RESEARCH PAPER | Microbiology | Volume : 4 | Issue : 2 | Feb 2014 | ISSN - 2249-555X

# Inorganic Phosphate Solubilization in Soil

KEYWORDS: Phosphate solubilizer, Tri calcium phosphate (TCP),Udaipur Rock Phosphate (URP), Citrobacter freundii

Pragya Rathore

Sanghvi Institute Of Management & Science Pithampur Bypass, Pigdamber, Rau- 453331, Indore

**ABSTRACT** *Indian agriculture is mainly dependent on the extensive use of chemical fertilizers to raise crop yield. Since 70 to 90 % of the supplemented super phosphate is not available for crops as a result of mineralization the requirements for this fertilizer are high. Hence there is need to increase the effectiveness of low grade Indian phosphate rocks. Phosphatic biofertilizers offer a viable alternative as these organisms can be inoculated directly along with the low grade rock phosphate and applied to soils. In the present study an attempt has been made to isolate phosphate solubilizing bacteria from soil, study their phosphate solubilizing activity and their utility as biofertilizer. The organism, Citrobacter freundii, has been screened from the local soil samples of Malwa region and was selected for the further studies as it shows efficacious solubilization of phosphates and being indigenous it is well adapted to the environment where it can be used as a biofertilizer.*

## Introduction

It is very important, particularly for poor countries often gravely short of phosphate in their soils, to know in what conditions it may be profitably used (Nye and Kirk, 1987). Rock phosphates are the nonrenewable resources for phosphate fertilizers. The long term application of P fertilizers has resulted in the accumulation of total soil P, most of which is poorly soluble. For this reason, the possibility of practical use of rock phosphate as a fertilizer has received significant interest in recent years. Recent researches in this regard are diverted towards more practical approach, i.e. the application of phosphate solubilizing microorganisms for solubilizaton of rock phosphates (Vasanthrajan, 1964; Vassilev et al., 1996). Other workers have also reported phosphate solubilization by Citrobacter species when provided with fructose as carbon source (Patel et al. 2008). The holistic P management involves a series of strategies involving manipulation of soil and rhizosphere processes, development of P efficient crops and improving P recycling efficiency (Seema B et al, 2013).

## Materials & Methods

### Solubilization of inorganic phosphates in soil:

A set of 12 flasks containing 500 mg of TCP, URP and BM in quadruplicate were inoculated individually with 100 g garden soil and the mixture was moistened. 6 flasks were steam sterilized for 3 consecutive days at 121°C for 2 hours each day. The remaining 6 flasks were unsterilized set. Except uninoculated control in each series the flasks were inoculated in duplicate in each series with 5 ml inoculum per flasks. The uninoculated flasks in each series served as control. In these flasks 5 ml of sterile distilled water instead of an inoculum. Loss of moisture was made up on every alternate day.

### Estimation of Phosphorus:

The concentration of phosphorus in soil was determined on 7th and 14th day by the $Na_2CO_3$ fusion method described by Jackson (1973). Various aliquots of 2 and 20 ppm standard stock solution of $KH_2PO_4$ were taken in 50 ml volumetric flask. A graph was plotted for concentrations of phosphorus against O.D. and the amount of phosphorus in the soil was extrapolated from the curve.

### Pot culture study:

The experiment was conducted in Kharif season using soybean as an experimental crop. A set of pots containing autoclaved and unautoclaved garden soil were kept. Each pot was filled with 9 Kg soil. The autoclaved soil used in the experiment was sterilized for 3 consecutive days at 121 °C for 2 hour each day. TCP and URP were applied to each pot so as to have 50 mg $P_2O_5$ /Kg of soil. The pots kept as controls for autoclaved and unautoclaved soil were kept without addition of TCP and URP.

### Seed Bacterization with Citrobacter freundii:

The seeds of soybean were surface sterilized by dipping in 0.1 % $HgCl_2$ followed by thorough washing with sterile distill water and dried overnight under sterile airflow (Brown, 1974). Then the seeds were bacterized by the method of Weller and Cook (1986). The treated seeds were examined for cfu on Pikovaskaya's agar (Pikovskaya 1948).

The bacterized seeds were sown in pots containing local unsterilized and sterilized soils in the set of experiment as shown below. For control seeds were treated with a sterile 1 % CMC suspension and grown under similar conditions. Each set of pot was performed in three replicas as per the scheme presented in **Table 1.**

**Table 1**
**Pot Culture study**

| S.N. | Autoclaved soil | Unautoclaved soil |
|---|---|---|
| 1 | Control | Control |
| 2 | Soil + TCP | Soil + TCP |
| 3 | Soil + TCP + *Citrobacter* | Soil + TCP + *Citrobacter* |
| 4 | Soil + URP | Soil + URP |
| 5 | Soil + URP + *Citrobacter* | Soil + URP + *Citrobacter* |

### Soil inoculation study:

Tropical soils are deficient in available phosphorus and thus make it unavailable for plant growth (Russel and Russel 1950; Somani *et al.*, 1990). Soil comprises both living and inert matter and various kinds of interactions are known to occur in it, it is mandatory to conduct soil inoculation studies to determine the actual impact of phosphate solubilizing microbes. It was suggested by Rose (1957) that the phosphate solubilizing capacity of different soils may be tested by inoculating a liquid medium with soil suspension. This may give an idea regarding a soil's total activity of phosphate solubilizing microorganisms. The solubilization of TCP in soil by inoculation of some *Pseudomonas sp.* was reported by Ostwal and Bhide (1972).

Volume : 4 | Issue : 2 | Feb 2014 | ISSN - 2249-555X

**RESULTS AND DISCUSSIONS**

In the present study the effect of *C. freundii* for solubilizing phosphates in soil was studied. For these studies autoclaved as well as unautoclaved soil was considered. Maximum phosphate solubilization was observed on 14th day for all the inoculated sets. The effect of C. freundii in phosphate solubilization of sterile and non – sterile soil was studied and was observed that the organism effectively solubilizes phosphorus in non-sterile soil. The organisms with good competitive ability colonize the non-sterile soil hence it is difficult to predict the competence of an organism based on taxonomic information (Milus and Rothrock, 1993). Since the organism in this study effectively solubilizes phosphate it can be said that it is competitive enough to work in consortia in non-sterile soil.

**Pot culture study:**

To check the efficiency of microorganisms in soil they were bacterized on seeds and their effects were studied. The seeds were sown (10 seeds per pot) in respective pots at suitable distance and watered everyday till the seedling appeared after which watering was done after every two days. At an interval of one month 3 plants from each pot were uprooted and washed repeatedly with running tap water and finally rinsed thoroughly in distilled water and blot dried. Different parameters of growth viz. root and shoot lengths, fresh weight and dry weight were recorded. Dry weight was measured by keeping the entire plant in a paper bag in an incubator at 70 °C for 24 hours (Chabot *et al.*, 1996).

The treated seeds were examined for colony forming units (CFU) on Pikovskaya's medium and approximated so as to give 8.9 x $10^6$ CFU / seed. The use of *C. freundii* as seed inoculant showed increased percent of root length; shoot height, fresh and dry weights and phosphorus uptake. Available phosphorus in the soil also increased. Enhanced root and shoot length on seed inoculation in both autoclaved and unautoclaved soil with both phosphate fertilizers (TCP and URP) was observed. The plant growth increased till the end of the experiment (90 days).

The organism proved superior for TCP to URP in terms of root and shoot lengths. URP proved better in autoclaved soil than in unautoclaved soil. With URP percent rise of root and shoot lengths was 1.8 times more in autoclaved soil than in unautoclaved soil.

**Available P in the soil:**

Total phosphorus of soil was analyzed as 10.61 mg% and the available phosphorus of soil came out to be 5.00 µg/g.

It is evident from the Table 2, available P in soil increased with addition of both the phosphate fertilizers and further increased with seed inoculation with autoclaved soil as well as unautoclaved soil. Enhanced plant growth vigour was observed in soybean inoculated with *C. freundii*.

**Table 2**
**Effect of seed bacterization with *C. freundii* on uptake of phosphorus**

| Treatments | P uptake by plant (mg P/plant), Day 90 | Available P on 90th day µg/g soil |
|---|---|---|
| Autoclaved | | |
| Soil | 36.71±0.91 | 8.32±0.24 |
| Soil+TCP | 45.32±0.80 | 8.76±0.17 |
| Soil+TCP+C | 53.63±0.09 | 13.35±0.49 |
| Soil+URP | 31.68±0.65 | 8.18±0.41 |
| Soil+URP+C | 50.17±0.37 | 11.68±0.35 |
| Unautoclaved | | |
| Soil | 32.83±0.25 | 8.21±0.07 |
| Soil+TCP | 36.15±0.32 | 8.96±0.21 |
| Soil+TCP+C | 37.31±0.68 | 13.73±0.28 |
| Soil+URP | 29.03±1.60 | 7.39±0.51 |
| Soil+URP+C | 43.02±0.55 | 9.18±0.53 |

**CONCLUSIONS**

The organism solubilizes phosphorus to higher extent in liquid medium as compared to soil because in broth readily metabolizing substrates are easily available while in soil the organism has to depend on the limited energy that is readily available rest it has to extract by spending its energy.

Non-sterile soil gave better solubilization as microorganisms work in consortia and various microbial interactions exert positive effect on solubilization.

When the seeds bacterized with *Citrobacter freundii* were inoculated in soil and various parameters were considered taking uninoculated seeds as control it was found that bacterized seeds could bring increase in fresh and dry weight of plant and also increase in root length and shoot height was observed.

Results of present work indicate promising use of the isolate for phosphate solubilization under field conditions in local soils.

**ACKNOWLEDEMENTS**

I extend my gratitude to Dr. H.H.Patel, Emeritus Scientist, Bhavnagar and Dr.K.C.Patel, Dept. Of Biosciences, VV Nagar, Gujarat for their valuable suggestions and positive support in all my endeavours.

**REFERENCE**

[1] Brown, M. E. (1974), "Seed and root bacterization", Ann. Rev. Phytopathol.12: 181-197. | [2] Chabot, R., Antoun, H., Kloepper, J. W. and Beauchamp, C. J. (1996), "Root colonization of maize and lettuce by bioluminescent Rhizobium leguminosarum biovar phaseoli.", Appl. Environ.Microbiol. 62: 2767-2772. | [3] Jackson, M. L. (1973), In: "Soil Chemical Analysis. Prentice Hall of India Pvt. Ltd." New Delhi, pp 134-182. | | [4] Milus, E. A. and Rothrock, C. S. (1993), "Rhizosphere colonization of wheat by selected soil bacteria over diverse environments",Can. J. Microbiol. 39: 335-341. | [5] Nye, P. H. and Kirk, G. J. D. (1987),"The mechanism of rock phosphate solubilization in the rhizosphere", Plant and Soil, 100: 127-134. | [6] Ostwal, K. P. and Bhide, V. P. (1972), "Solubilization of tricalcium phosphate by soil Pseudomonas", Ind. J. Exptl. Biol. 10: 153-154. | [7] Patel, D.K., Archana, G. and Kumar, G.N. (2008), "Variation in the nature of organic acid secretion and mineral phosphate solubilization by Citrobacter sp. DHRSS in the presence of different sugars",Curr Microbiol 56, 168–174. | [8] Pikovskaya, R.I. (1948), "Mobilization of phosphorus in soil in connection with vital activity of some microbial species",Microbiologiya. 17: 362- 370. | [9] Rose, R. E. (1957), "Techniques of determining the effect of micro-organisms on insoluble inorganic phosphates", N. Z. J. Sci. Technol.38: 773-780. | [10] Russel, E. and Russel, E. W. (1950), "In: Soil conditions and plant growth (8th Ed.), Longman Green & Co.", New York. pp 449. | [11] Seema B Sharma, Riyaz Z Sayyed, Mrugesh H Trivedi and Thivakaran A Gobi (2013), "Phosphate solubilizing microbes: sustainable approach for managing phosphorus deficiency in agricultural soils", Springer plus.2:587. | [12] Slininger, P. J., van Cauwenberge, J. E. , Bothast, R. J., Weller, D. M., Thomashow, L.S. and Cook, R.J. (1996), "Effect of growth culture, physiological state, metabolites and formulation on the viability, phytotoxicity and efficacy of the take- all biocontrol agent Pseudomonas fluorescens 2-79 stored encapsulated on wheat seeds", Appl. Microbiol. 45: 391-398. | [13] Somani, L. L., Bhandari, S. C., Vyas, K. K. and Saxena, S. N. (1990), "In: Biofertilizers. Scientific Publishers, Jodhpur", India. | [14] Vasanthrajan, V. N. (1964),"Inhibition of plant pathogenic fungi by Bacillus circulans solubilizing phosphorus", Indian J. Microbiol.4: 27. | [15] Vassilev, N., Vassileva, M. and Azcon, R. (1996), "Solubilization of rock phosphate by immobilized Aspergillus niger", Am. J. Microbiol.11: 1-4. | [16] Weller, D. M., and Cook, R. J. (1986), "Increased growth of wheat by seed treatments with fluorescent pseudomonads, and implications of Pythium control",Can. J. Plant Pathol. 8:328-334. |