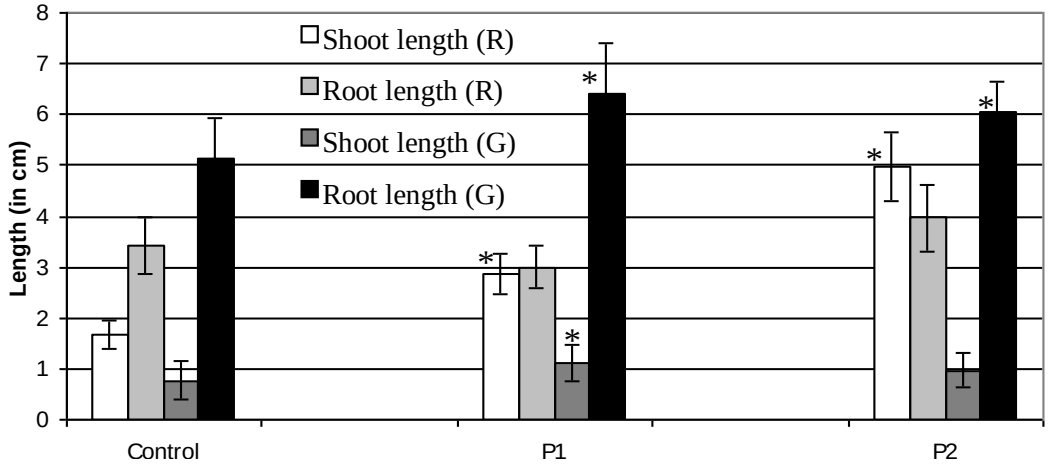

Figure 1. Effect of seed bacterisation on seedling growth over a period of 3 days. *Bacillus* sp. strains P1 and P2 are the isolates under study; R, Red Gram (*Cajanus cajan*); G, Green Gram (*Vigna radiata*) (n=25); *significant differences with respect to the control ($p<0.05$).

reported (Beneduzi et al., 2008; Calvo et al., 2010).

Based on the high values of phosphate solubilization by the isolates, only *Bacillus* sp. strains P1 and P2 were selected for experiments with seedling germination and plant growth in pots. Seeds of red gram, green gram, okra and chilli, bacterized with both strains of *Bacillus* sp., were incubated for 3 days under dark, and shoot and root lengths measured. Bacterization was seen to have no effect on the germination of okra and chilly seeds (data not shown). With respect to root length, only green gram (*V.radiata*) seedlings treated with *Bacillus* sp. strain P2 displayed significant enhancement when compared with control seeds (Figure 1).

However, the shoot length of red gram (*C.cajan)* seeds bacterized with *Bacillus* sp. strains P1 and P2 showed a considerable increase over the control seeds ($p<0.05$). The increase was approximately 70% with strain P1 and 200% with

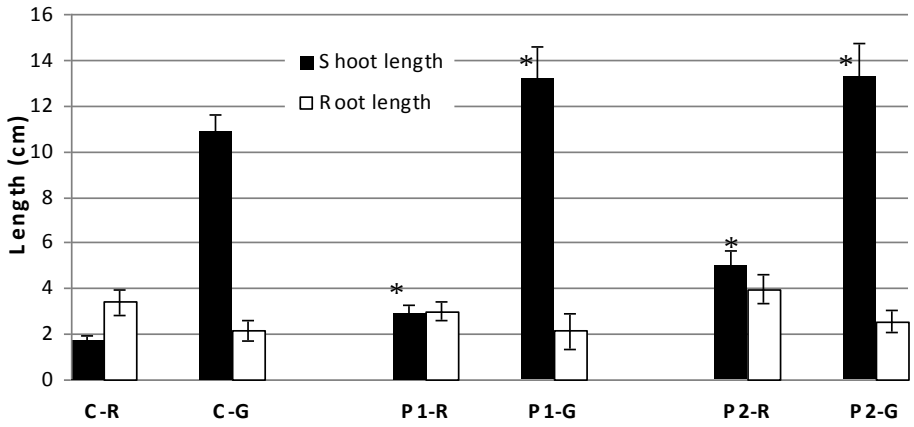

Figure 2. Effect of seed bacterisation on plant growth in pot experiments over a period of 3 weeks. *Bacillus* sp. strains P1 and P2 are the isolates under study; R, red Gram (*Cajanus cajan*); G, green Gram (*Vigna radiata*); C, Control (n=10); *significant differences with respect to the control ($p<0.05$).

strain P2. For *V.radiata,* the increase in shoot length was approximately 44% with P1. On performing the plant growth experiment in pots under controlled conditions, as with seedling germination results, no significant promotion of root length was observed. The percent increase of shoot length with *C. cajan* imitated the values obtained for seedling germination, viz. approximately 70% with *Bacillus* sp. strain P1 and 200% with strain P2. However, with *V. radiata,* the observed increase was 21% with both strains (Figure 2).

The experiments were performed in controlled conditions. Such studies need to be extended to field conditions, to evaluate the efficacy of these potential bioinoculants under variable natural conditions and to evaluate their response to indigenous rhizospheric population of crops. The present study opens a vast area of significance of weed rhizospheric community, which has immense biotechnological potential. Alternatives to the current practice of burning and uprooting *P. hysterophorus*, to manage weed invasion and removing its residues, which has been reported to make the soil deficient in organic matter (Singh et al., 2003), need to be sought. Strategies, to support the beneficial microflora residing in weed's rhizosphere so as to enhance growth of subsequent crop, need to be framed.

ACKNOWLEDGEMENTS

The work was supported partly by the Department of Biotechnology, Government of India, and by a grant given to B.A. by IIT Delhi, Alumni Association, New Delhi.

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