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Effect of Various pH Values on the Efficacy of Phosphate Solubilising Microbes Isolated from Rhizosphere of Rose and China Rose

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ABSTRACT

Phosphate Solubilising Microbes (PSMs) were isolated and screened from the rhizosphere of Rosa and Hibiscus rosa sinensis. These microbes were tested to see their potentials to solubilise Tricalcium Phosphate (TCP) at different pH values (pH 4, 6, 7, 9 and 11) on Pikovskaya (PVK) agar media and broth. It was observed that bacterium (Micrococcus) showed its maximum efficacy to solubilse TCP under neutral to alkaline conditions while fungi under neutral to acidic conditions. Of the total PSF found, Non sporulating sterile formed biggest halo zone (12.2 mm) followed by Penicillium sp. (11 mm) and Aspergillus niger (7.3 mm) on PVK agar media. The Non sporulating sterile Fungus also dissolved maximum TCP (28.5 µg/ml) in PVK broth and decreased the pH of filterate (3.2) at pH 6 of media. Phosphate solubilising bacterium Micrococcus showed its maximum halo size (8.1 mm) and TCP dissolution (27.3 µg/ ml) at pH 9. This bacterium decreased the pH of filtrate to 6.4 at pH 9 of PVK broth.

Key words: *PVK, TCP, Phosphate solubilising microbes, pH values.*

INTRODUCTION

Soil is an uppermost layer of earth crust and is a mixture of organic matter, minerals and forms the intermediate zone between the atmosphere and the rock cover of the earth; the lithosphere. It also forms the interface between water bodies and the lithosphere, thus forming the biosphere[2]. It contains various beneficiary microbes which increase the soil fertility and enhance the growth of plants. Phosphorus is an essential macronutrient required by plants in abundance and for performing various functions such as nitrogen fixation, respiration, photosynthesis, cell division and many more[4]. The large amount of phosphorus present in soil as insoluble form, approximately 20% of phosphorus is available to plants[3].

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These Phosphate solubilising microbes solubilise precipitated Phosphate and mineralize Organic Phosphate to make it in available form to soil and produce organic acids such as oxalic acid, acetic acid, formic acid, citric acid etc. which results in a decrease in soil pH[5]. Soil pH is a major factor influencing the availability of elements in the soil for plant uptake. Bacteria growing in alkaline soils in India during the summer season are subjected to high salt, high pH, high temperature stress. In the alkaline soils of the tropics, pH may be as high as 10.5[6]. The phosphate Solubilising Microbes provide an eco friendly environment and have a positive impact on human health.

MATERIAL AND METHODS

Phosphate Solubilising Bacterium (PSB) (*Micrococcus*) and Phosphate Solubilisng Fungi (PSF) viz. *Aspergillus niger*, *Aspergillus fumigatus* and *Penicilium sp.* were isolated from rhizosphere of *Rosa* and *Hibiscus rosa sinensis* on Pikovaskaya (PVK) agar media. These P solubilisers were tested for their potentials to dissolute TCP on PVK agar on the basis of their halo sizes and in broth at different pH values (4, 6, 7, 9 and 11).

Determination of halo size:

The isolated Phosphate Solubilising Bacterial and Fungal strains were cultured on PVK agar media supplemented with Tricalcium Phosphate (TCP) at different pH values (4-11). The plates were incubated at 28±1°C for 3 days for bacterial and 6 days for fungal cultures. The solubilisation of Phosphate by microbes resulted with clear zone formed around the colony and its size was measured in mm. The efficacy of microbes to solubilise Phosphate at different pH value (4, 6, 7, 9 and 11) was determined. The experiment was done in triplicates.

Quantitative estimation of P solubilisation in culture broth:

10^6 bacterial cells and 30×10^5 fungal spores per ml were inoculated in 100 ml PVK broth supplemented with and TCP at different pH values (4-11) for 3 days for bacteria and 6 days for fungi under shake at 250 rpm. Uninoculated broth served as control. The solubilised phosphate in clear filterate was determined using Ascorbic acid method[1]. Then the intensity of blue colour was measured on spectrophotometer at 730 nm and the quantity of solubilised P was expressed as µg/ml. The final pH of culture filtrate and microbial biomass was also determined.

RESULTS AND DISCUSSION

Efficacy of Phosphate Solubilising Microbes in P solubilisation at different pH ranges (4-11)

pH had a diverse impact on the capabilities of Phosphate solubilisers to solubilise inorganic P. The efficacy of isolated phosphate solubilising microbial cultures were tested at different pH ranges (4 -11) in PVK agar and in PVK broth. It was found that as the pH value became more basic (pH 11), the growth of all PSM decreased and due to extremophilic conditions and the rate of TCP dissolution also decreased. On the other hand, as the pH value became more acidic (pH 4-6), the growth of PSF was increased and TCP got easily solubilised. But as the pH value was increased (pH 7-9), the activity of PSB to solubilise TCP accelerated. Hence, due to some alterations in pH value the activity of PSM was affected. Different PSMs have varied levels of inorganic P solubilisation at different pH ranges depending on their capabilities and nature.

P solubilisation activity on PVK agar media

Isolated Phosphate solubilising bacterial culture viz. *Micrococcus* formed large clear halo zone (8.1 mm) at pH 9 by solubilising inorganic phosphate on PVK agar supplemented with TCP. The size of the halo zone gradually increased from pH 4 (4 mm), pH 6 (5.2 mm), pH 7 (7.6 mm), pH 9 (8.1 mm) and decreased at pH 11. This is due to the extremophilic conditions that do not favour the growth of bacterial culture (Table 1) (Fig.1 A). On the other hand, in case of isolated Phosphate solubilising fungal cultures, Non sporulating sterile was found to formed large halo zone (12.2 mm) at pH 6 followed by *Penicilium sp.* (11 mm) and *Aspergillus niger* (7.3 mm). It was observed that in all the fungal cultures the size of the halo zone gradually increased from pH 4 to pH 6 by solubilising TCP and decreased as the pH became more alkaline (pH 7 to pH 11) (Table 1) (Fig.1B)

P Dissolution activity in broth

The isolated PSB *viz. Micrococcus* showed the maximum TCP dissolution (27.3 μg/ ml) at pH 9. The highest decrease in pH (6.4) was also reported at pH 9 by *Micrococcus*. Phosphate Solubilising Fungal (PSF) isolates were also tested for their ability to solubilise TCP in PVK broth. Among all the fungal isolates, Non sporulating sterile was found to be highly efficient with maximum TCP solubilisation (28.5 μg/ml) followed by *Penicillium sp*. (25.2 μg/ml) (Fig.2 B) and *Aspergillus niger* (23.5 μg/ml) (Fig.2 A) at pH 6. Moreover Non sporulating sterile also showed maximum decrease in pH of filtrate (3.2) followed by *Penicillium sp.* (3.3) and *Aspergillus niger* (3.5) at pH 6 (Table 2). The microbial biomass produced was found to be maximum in case of fungi Non sporulating sterile (0.65 g) as compared to bacterium *Micrococcus* (0.05g) (Table 3). It was observed that the fungal isolates showed maximum P solubilising efficacy in acidic conditions (pH 4-6) while PSB showed it from neutral to alkaline value (pH 7-9). The similar results were observed by Gaind and Gaur[7], Chatli *et al.*[8].

Table 1: Effect of various pH values (4 -11) on halo zone (mm) formation by Phosphate solubilisers on PVK agar

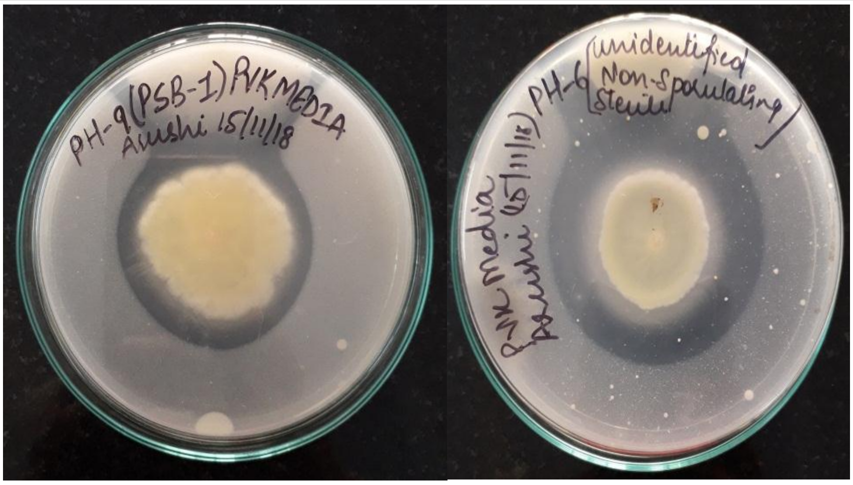
Phosphate solubilising Microbal cultures	pH values				
	4	6	7	9	11
	Halo zone (mm)				
Aspergillus niger	5.5	7.3	4.5	2.6	1.4
Penicillium sp	9.3	11	7.2	5.3	2.2
Unidentified non-sporulating sterile	11.1	12.2	10.3	6.8	4.6
Micrococcus	4	5.2	7.6	8.1	6.7

Table 2: TCP solubilisation (μg/ml) and Decrease in pH in PVK broth by Phosphate solubilisers at different pH values (4-11)

Phosphate solubilising Microbial cultures	pH values					
		4	6	7	9	11
Aspergillus niger	TCP solubilised	22.8	23.5	21.1	20.4	14.32
	Decrease in pH	3.7	3.5	5.7	6.6	10.5
Penicillium sp	TCP solubilised	23.5	25.2	21.8	20.7	17.7
	Decrease in pH	3.5	3.3	5.1	5.8	10.4
Unidentified non-sporulating sterile	TCP solubilised	24.0	28.5	23.2	21.8	19.1
	Decrease in pH	3.4	3.2	4.9	5.2	10.2
Micrococcus	TCP solubilised	23.5	24.9	26.0	27.3	19.7
	Decrease in pH	3.8	5.2	6.0	6.4	10.7

Table 3: Microbial biomass (g/100ml) in PVK broth after TCP solubilisation at different pH values (4-11)

Phosphate solubilising Microbial cultures	pH values				
	4	6	7	9	11
Aspergillus niger	0.45	0.52	0.26	0.23	0.14
Penicillium sp	0.42	0.57	0.30	0.28	0.17
Unidentified non-sporulating sterile	0.49	0.65	0.38	0.23	0.18
Micrococcus	0.04	0.05	0.06	0.08	0.05



(A) (B)

Fig. 1: Solubilisation of TCP by (A) Micrococcus at pH 9 and (B) Non-sporulating sterile at pH 6 on PVK agar media

(A) (B)

Fig. 2: Dissolution of TCP by (A) *Aspergillus niger* and (B) *Penicillium* at pH 6 in PVK broth

CONCLUSION

Soil that covers the earth's crust contains diverse varieties of microbes, macronutrients, micronutrients which facilitate the growth of plants The Phosphate Solubilising Microbes (PSMs) help in solubilisation of inorganic Phosphate for the growth and development of plants. The efficacy of Phosphate Solubilising Bacteria to solubilise inorganic Phosphate was found to be maximum at pH 9 (alkaline) while Fungi showed their highest efficacy of TCP solubilisation at pH 6 (acidic).

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