



THE PHOSPHATE SOLUBILIZING POTENTIAL OF *CITROBACTER FREUNDII* UNDER VARIOUS PHYSICOCHEMICAL CONDITIONS

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ABSTRACT

There are considerable evidences that support the specific role of phosphate solubilization in the enhancement of plant growth by phosphate solubilizing microorganisms. In this regard we had isolated a bacterial stain which could solubilize phosphate to a considerable level, hence we selected the organism and subjected it to further studies involving different parameters. The effects of different conditions on phosphate solubilization by *Citrobacter freundii* from local soils of Indore were studied. The optimum concentration of phosphorus for maximum % solubilization was found to be 50 mg% for tricalcium phosphate (TCP). Studies on effect of temperature and shake and static conditions revealed that the organism gives maximum solubilization of phosphorus at $28^{\circ}\text{C} \pm 2^{\circ}\text{C}$ and under static condition of incubation. Maximum solubilization of phosphorus was reported between pH ranges 7-8.

KEY WORDS: Phosphate solubilization, *Citrobacter freundii*, pH, temperature

INTRODUCTION

In soil microorganisms play various roles and are also responsible for enhanced crop yields. Phosphorus is an essential element and its solubilization is one of the factors that help in increasing soil yield. In soil, phosphorus is found in a multitude of organic and inorganic compounds. Of inorganic forms much is in minerals where phosphate is part of structure or as orthophosphates: PO_4^{-3} , HPO_4^{-2} , $\text{H}_2\text{PO}_4^{-}$ and generally availability of these ions to the plants is in the order $\text{H}_2\text{PO}_4^{-} > \text{HPO}_4^{-2} > \text{PO}_4^{-3}$ (Kapoor *et al*, 1989). Microorganisms in soil are involved in the solubilization of inorganic phosphates and make it available to plants. The use of indigenous organisms

always proves to be more useful than the introduced ones in this regard. The phosphate solubilizing potential of the isolated organism under various physicochemical conditions on TCP and URP sources was studied. The solubilizing potential of the organism was demonstrated when culture media supplemented with insoluble tricalcium phosphate gave transparent zone around the colony after few days of incubation. Then the optimum concentration of phosphorus for maximum solubilization by the organism was determined. Temperature and incubation conditions were also optimized. Effect of different initial pH on solubilization was studied.

MATERIALS AND METHODS

1. *Organism*

Screening and isolation of phosphate solubilizing bacterial strains was carried out using Pikovskaya's agar (Pikovskaya R I, 1948) from soil samples collected from agricultural fields of Indore and surrounding area. Out of many isolates, a culture showing maximum solubilization during screening was selected for further studies and was identified as *Citrobacter freundii* MTCC 6738 by MTCC, Imtech, Chandigarh. The culture was maintained on Pikovskaya's agar pH 7.2 at 4°C and subcultured every month.

2. *Inoculum and Incubation*

Overnight grown culture of *Citrobacter freundii* in nutrient broth was harvested by centrifugation and resuspended in broth to have 1 O.D. mL⁻¹ at 660nm. Inoculum thus prepared was transferred under aseptic conditions to 100 mL Pikovskaya's broth in 250 mL Erlenmeyer conical flasks. In one experimental set up the broth contained TCP 50 mg% equivalent to 114.5 mg P₂O₅ 100 mL⁻¹. To study the phosphate solubilization from URP (27.6 mg% P₂O₅), it was added equivalent to 50 mg P₂O₅ 100 mL⁻¹. The flasks were incubated at 28°C ± 2°C under static conditions with intermittent shaking at 12hr interval up to 7 and 15 days for TCP and URP respectively.

3. *Phosphate estimation*

Soluble phosphorus from the broth was estimated using chlorostannous reduced molybdophosphoric acid blue method as it is reported to be highly sensitive and satisfactory method.

RESULTS AND DISCUSSION

1. *Effect of various physicochemical parameters on phosphate solubilization*

All the experiments were performed in triplicates and results are presented as mean SD (Standard Deviation) values.

1.1.1 *Different concentration of phosphorus*

The solubility of Calcium phosphates in water is a function of Ca:P ratio. Many types of calcium

4. *Reagents used*

Phosphorus standard: Primary (50 ppm of phosphorus) and Secondary (2 ppm of phosphorus), Sulphomolybdic acid solution, 2.5% and Chlorostannous acid reductant, 5%.

5. *Development of molybdophosphoric blue color*

The test solution aliquot is placed in 50 mL volumetric flask and then 2 mL of the sulphomolybdic acid solution is added with the pipette. 3 drops of chlorostannous acid reductant solution is added, mixed thoroughly, color intensity develops in 3-4 minutes and fades in 10-12 minutes. It is read photometrically with a 660nm light maxima, within the interval (Jackson M L, 1973).

6. *Calibration curve*

Since the phosphate concentration was measured as a function of absorbance, a standard curve of absorbance versus known phosphate concentration was prepared. The curve was prepared by plotting concentration of P vs. O.D. The value of phosphorus in the sample was obtained from the standard curve. This value of phosphorus was converted to P₂O₅ by multiplying it with the factor 2.29.

7. *Determination of pH*

The pH of supernatant was read on digital pH meter.

phosphate compounds can be found in soils. These compounds have a wide range of solubilities, which in general, follow an inverse relationship with the Ca/P ratio. For example, monocalcium phosphate [Ca (H₂PO₄)₂, Ca/P= 0.50] has a water solubility of 150,000 ppm at pH 7 whereas fluoroapatite [Ca₁₀ (PO₄)₆F₂, Ca/P= 1.66] has a water solubility of 0.003 ppm. Poorly soluble mineral phosphates such

as fluorapatite (rock phosphate ore) or hydroxyapatite can only be effectively dissolved in aqueous solution under acidic conditions. This dissolution is the result of acid-mediated proton substitution for calcium. Therefore, rock phosphate ores and a number of these commonly occurring soil calcium phosphates are of such low solubility as to be considered insoluble from the practical standpoint of a fertilizer material for an annual crop plant. Therefore, for such insoluble phosphates that have high Ca/P ratios, dissolution can be obtained by inoculating mineral phosphate solubilizing bacteria that secrete organic acids whose protons can be substituted for calcium ions (Goldstein A H, 1995). P solubilization was studied by using increasing concentration of TCP in the medium. This parameter was studied in order to find out optimum concentration of P that causes maximum solubilization because it has been observed that the lower the quantity of phosphate added to the

medium, the greater is the conversion to soluble form. Various concentration of TCP and URP were inoculated with the organism and when the % solubilization was calculated it was observed that 50 mg% TCP which is equivalent to 114.5 mg% P_2O_5 had released 72.1 mg % P_2O_5 in the medium showing 41.97 % solubilization which is optimum concentration for solubilization of P. URP showed maximum solubilization of 18.8mg% when used at 50 mg % P_2O_5 concentration in the medium. Increase in the concentration of URP in medium again decreased the extent of P solubilization (Table 1). In similar studies by Panhwar Q. A. et al(2012) Significantly, the highest available P (31.75 mg kg^{-1}) and P uptake (0.78 mg P Pot^{-1}) was found with their isolate PSB16 inoculated treatments at 60 kg $P_2O_5 ha^{-1}$, where as, highest biomass (82.25 mg $plant^{-1}$) and root growth obtained at 30 kg of $P_2O_5 ha^{-1}$.

Table 1 Effect of different concentration of P on TCP solubilization

Conc. of TCP (mg %)	Max P solubilized as P_2O_5 (mg %)	pH
50	72.1 (3) \pm 0.02	3.48 \pm 0.04
100	58.9 (6) \pm 1.08	3.76 \pm 0.35
150	60.1 (6) \pm 1.29	3.86 \pm 0.39
200	99.81(7) \pm 0.58	3.85 \pm 0.23
250	94.69(5) \pm 1.86	3.41 \pm 0.08
300	103.2 (7) \pm 1.90	3.51 \pm 0.01

1.1.2 Temperature, Shake and Static condition

Temperature is a vital factor for the growth and activity of microorganisms. 28°C \pm 2°C temperatures proved the best for TCP solubilization. pH was reduced up to 3.0 to 4.5 at all temperature ranges that permitted solubilization. Maximum solubilization of 72.1mg% occurred at 30° C after third day of inoculation. Above 40° C no turbidity was observed as the organism failed to grow above this temperature, which resulted in very less amount of P released in the medium. URP also displayed maximum solubilization of 18.8mg% at 28°C \pm 2°C. At 40 °C solubilization up to 10.09 mg% was

obtained for URP (Figure1). Above this temperature the organisms failed to grow. Since the organism is aerobic there was a need to study the effect of shake and static condition on phosphate solubilization. For both TCP and URP better solubilization up to three times occurred when the flasks were incubated under static conditions (Figure2).

1.1.3 Effect of pH

The pH is considered as one of the main factors responsible for efficient solubilization of inorganic phosphate. Media with different initial pH showed varied response. It did not show any relation between low pH and maximum solubilization. The

medium with initial pH 7.0 could solubilize TCP to the maximum extent. It could solubilize 72.1 mg % of P_2O_5 on third day of incubation. Next highest solubilization of TCP up to 71.38mg% by *C. freundii* was obtained at pH 8.0. Maximum solubilization of URP was obtained at pH 7.0 on 12th day. Next highest solubilization of URP was obtained at pH 5 and pH 6. Other pH range i.e., pH

3, 4, 8 and pH 9 did not show significant solubilization of URP. At all pH ranges fall in pH from the initial value was noticed and the changes in pH values obtained after solubilization lay in between pH 4.87 to 3.5. For both TCP and URP maximum solubilization was obtained at pH 7.0 (Figure3).

Figure 1 *Effect of temperature on maximum solubilization of TCP and URP*

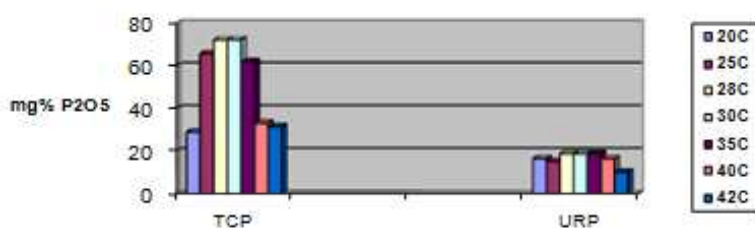


Figure 2 *Effect of aeration – agitation on maximum solubilization of TCP and URP*

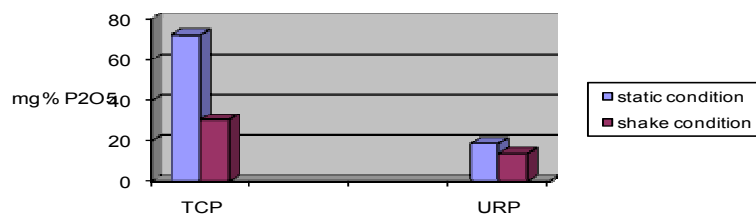
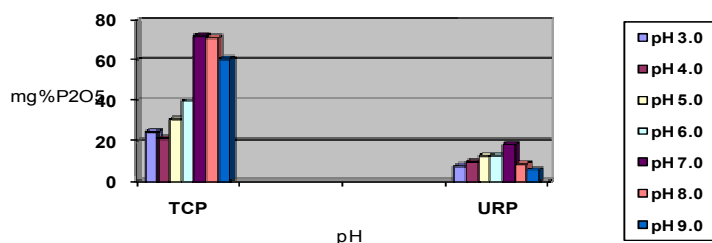


Figure 3 *Effect of different pH on maximum solubilization of TCP and URP.*



CONCLUSION

Phosphate is best solubilized at 50 mg% concentration, phosphorus limitation might positively affect the production of low molecular weight organic acids that in turn cause solubilization of phosphorus. In soil the fixation of applied phosphorus is believed to depend upon the concentration of P in soil solution (Marwaha B C, 1983). As the concentration of phosphorus in soil increases it results in fixation. A complete solubilization never occurs because some phosphate fractions of phosphate rocks are in such strongly bound form that even concentrated hydrochloric acid or sulfuric acid cannot bring them into solution. Good solubilization was observed when flasks were kept under static

condition. This suggests that under oxygen limitation this organism produces more amounts of organic acids which cause solubilization of phosphates. The organism seems to have better growth at the neutral pH. As the growth increased acid production in the medium also might increase bringing the pH to lower acidic side and thereby increasing solubilization with time. Initial acidic pH (or the alkaline pH) may not allow the efficient growth of the organism thereby affecting both acid production and phosphate solubilization. Hence the given strain is efficient phosphate solubilizer and its optimum temperature of solubilization is 30° C under static conditions and the optimum pH for solubilization is near neutral.

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