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Screening For Microorganisms Possessing Phosphate Solubilizing Potential

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ABSTRACT

Rhizosphere and non- rhizosphere soil samples from the agricultural fields of Indore and surrounding areas were collected for the isolation of phosphate solubilizing microorganisms. Soil samples which had been treated with herbicides, were also collected. Soils treated with herbicides like Pendimethalin, Triflusalin, Imaza 13, Squadron were screened for phosphate solubilizers. These soils contained very few bacterial species which could solubilize Phosphate. These species belonged mainly to the genus Bacillus and Pseudomonas. Among the fungal isolates from these soils were species of Aspergillus and Penicillium. The number of phosphate solubilizers in these treated soils was less as compared to untreated soils. However, Triflusalin treated soil had moderate number of phosphate solubilizers. Out of the 17 isolates 2 bacterial and 3 fungal cultures showing comparatively more solubilization ability during screening on Pikovskaya's agar and Pikovskaya's broth containing TCP were selected for their characterization

Keywords : screening, phosphate solubilizers, pikovskaya's media

Introduction

Phosphorus is one of the essential elements for all biological entities. It is associated with several vital functions and is responsible for several characteristics of plant growth such as utilization of sugars and starch, photosynthesis, nucleus formation and cell division, fat and albumin formation, cell organization and the transfer of heredity (Arnon 1956 and Mc Vicker et al. 1963). Microbial solubilization of inorganic phosphatic compounds is of great economic importance in plant nutrition. Phosphorus solubilizing bacteria and fungi play an important role in converting insoluble phosphatic compounds such as rock phosphate, bone meal and basic slag, and particularly the chemically fixed soil phosphorus, into available form (Narsian & Patel, 2000). Such organisms not only assimilate phosphorus but they also cause a large portion of soluble phosphate to be released in quantities in excess of their own requirements. The solubilization is not restricted to calcium salts but iron, aluminium, magnesium, manganese and other phosphates are also acted upon.

Several microorganisms responsible for solubilization of insoluble phosphates were found in great numbers in soil rhizosphere isolates (Sperber 1958, Azcon et al. 1976). High concentrations of P, Ca, and other elements were reported to occur in vesicular-arbuscular mycorrhizal (VAM) fungi (Strullu et al. 1981) as a result of oxalic acid production by the VAM fungi. The chelating effects of microbial products and other forms of soil organic matter were thoroughly reviewed by Kononova (1961).

Soviet Union for the first time used the culture of Bacillus megaterium var. phosphaticum as an inoculant for phosphorus solubilization. Canada used Penicillium bilaji with the trade name Provide. Phosphate solubilizing organisms thus were started to be used as inoculants to increase the availability of P to crop in these countries and has led to the development of inoculum preparation which is popularly known as phosphobacterin (Gaur, 1990).

In India, Sundara Rao and Paul first reported significant increase in the yield of berseem (Trifolium alexandrinum) due to inoculation of Phosphobacterin. Microbial inoculants can substitute almost 20-25% of the phosphorus requirement of plants. Most studies on phosphate solubilization were done by isolating microorganisms from soil and studying the extent of solubilization under in vitro conditions (Pandya and Saraf, 2010). Investigations on solubilization of insoluble phosphates under field conditions however, started later (Kapoor, Mishra et al, 1989).

Materials and Methods

Pikovskaya's medium with the following composition was used: Glucose10g, Tricalcium phosphate (TCP) 5g, Ammonium sulphate 0.5g, Sodium chloride 0.2g, Magnesium sulphate 0.1g, Potassium chloride 0.2g, Yeast extract 0.5g, Manganese sulphate and Ferrous sulphate Trace, Agar 15g,d/w 1liter, pH Adjusted to 7.0 ± 0.2.

Enrichment culture technique was employed for isolation of phosphate solubilizers. 1g of soil sample was added to a flask containing 100 ml Pikovskaya's broth (Pikovskaya, 1948). Three successive transfers were made at weekly intervals to enrich the medium. Then, phosphate solubilizers were isolated by streaking a loopful of the culture from the final flask on solid Pikovskaya agar medium. Colonies around which the clear zones of phosphate solubilization were obtained within 3-5 days were selected and transferred to fresh medium. Repeated subculturing was carried out till the pure cultures were obtained on the same media when grown at 30 °C. Pure cultures were maintained on Pikovskaya's agar slant at 4°C.

Screening of Phosphate Solubilizers:
Solubilization of TCP on Solid Medium:
All the isolated strains were inoculated separately on the Pikovskaya agar plates. These strains were spot inoculated on the plates under aseptic conditions. The plates were incubated at 28 ± 2 °C for 3-5 days and observed for solubilization zone around the colony. Those showing solubilization zone were presumed to be phosphate solubilizers and were further tested for solubilization in liquid medium.

Solubilization of TCP in Liquid Medium:
All the isolates selected from Pikovskaya agar and preserved on Pikovskaya slant, were inoculated separately in 250 ml Ehrlenmeyer flask containing 100 ml Pikovskaya broth with 1.0 ml of inoculum in each. All the flasks were incubated at 28 ± 2 °C for 21 days, under static condition and were shaken once at 12 hr interval. Uninoculated medium served as control. 10.0 ml medium was withdrawn on every third day and centrifuged at 10,000 rpm for 20 minutes. The supernatant was analyzed for its water soluble P content by the method given by Jackson (1973). The final pH of the medium was measured using digital pH meter.

Selection of Prominent Solubilizers:
Of the selected isolates, five organisms that showed maximum solubilization on the solid media as well as in the liquid broth were selected to determine their phosphate solubilizing activity in triplicate sets. Each flask was inoculated with 1.0 ml inoculum. Rest of the procedure was followed as discussed in solubilization of TCP in liquid medium.

Out of the five selected isolates, the one which gave maximum solubilization of phosphorus in broth was selected and characterized for further studies.

Maintenance of the Culture:
After sterilization of the Pikovskaya agar medium it was allowed to cool till the temperature dropped to 60°C, then 50 µg / ml of cycloheximide was added aseptically in order to avoid fungal contamination on plates and thus the culture was maintained on these plates.

Estimation of soluble Phosphorus:
Osmond method (Jackson, 1973) has been extensively adapted for phosphorus determinations.

Determination of pH:
The pH of the culture supernatant was measured using digital pH meter

Soils from rhizosphere of maize crop treated with various sources of nitrogen were also studied for phosphate solubilizers. Soil which had 100% recommended nitrogen through inorganic fertilizers were found to contain about 10 times more number of phosphate solubilizers as compared to untreated soil. Soils treated with nitrogen and phosphorus fertilizers were found to be 12-13 times rich in phosphate solubilizers. These data were calculated by comparing treated soil samples with untreated controls. Soil samples collected from rhizosphere and non rhizosphere soils of different crops from Indore and surrounding areas were inoculated separately in Pikovskaya's broth. For enrichment of phosphate solubilizers three successive transfers of this broth were made at weekly intervals. Then a loopful of culture was streaked on solid plates. Initially 17 different isolates were selected for P solubilization as all these isolates could produce zone of solubilization on modified Pikovskaya's agar plates (Gupta et al., 1994). Out of the 17 isolates 2 bacterial and 3 fungal cultures showing comparatively more solubilization ability during screening on Pikovskaya's agar and Pikovskaya's broth containing TCP were selected for their characterization.

Results and Discussions
On the basis of their morphological and cultural characteristics the two bacterial isolates were identified as the species of Bacillus and Citrobacter, while the three fungal species were identified as Aspergillus, Mucor and Penicillium.

Prior to selection of Citrobacter for further studies, the 5 isolates were spot inoculated on Pikovskaya's agar cotaining tricalcium phosphate. Phosphate solubilizers give clear zone around the colonies due to dissolution of phosphate. As depicted in Table 1, the zone of solubilizaton was largest for Citrobacter as compared to other four selected strains. Zone of solubilization was found to be 9 mm for Citrobacter, while for Bacillus it was 7 mm. Aspergillus produced 8 mm zone. For Penicillium and Mucor the zone size was 6 mm and 3 mm respectively.

Table 1
Phosphate solubilizing zone observed on Modified Pikovskaya's agar after 5 days by different isolates

Isolates	Colony diameter (mm)	Phosphate solubilizing zones (mm)
Bacillus	8	7
Citrobacter	10	9
Aspergillus	12	8
Mucor	12	3
Penicillium	15	6

Solubilization in liquid medium with TCP as source of P was also studied for the 5 organisms. TCP solubilization trend followed by these 5 isolates was as follows (Table 2).

TCP (mg % P_2O_5):
Citrobacter (167.70) > Penicillium (86.75) > Bacillus (64.80) > Aspergillus (59.16) > Mucor (55.58)

Table 2
Solubilization of TCP by 5 different isolates during growth on Pikovskaya's broth

Organism	(mg % P_2O_5)	pH of the broth	Day of max Solubilization
Bacillus		4.91	3
Citrobacter	64.8	4.13	6
Aspergillus	167.7	5.40	3
Mucor	59.16	4.69	9
Penicillium	55.58 86.75	5.13	9

Conclusions
The organism was screened from various soil samples from the local region. Among the various isolates obtained the one which gave maximum phosphate solubilizing activity was selected for further studies. An attempt was made to identify the organism by studying its morphological and biochemical properties and was identified as Citrobacter species. Later it was identified as Citrobacter freundii MTCC 6738 by MTCC, (IMTECH) Chandigarh. The screening of organism from the indigenous soils may prove to be better inoculants as compared to the introduced ones.

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