

Effect of Chelating Agent on the Efficiency of Phosphate Solubilisers

Arushi Makkar^{1*}, Anshu Sibbal Chatli², Navdeep Kaur¹ and Parneet Kaur²

P.G. Students, Department of Biotechnology, Guru Nanak Girls College, Model Town, Ludhiana (Punjab)-, India¹

Assistant Professor, Department of Microbiology, Guru Nanak Girls College, Model Town, Ludhiana (Punjab), India²

ABSTRACT: Phosphate Solubilising Microbes (PSM) were isolated from Rhizosphere of *Rosa* and *Hibiscus rosa sinensis* from two different areas of Ludhiana (Urban Estate and Gorahoor). These microbes were tested to see their potentials to dissolve Tricalcium Phosphate (TCP) on Pikovskaya agar (PVK), MPVK agar and PVK broth supplemented with different EDTA concentrations (0.2 g, 0.5 g, 0.8 g, 1.0 g /250 ml). The decrease in pH and microbial biomass was also determined. Among microbial isolates Nonsporulating sterile was found to form maximum halo size followed by *Micrococcus*, *Penicillium sp.*, *Aspergillus niger* and *Aspergillus fumigatus* on MPVK agar at 0.8 g/250 ml. The increase in P solubilisation and microbial biomass with decrease in pH of filtrate was also observed.

KEYWORDS: Phosphate Solubilising Microbes, EDTA, PVK

I. INTRODUCTION

Soil harbours various beneficiary microorganisms which are required to improve its fertility. These microbes work incognito by mutual interaction to maintain soil ecological balance by active participation in natural nutrient cycling (Talwar and Chatli, 2018). Phosphorus is the major essential nutrient present in the soil. Being a macronutrient, it is needed in abundance by plants. Phosphorus (P) increases the metabolic processes in plants including nitrogen fixation, respiration, photosynthesis, cell division and many more (Sharma *et al*, 2011). Although the amount of Phosphorus is abundant in soil but available in insoluble form which can not be taken up directly by the plants. The availability of soluble Phosphorus is approximately 20% (Chonker and Taraeder, 1984). So inoculation of various efficient P solubilisers in soil is mandatory to dissolve rest 80% of insoluble P. The deficiency of Phosphorus may cause various plant diseases such as browning of leaves, weak stem, poor development of plants and small leaves. So to meet up the required amount of Phosphorus by the plants, it should be present in available form. To increase the soil fertility and to make the environment eco-friendly, rather than using fertilizers as the source of Phosphate, plant growth promoters can be used. Solubilisation of insoluble sources of P is also affected by various abiotic stresses such as pH, temperature, salt concentration, moisture etc. In acidic soils certain chelating agents such as siderophores, organic acids etc also bind with Calcium of soil rock phosphate and hence render the availability of P in soluble form. Certain Amino acids are also used as chelating agents to provide numerous benefits to plants (Korat *et al*, 2001). EDTA (Ethylenediaminetetraacetic acid), a chemical chelating agent also forms a Calcium-EDTA complex and make Phosphorus free for plants (Johnston, 1956).

II. RELATED WORK

Parma and Dadarwala (1997) observed that in the surrounding rhizosphere the occurrence of TCP dissolving bacteria was maximum on the roots of Chickpea, Mustard, Watermelon, Lucerne and Maize. Chatli (2005) studied the effect of chelating agent Disodium EDTA on P solubilisation. It was observed that the addition of EDTA upto 0.8 mg/ ml resulted in increase in P solubilisation and fungal biomass with decrease in pH of culture filtrate. Goswami *et al* (2018) tested the efficacy of screened Phosphate solubiliser by using PVK and MPVK agar supplemented with Tricalcium Phosphate (TCP) as insoluble phosphate source. These solubilisers formed yellow halos around their colonies on

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MPVK agar. Mardad *et al* (2014) demonstrated the effect of Carbon, Nitrogen sources and Abiotic stress on P solubilisation by Bacterial strains. It was observed that the addition on 1 mg/ ml of EDTA increased the levels of Phosphate released.

III. MATERIAL AND METHODS

Isolated Phosphate Solubilising Bacterium (PSB), *Micrococcus* and Phosphate Solubilising Fungi (PSF), *Aspergillus niger*, *Aspergillus fumigatus* and *Penicillium sp.* from different areas of Ludhaiana (Urban Estate and Gorahoor) on Pikovskaya (PVK) and Modified Pikovskaya (MPVK) agar media were tested for their efficacy to solubilise P at different EDTA concentrations.

Determination of halo size:

Bacterial and Fungal isolates were cultured on PVK and MPVK agar media supplemented with Tricalcium Phosphate (TCP) as insoluble inorganic Phosphate source and different EDTA concentrations (0.2 g, 0.5 g, 0.8 g, 0.1 g / 250 ml). The plates were incubated at $28\pm 1^\circ\text{C}$ for 3 days for bacterial and 6 days for fungal cultures. The solubilisation of Phosphate by microbes was indicated by clear zone formed around the colony and its size was measured in mm. The halo size around the colony was calculated by subtracting the colony size from total size. The efficacy of microbes to solubilise Phosphate at different EDTA concentration 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 with 100 ml PVK broth supplemented with TCP and 0.8 g EDTA for 3 days in bacteria and 6 days in fungi under shake at 250 rpm. Uninoculated broth served as control. The solubilised phosphate in clear filtrate was determined using Ascorbic acid method (Watanobe and Olsen, 1965). Then the intensity of blue colour was measured on spectrophotometer at 730 nm and the quantity of solubilised P was expressed as $\mu\text{g/ml}$. The final pH of culture filtrate and microbial biomass was also determined.

IV. RESULTS AND DISCUSSION

Effect of EDTA on phosphate solubilisation

The effect of chelation on solubilisation of inorganic phosphate by PSM was studied by adding 0.2, 0.5, 0.8, 1.0 g/250 ml of EDTA to PVK agar and broth.

P solubilisation activity on agar media

Addition of 0.8g/250 ml of EDTA formed large halo zone after dissolving TCP in case of Non sporulating sterile (10.4 mm) (Fig.2) followed by *Micrococcus* (9.8 mm) (Fig. 3), *Penicillium sp.* (9.5 mm), *Aspergillus niger* (7 mm), *Aspergillus fumigatus* (5 mm) in PVK agar (Table 1). This could be due to the formation of calcium EDTA complex, due to chelation of Ca^{2+} ions with EDTA. Increase in EDTA concentration beyond 0.8 g/250 ml caused decrease in halo size. It was observed that there was large halo zone on MPVK media than PVK, due to the interaction of bromophenol blue dye with that of genes of organism which resulted in increase in phosphatase activity. It was observed that when *Micrococcus* was kept under refrigeration condition at $4-7^\circ\text{C}$, the zone size was increased, indicating more P solubilisation and its Psychrophilic nature (Fig. 4).

P solubilisation activity on broth

The isolated Non sporulating sterile showed maximum TCP solubilisation (45.71) at 0.8 g/250 ml of EDTA concentration followed by *Micrococcus* (40.94), *Penicillium sp.* (36.16), *Aspergillus niger* (27.29), *Aspergillus fumigatus* (22.51) in PVK broth (Table 2) (Fig. 1). Moreover these microbial cultures showed decrease in pH of

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filtrate and obtained maximum microbial biomass (Table 3) at 0.8 g/ 250ml EDTA concentration. The similar results were observed by (Chatli, 2005; Nautiyal *et al*, 2000)

TABLE 1: Effect of EDTA on halo zone formation (mm) after solubilisation of inorganic phosphate (TCP) by Phosphate solubilising microbes on Pikovskaya (PVK) and Modified Pikovskaya (MPVK) agar

Phosphate Solubilising Microbial cultures	Concentration of EDTA (g/250 ml)							
	0.2		0.5		0.8		1.0	
<i>Aspergillus niger</i>	HALO ZONE (mm)							
	PVK	MPVK	PVK	MPVK	PVK	MPVK	PVK	MPVK
	2.5	3	4.5	5.2	6	7	3	3.2
<i>Aspergillus fumigatus</i>	1.5	2.2	3	3.5	4.3	5	2	2.5
<i>Penicillium sp.</i>	2.6	3.4	5.5	6.3	8	9.5	3.5	3.7
Unidentified non-sporulating sterile	4.2	5	6	7.5	9.8	10.4	4	4.5
<i>Micrococcus</i>	3.5	4	5.8	6.6	9	9.8	3.7	4.2

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TABLE 2: Effect of EDTA (0.8 g/250 ml) on TCP solubilisation and Decrease in pH by Phosphate solubilising microbes in Pikovskaya broth (PVK)

Phosphate Solubilising Microbial cultures	TCP solubilise ($\mu\text{g/ml}$)	Decrease in pH
<i>Aspergillus niger</i>	27.29	4.6
<i>Aspergillus fumigatus</i>	22.51	4.75
<i>Penicillium sp</i>	36.16	4.45
Unidentified non-sporulating sterile	45.71	4.2
<i>Micrococcus</i>	40.94	4.32

Initial pH of broth = 6.8

TABLE 3: Microbial biomass (g/100 ml) in PVK broth after TCP solubilisation supplemented with EDTA (0.8 g)

Phosphate solubilising Microbial cultures	Microbial biomass (g/100ml)
<i>Aspergillus niger</i>	0.28
<i>Aspergillus fumigatus</i>	0.23
<i>Penicillium sp</i>	0.32
Unidentified non-sporulating sterile	0.38
<i>Micrococcus</i>	0.10

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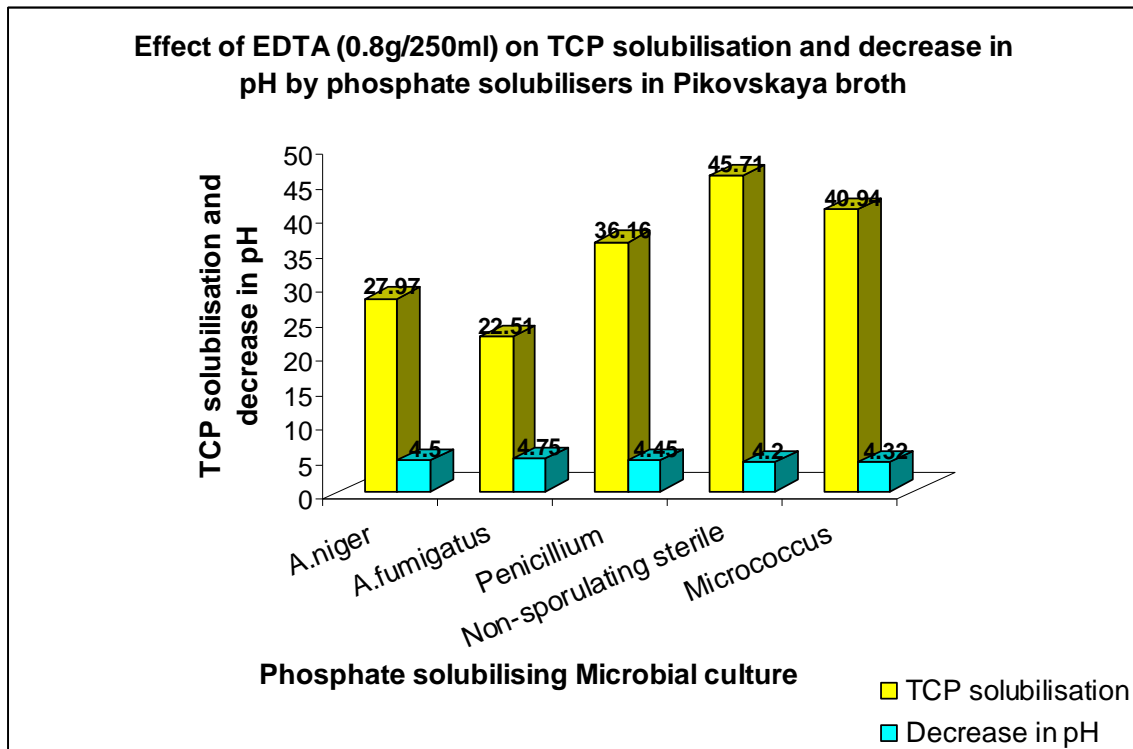


Fig.1: Effect of EDTA (0.8 g/250ml) on TCP solubilisation and Decrease in pH by phosphate solubilisers in Pikovskaya broth

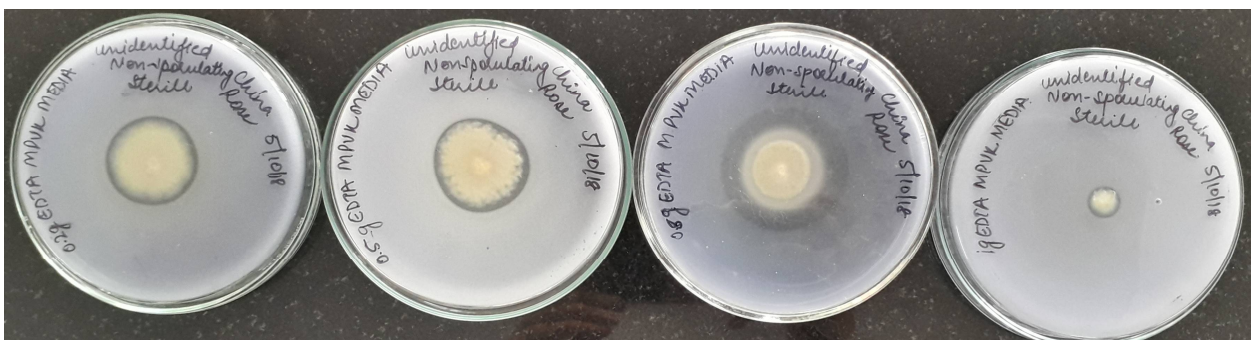


Fig. 2 : Solubilisation of TCP by Nonsporulating sterile on MPVK agar supplemented with different EDTA concentrations (0.2, 0.5, 0.8, 1.0 g/250 ml)

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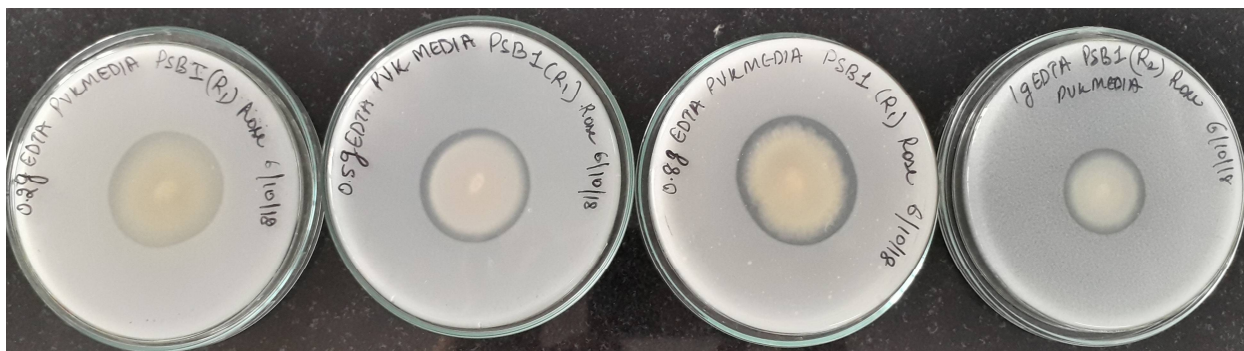


Fig. 3: Solubilisation of TCP by *Micrococcus* on PVK agar supplemented with different EDTA concentrations (0.2, 0.5, 0.8, 1.0 g/250 ml)

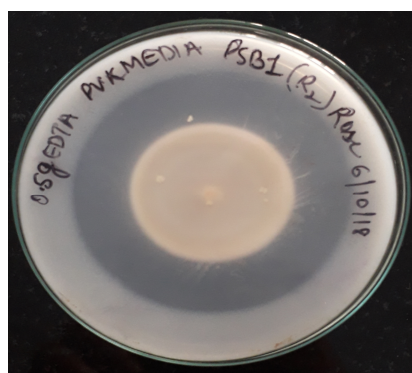


Fig. 4 : Increase in Halo Size by Phosphate solubilisers under refrigeration in the presence of EDTA

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