

A Study on the In-Vitro Assessment of the Efficacy of Phosphate Solubilizing Microorganisms



Agriculture

KEYWORDS: Robinia pseudoacacia, Tricalcium phosphate, Rock phosphates, P solubilisers

Anshu S. Chatli

Department of Microbiology, Guru Nanak Girls College, Ludhiana

Viraj Beri

Department of Soils, Punjab Agricultural University, Ludhiana

ABSTRACT

The solubilisation efficacies of a single or combination of three Phosphate dissolving microorganisms RBC2 (unidentified), RBC4 (unidentified) bacteria & *Penicillium* sp. isolated from rhizosphere of *Robinia pseudoacacia* were tested by using different sources of insoluble inorganic phosphates viz. tricalcium phosphate (TCP), Mussoorie Rock Phosphate (MRP) & Udaipur Rock Phosphate (URP) in Pikovskaya (PVK) & National Botanical Research Institute (NBRIP) broths. Among all the P-solubilisers studied, fungus *Penicillium* sp. solubilised maximum P while RBC2 (unidentified) bacterium the least. RBC4 (unidentified) & RBC2 (unidentified) bacteria & fungus *Penicillium* sp. in combination resulted in less P solubilisation than that by fungus alone, though it was more than the efficacy of bacteria alone. The fall in pH of broth during P dissolution was due to the production of oxalic & citric acids by microbes.

Introduction

Phosphate is an essential nutrient required by the plant & it is generally present in fixed forms. Hence, the large amount of phosphorus present in inorganic form is not available to plants & is often considered as a limiting factor of the plant growth. Phosphate solubilizing microorganisms (PSM) present in soil have the unique quality of bringing the immobile P into soluble form (Chatli et al, 2007; Panhwar et al., 2013; Chonker & Taraedar., 1984). PSM render the insoluble phosphates into soluble form through the process of acidification, chelation & exchange reactions. Rock phosphate (RP) is an economical source of phosphorus & hence, microbial solubilisation of RP & its use in agriculture is receiving greater attention. This process not only compensates for higher cost of production of fertilizers in industry, but also mobilizes the fertilizers added to soil (Chatli et al, 2008; Sharma et al, 2013; Roy Choudhary & Kaushik, 1989). In recent years, attempts have been made to increase the availability of naturally occurring cheap source of plant nutrients i.e. RP in soil. PSM have been used singly or in mixed inocula for improving the soil fertility. The dual effect of PSM can cater the needs of useful crops by enhancing the availability of P in soil. The present study was envisaged with the objectives to evaluate the phosphate dissolution abilities of PSM either singly or in combination using various sources of insoluble inorganic phosphates viz. tricalcium phosphate (TCP), Mussoorie rock phosphate (MRP) & Udaipur rock phosphate (URP) using Pikovskaya (PVK) & National Botanical Research Institute (NBRIP) broth.

MATERIALS & METHODS

Organisms & Maintenance

Three different phosphate solubilising microorganisms, RBC2 (unidentified), RBC4 (unidentified) bacteria & *Penicillium* sp. isolated from the rhizosphere of *Robinia pseudoacacia* of Indian Trans Himalayas, were screened on the basis of their P dissolution abilities in Pikovskaya (PVK) & National Botanical Research Institute (NBRIP) broth. These organisms were maintained on PVK agar. The purity of cultures was examined by growing them first in broth & then streaking on Pikovskaya agar containing TCP as source of phosphate at 28±2°C for 5 days.

Analysis of organic acids

The organisms were grown in PVK broth supplemented with TCP for 5 days under shake at 28±2°C. Qualitative analysis of organic acids was done by paper chromatography using solvent n-butanol: acetic acid: water (12:3:5) (Nordmann & Nordmann., 1960).

Estimation of phosphorus

10⁶ bacterial cells & 30x10⁵ fungal spores per ml were inoculated in 100 ml PVK & NBRIP broth, respectively, supplemented with various sources of soluble inorganic phosphates viz. TCP, MRP & URP.

The broths were incubated at 28±2°C for 13 days under shake at 250 rpm & the microbial vulnerability of these different sources of phosphates was examined. However, the P solubilising capabilities of these microbes in combination was also determined. The fungal culture along with bacteria were harvested by filtration through Whatman filter paper No.1 & then centrifugation at 15,000 rpm for 10 minutes. Uninoculated broths served as control. The solubilising P was determined in clear filtrate using Ascorbic acid method (Watanabe & Olsen., 1965). The intensity of blue color was measured on spectrophotometer at 730 nm & the quantity of the solubilised P was expressed as µg/ml. The final pH of culture filtrate was also determined. The each treatment was triplicated.

RESULTS & DISCUSSION

The screened microorganisms viz. RBC4 (unidentified), RBC2 (unidentified) & *Penicillium* sp. after incubation in PVK broth could solubilise TCP & produced mainly oxalic & citric acids (Table 1). These organic acids seem to be responsible for drop in pH. Our results are in consonance with that of Gaind & Gaur., 1989 & Singal et al., 1991.

Quantitative estimation of P solubilisation was carried out by RBC4 (unidentified), RBC2 (unidentified) & *Penicillium* sp. either singly or in combination by supplementing TCP, MRP & URP in PVK & NBRIP broth. Phosphorus of all the insoluble inorganic phosphate sources was vulnerable to microbial dissolution. Among all the P solubilisers studied, *Penicillium* sp. solubilised highest P ((93 µg/ml of TCP solubilised in PVK & NBRIP, 53.7 µg/ml of MRP solubilised in PVK & 54.7 µg/ml in NBRIP, 37.6 µg/ml of URP solubilised in PVK & 38.9 µg/ml in NBRIP) followed by RBC4 (unidentified) bacterium (83 µg/ml of TCP solubilised in PVK & 85 µg/ml in NBRIP, 49.1 µg/ml of MRP solubilised in PVK & 56.1 µg/ml in NBRIP, 28 µg/ml of URP solubilised in PVK & 30.1 µg/ml in NBRIP) & RBC2 (unidentified) bacterium (71.3 µg/ml of TCP in PVK & 72 µg/ml in NBRIP, 41.9 µg/ml of MRP solubilised in PVK & 49.9 µg/ml in NBRIP, 19.4 µg/ml of URP solubilised in PVK & 23.3 µg/ml in NBRIP). Singh et al (1984) also reported that the P dissolution ability of fungus is more than bacteria in broth under *in vitro* conditions. RBC4 (unidentified) & RBC2 (unidentified) bacterial isolates solubilised more P in combination (86 µg/ml of TCP solubilised in PVK & 87.9 µg/ml in NBRIP, 56.4 µg/ml of MRP solubilised in PVK & 59.5 µg/ml in NBRIP, 32.5 µg/ml of URP solubilised in PVK & 35.6 µg/ml in NBRIP) than individually indicating that the bacteria in combination can survive & solubilise P efficiently.

A combination of RBC4 (unidentified) bacteria & *Penicillium* sp. resulted in increased P solubilisation (84.9 µg/ml of TCP solubilised in PVK & 89.2 µg/ml in NBRIP, 54.2 of MRP solubilised in PVK & 58.2 µg/ml in NBRIP, 30.4 µg/ml of URP solubilised in PVK & 33.3 µg/ml in NBRIP) than that of bacteria alone, but it was less than that of

Penicillium sp. alone. RBC2 (unidentified) bacterium & *Penicillium* sp. also resulted in more P dissolution synergistically than bacteria alone but less than fungus alone. RBC4 (unidentified), RBC2 (unidentified) bacteria & fungus *Penicillium* sp. in combination resulted in less P solubilisation (86.1 µg/ml in PVK & 91.3 µg/ml in NBRIP) than that by fungus alone, though it was also more than the efficacy of bacteria alone. This could be due to the production of antifungal substances by bacteria. Singh *et al* (1984) also reported that *Pseudomonas striata* in combination with *Aspergillus awamori* inhibited fungal growth & its P solubilising efficiency. The decrease in pH of medium is due to the production of organic acids as a re-

sult of P solubilisation. A positive correlation has been reported between P solubilisation with the decrease in pH of filtrate.

Table 1 Detection of organic acids in culture filtrate of organism tested by paper chromatography

Test organism	Rf value	Organic acid
Bacteria		
RBC4 (Unidentified)	0.160	Oxalic acid
RBC2 (Unidentified)	0.170	Citric acid
Fungus		
<i>Penicillium</i> sp.	0.158	Oxalic acid

Table 2 Effect of P-solubilisers on the solubility of inorganic sources of phosphate in broth after 13 days of inoculation (supplemented with TC, MRP & URP)

	Treatment	P solubilised (µg/ml)	Final pH of broth
PVK	TCP		
	RBC4 (Unidentified)	83.0 ± 3.00	5.88 ± 0.026
	RBC2 (Unidentified)	71.3 ± 2.51	6.00 ± 0.015
	<i>Penicillium</i> sp.	93.0 ± 3.00	4.71 ± 0.015
	RBC4 (Unidentified) + RBC2 (Unidentified)	86.0 ± 0.152	5.80 ± 0.010
	RBC4 (Unidentified) + <i>Penicillium</i> sp.	84.9 ± 0.0577	5.82 ± 0.00
	RBC2 (Unidentified) + <i>Penicillium</i> sp.	75.1 ± 0.152	5.98 ± 0.0152
	RBC4 (Unidentified) + RBC2 (Unidentified) + <i>Penicillium</i> sp.	86.1 ± 0.435	5.81 ± 0.0152
	MRP		
	RBC4 (Unidentified)	49.1 ± 0.860	6.11 ± 0.01
	RBC2 (Unidentified)	41.9 ± 1.58	6.15 ± 0.005
	<i>Penicillium</i> sp.	53.7 ± 0.660	6.04 ± 0.037
	RBC4 (Unidentified) + RBC2 (Unidentified)	56.4 ± 0.230	6.08 ± 0.0152
	RBC4 (Unidentified) + <i>Penicillium</i> sp.	54.2 ± 0.321	6.10 ± 0.0115
	RBC2 (Unidentified) + <i>Penicillium</i> sp.	45.5 ± 0.416	6.17 ± 0.01
	RBC4 (Unidentified) + RBC2 (Unidentified) + <i>Penicillium</i> sp.	50.2 ± 0.360	6.13 ± 0.0
	URP		
	RBC4 (Unidentified)	28.0 ± 0.350	6.18 ± 0.011
	RBC2 (Unidentified)	19.4 ± 1.10	6.22 ± 0.025
	<i>Penicillium</i> sp.	37.6 ± 1.51	6.17 ± 0.015
	RBC4 (Unidentified) + RBC2 (Unidentified)	32.5 ± 0.458	6.20 ± 0.00577
	RBC4 (Unidentified) + <i>Penicillium</i> sp.	30.4 ± 0.458	6.26 ± 0.0115
	RBC2 (Unidentified) + <i>Penicillium</i> sp.	24.5 ± 0.458	6.33 ± 0.00577
	RBC4 (Unidentified) + RBC2 (Unidentified) + <i>Penicillium</i> sp.	33.3 ± 0.472	6.27 ± 0.0115

NBRIP	TCP		
	RBC4 (Unidentified)	85.0 ± 3.00	5.88 ± 0.020
	RBC2 (Unidentified)	72.0 ± 2.64	5.92 ± 0.015
	Penicillium sp.	93.0 ± 2.64	4.61 ± 2.56
	RBC4 (Unidentified) + RBC2 (Unidentified)	87.9 ± 0.152	5.74 ± 0.011
	RBC4 (Unidentified) + Penicillium sp.	89.2 ± 0.321	5.79 ± 0.015
	RBC2 (Unidentified) + Penicillium sp.	78.4 ± 0.264	5.91 ± 0.015
	RBC4 (Unidentified) + RBC2 (Unidentified) + Penicillium sp.	91.3 ± 0.435	5.76 ± 0.011
	MRP		
	RBC4 (Unidentified)	56.1 ± 2.00	6.08 ± 0.015
	RBC2 (Unidentified)	49.9 ± 1.10	6.11 ± 0.025
	Penicillium sp.	54.7 ± 0.305	5.98 ± 0.015
	RBC4 (Unidentified) + RBC2 (Unidentified)	59.5 ± 0.416	6.04 ± 0.025
	RBC4 (Unidentified) + Penicillium sp.	58.2 ± 0.305	6.07 ± 0.011
	RBC2 (Unidentified) + Penicillium sp.	48.3 ± 0.305	6.13 ± 0.005
	RBC4 (Unidentified) + RBC2 (Unidentified) + Penicillium sp.	52.2 ± 0.305	6.11 ± 0.015
	URP		
	RBC4 (Unidentified)	30.0 ± 0.150	6.11 ± 0.020
	RBC2 (Unidentified)	23.3 ± 1.85	6.18 ± 0.026
	Penicillium sp.	38.9 ± 2.94	6.12 ± 0.00
	RBC4 (Unidentified) + RBC2 (Unidentified)	35.6 ± 0.400	6.18 ± 0.017
	RBC4 (Unidentified) + Penicillium sp.	33.3 ± 0.264	6.23 ± 0.010
	RBC2 (Unidentified) + Penicillium sp.	26.3 ± 0.305	6.30 ± 0.005
	RBC4 (Unidentified) + RBC2 (Unidentified) + Penicillium sp.	35.3 ± 0.472	6.19 ± 0.010

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