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RESEARCH ARTICLE

REVELATION OF THE PHOSPHATE SOLUBILIZING ABILITIES OF SOIL BACTERIA AND ITS ROLE IN PLANTS GROWTH PROMOTION

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ABSTRACT

Phosphate solubilizing bacterial strains GRS_3, GRP_5, GPP_3, GRZ_3, GCP_1, GCP_2, GCP_3 and GCN_3 were isolated from the rhizosphere soils of different altitude and longitudes of Uttarakhand state of India. All eight strains demonstrated diverse levels of phosphate solubilization activity under *in vitro* conditions in the presence of tribasic calcium phosphate as a sole phosphorus source. At the same time, there is a reduction in pH of the medium and release of soluble phosphate. Acid production may have contributed to phosphate solubilization, but was not the only reason for phosphate release into the medium. Among the eight strains, GRS_3, GRP_5 and GCP_1 were the most efficient in terms of phosphorus solubilization in NBRIP broth (pH 7.0) at 30 ^{0}C. The growth promotory abilities of potential candidate GRS_3, GRP_5 and GCP_1 was documented under *in situ* condition green house experiment using different leguminous crops lentil, mungbean, gram, black gram and soybean respectively.

INTRODUCTION

Phosphorus (P) is one of the major nutrients to plants as well as micro-organisms and being involved in major physiological processes, second only to nitrogen in requirement. However, a greater part of soil phosphorus, approximately 95–99% is present in the form of insoluble phosphates and hence cannot be utilized by the plants (Vassileva *et. al*, 1998). Organic phosphorus constitutes a large proportion of the total phosphorus in many soils. Inositol phosphate (soil phytate) is the major form of organic phosphorus in soil, and other organic P compounds in soil are in the form of phosphomonoesters, phosphodiesters including phospholipids, nucleic acids, and phosphotriesters respectively.

However, plants can only utilize P in inorganic form. Mineralization of most organic phosphorus compound is carried out by means of phosphatase enzymes. The major source of phosphatase activity in soil is considered to be microbial origin. To increase the availability of phosphorus for plants, now a day's large numbers of bacteria are used for the conversion of soil organic phosphorus to the soluble inorganic form, which is known as 'Phosphate Solubilizing Bacteria (Asea, 1988; Das *et al.* 2003).

Rhizosphere bacteria that favorably effect plant growth and yield of commercially important crops are dominated plants growth promoting rhizobacteria (PGPR) which include bacteria belonging to the some important genera i.e. *Azotobacter, Azospirillum, Arthrobacter, Bacillus, Burkholderia, Enterobacter, Klebsiella, Pseudomonas, Xanthomonas* and *Serrratia*. Other microorganisms that are known to be beneficial to plants are the phosphate solubilizers, plant growth promoting pseudomonads and mycorrhizal fungi. A majority of agricultural soils contain large reserves of phosphorus, of which a considerable part is accumulated as consequence of regular applications of P-fertilizers (Katiyar and Goel, 2004). The phenomenon of fixation and precipitation of P in soils is dependent on pH. In acidic soils P is precipitated as Al and Fe phosphates, whereas, in calcareous soils high concentration of Ca results in P precipitation. Diverse groups of organisms in soil employ variety of solubilization reactions to release soluble phosphorus from insoluble phosphates (Illmer *et al.,* 1995; Singh and Kapoor, 1998).

MATERIALS AND METHOD

The organisms and culture conditions

The phosphate solubilizing bacterial strains used in this study were isolated from rhizospheric soil of different longitude and altitude sites of Uttarakhand state of India. Approximately 50 g of soil sample was taken from the upper 30 cm of the soil profile aseptically. The soil pH was 6.8. Soil contained 59.1% of sand, 11.65% clay and 29.3% silt. The serially diluted soil samples were placed on standard agar medium (pH 6.8–7.0) at 4^0C and 30^0C.

Screening and Characterization

All the isolates recovered were characterized morphologically by gram staining shows all are gram negative and small rods except GCP_2 and GCN_3 were gram negative and long rods (table 1).

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Table 1 Morphological characteristic of isolated strains

S.No.	Strains	Morphological characteristics
1.	GRS3	Gram –ve, small rods, single and light yellow colour colony
2.	GRP5	Gram –ve, small rods, single and white colour colony
3.	GPP3	Gram –ve, small rods, single and light yellow colour colony
4.	GRZ3	Gram –ve, small rods, single and yellow and convex colony
5.	GCP1	Gram –ve, small rods, single and brown colour colony
6.	GCP2	Gram –ve, long thin rods, single, filamentous and brown
7.	GCP3	Gram –ve, small rods, single and yellow colour colony
8.	GCN3	Gram –ve, long rods, single and light yellow colour colony

Screening was based on phosphate solubilizing potential of isolates. 'P' solubilization was checked on Pikovskaya agar medium containing (g/l): Yeast extract.0.5; Dextrose, 10.0; $Ca_3(PO_4)_2$, 5.0; $(NH_4)_2SO_4$, 0.5, KCl, 0.2; $MgSo_4$, 0.1; $MnSO_4$, 0.001: $FeSO_4$, 0.001 and Agar 15.0, pH 7.0±0.2. For quantitative estimation of phosphate selected isolates were checked in NBRIP broth (Das *et al*. 2003), where tri calcium phosphate (5%) was used for 10 days. The pH of the culture media was also recorded with 24 h time interval. Eight phosphate solubilizing bacterial strains thus screened were selected for further analysis.

Growth Characteristics

All the strains were grown in 50 ml nutrient broth from 1 ml of overnight grown culture. The cultures were incubated at 10, 20, 30 and 37^{0}C with shaking at 150 rpm for 24 h and O.D. was recorded at 600 nm at different times. The optimum pH for all the isolated strains were recorded in different pH medium varies from 3-10 by incubated at 37^{0}C for 24 h.

Agronomical parameters

The growth promoting activity of three strains GRS_3, GRP_5 and GCP_1 were quantified and compared by measuring the effect of the bacterium on the agronomical parameters like root and shoot elongation, fresh and dry weight of the developed leguminous plants. The cultures were grown overnight at 30^{0}C and 150 rpm were used for seed bacterization. The seeds were surface sterilized by soaking in 0.1% $HgCl_2$ solution for 1 min, and then washed four times with distilled water. Carboxy methyl cellulose (CMC) was mixed at the rate of 100 mg/l in culture medium and the resulting culture suspension was used for coating the seeds which were then dried at room temperature. Three replicates of pots with 10 seeds with each treatment and control were used for each experiment. The pots were placed in poly house for 60 days. The pre sowing and post sowing data were recorded after time to time.

Biochemical Parameters

Chlorophyll content of leaves of control and treated plants was measured after 45 days of sowing by the method of Hiscox and Israelstam (1971). P content of the soil in which experiment was done was estimated after 45 days of sowing by Olsen's ($NaHCO_3$) method (1954).

RESULT AND DISCUSSION

The isolated phosphate solubilizing bacterial strains GRS_3, GRP_5 and GCP_1 among all eight strains demonstrated diverse levels of phosphate solubilization activity under *in vitro* conditions. The phosphate-solubilizing activity of the isolates was first qualitatively evaluated by the formation of halos (clear zones) around the colonies growing on solid medium containing tribasic calcium phosphate as a sole phosphorus source (fig.1).

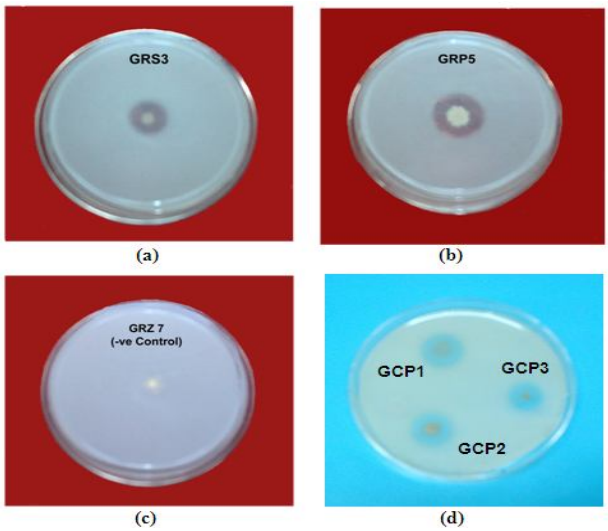

Fig. 1 Qualitative test for P solubilization (a) GRS_3, (b) GRP_5, (c) -ve Control and (d) GCP_1

Spectrophotometric quantification of phosphate solubilization documented that all bacterial strains solubilized insoluble phosphate well in a liquid medium, and that GRS_3, GRP_5 and GCP_1 are the most active solubilizing strains among all eight isolates which maximum P solubilization was 482.86µg/ml, 433.31µg/ml and 456.54µg/ml respectively in 10 days (Fig. 2).

Table 2 Effect of P solubilizing strains on total soil phosphorous content on different plants (µg/gm) after 60 days of sowing under green house condition

Strains	Crop	Total Soil Phosphorus (µg/gm)[a]		
		Control	Treatment	% Increase
GRS3	Lentil	56.96±0.26	60.07±0.34	5.5%
	Mungbean	54.91±0.28	54.99±0.22	0.15%
	Gram	52.82±0.20	58.81±0.44	11.3%
GCP1	Lentil	53.2±0.34	57.92±0.41	8.8%
	Mungbean	52.97±0.09	55.32±0.11	4.4%
	Gram	49.80±0.29	53.82±0.21	8.0%
GRP5	Soybean	67.27±0.27	70.133±0.27	4.2%
	Black gram	63.79±0.13	71.00±0.27	11.3%

[a] Mean of ten replicate samples
± SEM

The P solubilization by these strains were found maximum increase in 4-5 days and there was a significant decrease in pH of the medium (up to 3.5) in 10 days of incubation (fig. 3).

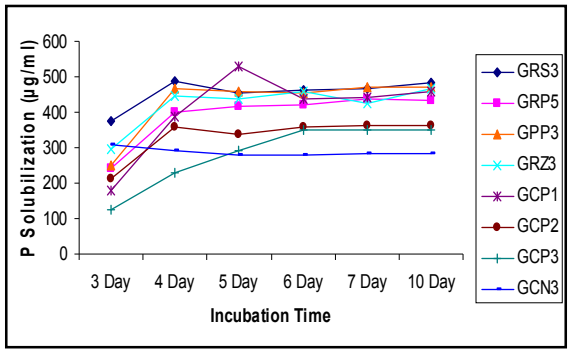

Fig. 2 Quantitative estimation of phosphorus by isolated strains at 30^{0}C at different time interval

Table 3 Effect of P solubilizing strain (GRS_3) on leguminous crops in a greenhouse experiment at 30^0C after 60 days

Crop		Fresh Weight[a] (g)	Dry Weight[a] (g)	Root length[a] (cm)	Shoot length[a] (cm)	Chlorophyll content (µg/g)[a]
Lentil	Mean control	1.076 ±0.623	0.352±0.047	19.45±0.377	19.25±0.908	2.762±0.0923
	Mean treated	1.428±0.09	0.689±0.0277	22.0±0.489	26.05±0.383	3.195±0.0466
	% Increase	32.7%[b]	95%	13.1%	35%	15.6%
Mungbean	Mean control	1.273±0.119	0.325±0.013	21.1±0.33	10.25±0.437	1.565±0.0504
	Mean treated	2.122±0.1	0.503±0.0267	22.5±0.30	12.95±0.61	2.076±0.00227
	% Increase	66%	54%	6.6%	26.3%	32.6%
Gram	Mean control	5.76±0.253	1.10±0.0657	20.20±0.502	17.35±0.526	4.433±0.0256
	Mean treated	8.495±0.328	1.417±0.0692	22.2±0.453	21.7±0.80	4.811±0.012
	% Increase	47.7%	28.8%	10%	25%	8.5%

[a] Mean of ten replicate samples
± SEM
[b] values in parenthesis indicate the % increase over the respective control.

Table 4 Effect of P solubilizing strain (GCP_1) on leguminous crops in a greenhouse experiment at 30^0C after 60 days

Crop		Fresh Weight[a] (g)	Dry Weight[a] (g)	Root length[a] (cm)	Shoot length[a] (cm)	Chlorophyll content (µg/g)[a]
Lentil	Mean control	0.6054±0.066	0.25±0.013	21.0±0.374	21.5±0.79	2.555±0.0863
	Mean treated	1.1156±0.055	0.508±0.023	21.85±0.347	24.6±0.924	3.273±0.0066
	% Increase	84%[b]	103%	4.0%	14.4%	28%
Mungbean	Mean control	1.599±0.0372	0.51±0.0196	15.25±1.03	12.85±0.728	1.885±0.00433
	Mean treated	2.091±0.1344	0.61±0.0186	20.4±0.504	14.75±1.21	2.131±0.0008
	% Increase	30%	19.6%	33.7%	14.8%	13%
Gram	Mean control	3.812±0.157	1.098±0.038	18.5±0.922	22.2±1.56	2.842±0.0091
	Mean treated	5.331±0.328	1.178±0.097	23.2±0.998	23.65±0.824	3.531±0.00746
	% Increase	39.8%	7.3%	25.4%	6.5%	24%

[a] Mean of ten replicate samples
± SEM

Table 5 Effect of P solubilizing strain (GRP_5) on leguminous crops in a greenhouse experiment at 30^0C after 60 days

Crop		Fresh Weight[a] (g)	Dry Weight[a] (g)	Root length[a] (cm)	Shoot length[a] (cm)	Chlorophyll content (µg/g)[a]
Soybean	Mean control	5.922±0.168	2.035±0.070	29.25±0.279	18.62±0.778	3.703±0.0001
	Mean treated	7.019±0.38	2.219±0.071	26.55±0.975	18.6±0.965	4.430±0.00084
	% Increase	18.5%[b]	9.0%	-10%	0.0%	19.6%
Blackgram	Mean control	2.354±0.128	0.642±0.046	17.2±0.46	11.0±0.316	2.323±0.0717
	Mean treated	2.664±0.384	0.684±0.0368	21.35±0.583	12.5±0.519	4.725±0.0077
	% Increase	13.2%	6.5%	24%	13.6%	3.4%

[a] Mean of ten replicate samples
± SEM
[b] values in parenthesis indicate the % increase over the respective control

The phosphate-solubilizing potential of the rhizosphere microbial community in legumes was demonstrated under in situ condition when seeds were bacterized with GRS_3, GRP_5 and GCP_1 and sown in pots under green house.

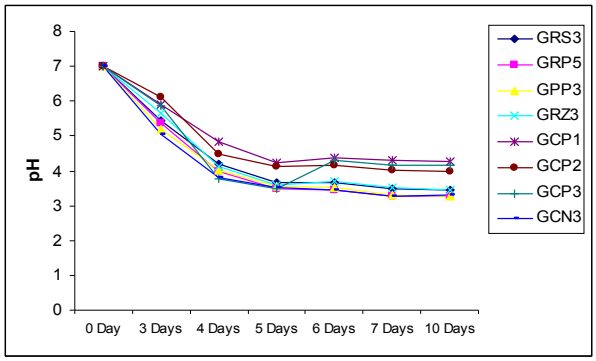

Fig. 3 Effect of P solubilization on pH of the medium at 30^0C at different time interval

Effect became transparent after a few days of sowing. The agronomical parameters were measured after 60 days of sowing, there was a significant increase found in root and shoot length, fresh and dry weight in treated plants compared to untreated plants (table 3, 4, and 5).

These three strains GRS_3, GRP_5 and GCP_1 showed better result for lentil, gram and mung-bean plants which is comparable to treated plants by improving the physiology of the plants as chlorophyll content (table 3,4 and 5) and increase in P-content of soil [11.3%] (table 2) over control (non-bacterized seeds). A significant increase in chlorophyll content of leaves and P content of soil was found in treatment having GRS_3, GRP_5 and GCP_1 as a phosphatic fertilizer (P.F.)

It is clear that these three strains are positive for 'P' solubilization which is considered as essential property of Plant Growth Promotory Rhizobacteria (PGPR) strains (Kloepper *et al.,* 1989).

CONCLUSION

It is concluded that these isolated strains influences the phosphate solubilizing ability of soil organic phosphorus. The results are discussed in relation to the possible mechanisms involved in solubilization and the potential benefits of phosphate solubilization to leguminous plants.

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