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RESEARCH ARTICLE

Phosphate Solubilization by Rhizosphere Bacteria Isolated from Rose Garden Soils of Satkhol, India

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Abstract

In many of the agricultural soils, only a part of the phosphorus fertilizer applied is taken up by crops immediately necessitating its excess application by the farmers. Surplus phosphorus is stored in the soil for a long time in an unavailable form. With an objective of amending the phosphorus deficient agricultural soils, fertile garden soil from Satkhol were collected from the rhizospheres of rose plants. Five types of bacteria were consistently present in the soil suspensions plated on the Pikovskaya medium showing clearance, implying the degradation of rock phosphate. There were cocci and rods, some being gram positive and some gram negative. The strain S1.3 showed maximum solubilization of tricalcium phosphate with a significant increase over the control strain used. The findings indicate that the use of these promising phosphate solubilizers would release the unavailable form of phosphorus to crops in agricultural soils.

Keywords: Phosphorus fertilizer, rock phosphate, rhizospheres, rose plants, solubilization.

Introduction

Phosphorus is an indispensible mineral nutrient for the growth and yield of plants. Large amounts of chemical fertilizers are applied to provide inorganic phosphate to the soil. It is estimated that 75% of the applied phosphate fertilizer may be lost without reaching the plant sap (Goldstein, 1986). Phosphate solubilization is mainly due to the acid production of phosphate solubilizing bacteria that release in the medium, acids such as malic, glyoxalic, succinic , fumaric, tartaric, alpha, keto butyric, oxalic, citric, 2-ketogluconic and gluconic acid bringing the medium pH to as low as 2.0 (Kpomblekou and Tabatabai, 1994). Although many organisms have been reported to solubilize the bound form of phosphate, the environmental factors also influence the conversion of insoluble phosphorus to soluble one (Halder *et al.,* 1990). Apart from this environmental factor, the genes involved in solubilizing the locked up phosphorus in soil has been extensively studied (Miller *et al.,* 2010). In this study, we report solubilization of tricalcium phosphate by three different bacterial isolates from the garden soils of Satkhol, Uttarkhand.

Materials and methods

Sample collection and screening: Soil samples of Satkhol, Uttarakhand, collected from the rhizospheres of rose plants growing in three different gardens were used for making soil suspensions. One gram of soil was added to 9 mL of distilled water to make a suspension that was spread plated on Pikovskaya (PVK) agar medium with a pH 7.0 (Pikovskaya, 1948) containing 0.5 g of tricalcium phosphate (TCP) as sole phosphorus source for selectively screening the bacteria which have the ability to release inorganic phosphate from tricalcium phosphate. All the colonies grown on this screening medium were positive for phosphate solubilization. The positive strains were subcultured and purified for glycerol stock preparation and further use. An aliquot of 50 µL from each of the culture was plated in the wells bored in the centre of Pikovskaya agar plates. These plates were incubated for 7 d to check for halo formation.

Quantitative determination of phosphate solubilization: The selected strains were inoculated in Luria Bertani broth. Overnight grown cultures were subcultured in PVK broth and kept on a shaker cum incubator for 7 d at 30°C and at 200 rpm. All the glasswares were washed with 1:1 diluted hydrochloric acid and rinsed with distilled water. The cultures were centrifuged and the supernatant were used for quantitation of solubilized phosphate by Watanabe and Olsen's method (1965). pH of the broth was checked before inoculation and quantitative analysis.

Results

Rose garden soils were collected from Satkhol to check for any bacterial strain that has an efficacy to solubilize tricalcium phosphate amended in the screening medium. Three soil samples numbered S.1, S.2 and S.3 were diluted and spread plated on Pikovskaya medium (S.3 is indicated in Fig. 2). The isolates namely S1.1, S1.2, S1.3 were obtained from the soil sample S.1. Isolate S2.1 was the only positive strain from the soil sample, S.2 and in soil sample S.3, S3.1 and S3.2 were positive for phosphate solubilization.

Table 1. General characteristics of the bacterial strains.

Isolate	Colony morphology	Gram's reaction	Cell shape	Motility
S1.1	Yellowish white	+ve	Cocci	Motile
S1.2	Yellow	+ve	Rods	Motile
S1.3	Yellow	-ve	Rods	Non-motile
S2.1	Yellowish white	-ve	Cocci	Motile
S3.1	Yellowish white	-ve	Cocci	Motile

Table 2. Phosphate solubilization by bacterial strains isolated from soil samples.

Isolates	µmol P released/mL
E. coli	0.009±0.012
S1.1	0.875±0.073
S1.2	1.265±0.074
S1.3	2.155±0.015
S2.1	1.240±0.256
S3.1	1.333± 0.05

Fig. 1. Quantification of phosphate released by bacterial isolates.

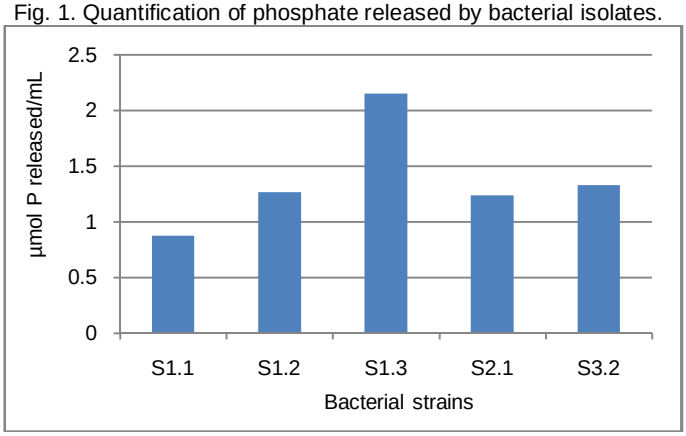
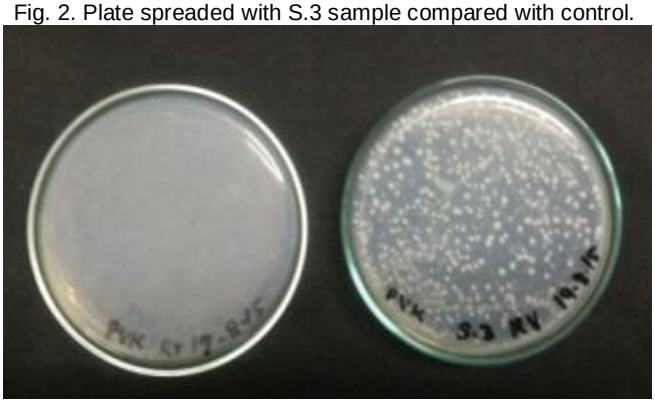


Fig. 2. Plate spreaded with S.3 sample compared with control.



Thus, a total of 6 bacterial strains distinct in colony morphology were recorded. Among these bacteria, S3.1 failed to grow subsequently. Hence, further analysis was carried out for the rest of the 5 strains. The general characteristics of the isolates are given in Table 1. The pH of the growth medium of all the strains drastically reduced at the end of study period. Initial pH was 7.0 and the final pH of each of the cultures is indicated in Table 1. In the blank treatment which was inoculated with *E. coli*, meager amounts of inorganic phosphate were detected and there was no drop in pH. Micromoles of phosphate released by the strains are shown in Fig. 1 and Table 2.

The quantity of phosphate solubilized by all the strains was significantly higher compared to the negative control, *E. coli*. The maximum efficacy of phosphate solubilization was observed in S1.3 culture. This was followed by S3.2 and S1.2. The rest of strains also were able to show remarkable solubilization of tricalcium phosphate. The pH of the samples was between 4.5 and 5.0, whereas that of *E. coli* was 6.8.

Discussion

Many soil bacteria and endophytic bacteria show phosphate solubilizing capacity. However, only restricted number of bacteria releases significant quantities of phosphate into the soil or plant sap. *Pseudomonas, Bacillus, Rhodococcus* and *Serratia* have been reported to be phosphate solubilizers by Wani *et al.* (2005). Nitrogen fixers have also been observed to solubilize phosphate by Zaidi *et al*. (2009). In a view to trap efficient phosphate solubilizers from fertile soils, garden soil samples were collected from Satkhol where the average prevailing temperature is around 20-28°C. The purpose of this study is to scale up the positive cultures and use them as biofertilizers for the agricultural fields which are poor in phosphate. S1.3 that showed higher amounts of phosphate release from insoluble phosphate (TCP) can be formulated so as to apply it to the commercial crops to increase the plant growth and yield. Contained field studies using this organism in combination with the plant growth promoting bacteria would reveal the potential of this organism in providing profit to farmers.

Conclusion

Almost all the PSB strains isolated from rhizosphere soils of rose plants showed efficient solubilization of insoluble phosphate compared to control strain which was *Escherichia coli*. Microbial solubilization of phosphate in soil is correlated with the ability of microbes in producing selected organic acids. It is generally accepted that the mechanism of mineral phosphate solubilization by PSB strains is associated with the release of low molecular weight organic acids.

The organic acids produced by the organism changed the pH of the growth medium to acidic. Further investigation is needed to exploit these bacterial strains as biofertilizers in agricultural soils.

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