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Phosphorus solubilizing activity of *Mycobacterium cosmeticum* under various cultural conditions

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Abstract

Effect of various cultural conditions on the phosphorus solubilizing activity of *Mycobacterium cosmeticum* was tested under different parameters such as carbon (Glucose, Fructose, Sucrose, Maltose and Lactose), nitrogen (Ammonium sulphate, Sodium nitrate, Potassium nitrate and Urea), pH (5.0, 7.0 and 9.0) and temperature (25°C, 35°C and 45°C) using Pikovskaya's medium amended with 0.5% tri calcium phosphate (TCP) as an insoluble source of P. Solubilization efficiency of TCP by *M.cosmeticum* varied with different carbon and nitrogen sources in both plate and broth assay. The results of plate assay revealed that P-solubilizing ability of the isolate was more in the presence of glucose (237.3%) as C-source and potassium nitrate (140.2%) as N-source. Whereas for the broth assay lactose was found to be the best (9.0 mg/l) and ammonium sulphate was the best nitrogen source (6.5mg/l). The isolate exhibited its maximum ability to solubilize TCP at the pH of 7.0 and at the incubation temperature of 35^{0}C in both the assays. Thus the study confirmed the efficiency of *M.cosmeticum* to solubilize insoluble form of P under various cultural conditions and can be used as a source of Bioinoculants to eradicate P deficiency in plants and this study was the first to report P-solubilizing activity of *M.cosmeticum*.

Keywords: *Phosphorus; Mycobacterium cosmeticum; solubilization; cultural conditions.*

1. Introduction

Phosphorus (P), the second important macro-nutrient plays a vital role in plant progression and is considered as the most significant growth limiting factor for many crop productions in India due to its limited availability in the soils. Due to the unavailability of phosphorus in soil, plants cannot meet their requirement which lead to the large utilization of phosphorus chemical fertilizers by farmers (Islam *et al.*, 2007). Approximately 70-90% of P fertilizer applied to the soil gets converted into insoluble forms due to the presence of Fe and Al in acidic soils and Ca in neutral and alkaline soils (Harris *et al.*, 2006) resulting in poor availability to plants and also adversely affect both environment and economy (Park *et al.*, 2010).

. Theoretical estimates have suggested that the accumulated phosphorus (P) in agricultural soils due to fixation is sufficient to sustain maximum crop yields world-wide for about 100 years (Khan *et al.*, 2010). During the last two decades knowledge on phosphate solubilizing microorganisms are increased significantly which mobilize insoluble mineral phosphate in a sustainable and eco-friendly manner (Richardson *et al.*, 2001).

High proportion of phosphate solubilising microorganisms is concentrated in the rhizosphere, and they are metabolically more active than other sources (Vazquez *et al.*, 2000). A considerably higher concentration of phosphate solubilizing bacteria is commonly found in the rhizosphere in comparison with non-rhizosphere in soil (Raghu and MacRae, 1966). Most of the bacterial species, those associated with the plant rhizosphere, are able to exert a beneficial effect upon plant growth (Hilda and Fraga, 1999). The major mechanism involved in the P solubilization is the production of organic acids and chelating oxo-acids from sugars ((Antoun and Kloepper, 2001).

Cultural conditions have a major impact on the growth of microbes and the production of microbial products. Although microorganisms could be used effectively for metal solubilization in the soil, the environmental factors that vary in time affect the metabolic activities of microorganisms responsible for metal solubilization. Understanding the importance, microorganisms isolated from the agricultural field must be optimized before it is exploited as Bioinoculant (Miao *et al.*, 2006).

In the present experimental study P-solubilizing activity of *Mycobacterium cosmeticum* using various cultural parameters such as carbon, nitrogen, pH and temperature was tested under *in*

vitro conditions by both qualitative (plate assay) and quantitative (broth assay) method.

2. Materials and Methods

Mycobacterium cosmeticum was isolated from the rhizospheric soil samples of maize field and was tested for its phosphorus solubilization efficiency by growing them in Pikovskaya's medium amended with 0.5% tri calcium phosphate as an insoluble source of P. The formation of halo zone around the colony in the agar medium indicated its P-solubilization efficiency. The isolate was identified using universal primer 16S rRNA at Yaazh Xenomics, Chennai. Effect of various parameters such as carbon sources, nitrogen sources, pH and temperature on the efficiency of P-solubilization by *M. cosmeticum* was tested qualitatively (Plate assay) and quantitatively (broth assay) by following methods.

Plate assay

Effect of various carbon sources (glucose, fructose, sucrose, maltose and lactose), nitrogen sources (ammonium sulphate, sodium nitrate, potassium nitrate and urea), Temperature (25°C, 35°C and 45°C) and pH (5.0, 7.0 and 9.0) on the phosphorus solubilizing activity of *Mycobacterium cosmeticum* were assessed using Pikovskaya's medium amended with 0.5% tricalcium phosphate as insoluble source by the method of Shahab and Ahmed, 2008. Inoculation was carried out by placing 10µl of culture at the centre of the Petri plates and was incubated at 37°C for five days. By measuring the diameter of clear zone and organism's growth P solubilization efficiency was tested (Nyugen *et al* 1999).

Broth assay

To find out the amount of P solubilized by *Mycobacterium cosmeticum* under various cultural conditions, the isolate was inoculated into the 100ml Erlenmeyer flasks containing 50ml of liquid basal medium amended with 0.5% tri calcium phosphate. Appropriate uninoculated controls were maintained. All the treatments were replicated. The bacterial cultures were withdrawn on the 5^{th}, 7^{th} and 9^{th} days of incubation at 37°C for the estimation of soluble P. The amount of P solubilized by *M.cosmeticum* in the cultural filtrate under varied cultural conditions would be assessed by the method of Bray and Kurtz, 1945. The P content was expressed as mg/l.

3. Results and Discussion

The phosphate solubilizaton activity of *Mycobacterium cosmeticum* in the presence of varying carbon, nitrogen, pH and temperature was studied by plate assay method (qualitative estimation) and broth assay method (quantitative estimation).

Carbon is the main constituents of cellular material and forms an important requirement for the organism's growth and metabolic activities. Therefore the P-solubilizing activity of *Mycobacterium cosmeticum* using different sources of carbon was studied. The study revealed that for qualitative estimation, glucose proved to be the best carbon source (237.3%). Whereas for the quantitative estimation lactose was found to be the best (9.0 mg/l) (Table: 1). This variation in the utilization of carbon sources is due to the fluctuation in their behavior of PSM. Sri and Kannapiran, 2011 reported that there was no correlation between P solubilization efficiency on solid and liquid medium. Thus, among different carbon sources tested glucose and lactose enhanced more solubilization than maltose.

One of the mechanisms that explain solubilization activity results from the secretion of protons associated with the uptake of ammonia (Roos and Luckner, 1984). The different source of nitrogen such as ammonium sulphate, sodium nitrate, potassium nitrate and urea were used to test the P-solubilizing activity of *Mycobacterium cosmeticum*. The study showed that for qualitative assay, potassium nitrate was found to be the best source of nitrogen for PSB-3 (140.2%) and for quantitative estimation ammonium sulphate was the best nitrogen source for PSB-3 (6.5mg/l) (Table: 2). Solubilization efficiency of TCP by *M.cosmeticum* varied with different nitrogen sources. This could be due to the production of inorganic acids by proton exchange mechanism in presence of NH_4^+ that cause accelerated phosphate solubilization (Halder *et al.*, 1991). Based on the experimental results ammonium sulphate was found to be best in reducing the medium pH to 3.75 and simultaneously increases solubilization.

pH plays a important role in solubilization of metal, as drop in pH indicates the solubilization potential of the isolates. The influence of pH on the P-solubilizing activity of *Mycobacterium cosmeticum* was studied at different pH levels 5, 7 and 9. The strain exhibited its maximum ability to solubilize TCP at pH 7.0 in both the assays. Rashid and Ryan, 2004 reported the existence of negative correlation between pH of the culture and solubilization of metals. Shahab and Ahamad, 2008 demonstrated that all the isolates solubilized the insoluble P in the pH range 5-7. The present result revealed that most of the microorganisms prefer neutral pH for their metabolic activities.

Micro organisms adapt to their indigenous environment which indicates the importance of temperature for their metabolic activities. The efficiency of the isolates varies with the incubation temperature. The study was conducted to find out the optimum temperature for the growth and solubilization efficiency of the selected isolates. In the present study, the isolate solubilized TCP more effectively at 35^0 C incubation temperature than at 25^0 C and 45^0 C in both the assay. The result was

concordance with Sayer and Gadd, 1997 who reported 25^0 C as the optimum temperature for better solubilization of insoluble metals. Whereas Shahab and Ahmad, 2008 reported that 20^0C was the best temperature for solubilization.

Table : 1 Effect of different carbon sources on the phosphorus solubilization activity of *Mycobacterium cosmeticum*

Carbon sources	**Solubilizing efficiency (%)**	**Amount of P solubilized (mg/l)**
Glucose	113.8 ± 1.4	**9.0 ± 1.5**[a]
Fructose	118.8 ± 1.5	4.0± 2.0[c]
Sucrose	107.9 ± 0.4	4.5 ± 0.5[d]
Maltose	103.7 ± 0.6	3.17 ± 0.76[bc]
Lactose	**237.3 ± 2.3**	8.5 ± 1.0[b]

Table : 2 Effect of different nitrogen sources on the P solubilization activity of *Mycobacterium cosmeticum*

Nitrogen sources	Solubilizing efficiency (%)	Amount of P solubilized (mg/l)
Ammonium sulphate	139.7 ± 1.9	6.5 ± 1.0[a]
Sodium nitrate	122.5 ± 1.1	4.2 ± 0.86[c]
Potassium nitrate	140.2 ± 1.4	5.5 ± 1.5[bc]
Urea	103.4 ± 0.6	3.8 ± 0.55[b]

Table : 3 Effect of different pH on the P solubilization activity of *Mycobacterium cosmeticum*

pH	**Solubilizing efficiency (%)**	**Amount of P solubilized (mg/l)**
5	126.1 ± 1.1	4.0 ± 0.85[c]
7	**130.8 ± 1.6**	**7.5 ± 0.7**[a]
9	120. 2 ± 1.9	5.0 ± 0.4[b]

Table : 4 Effect of different temperatures on the P solubilization activity of *Mycobacterium cosmeticum*

Temperature (°C)	**Solubilizing efficiency (%)**	**Amount of P solubilized (mg/l)**
25	128.3 ± 0.6	4.8 ± 0.76[b]
35	**128.6 ± 1.0**	**5.6 ± 0.60**[a]
45	114.4 ± 0.6	2.7 ± 0.15[c]

4. Conclusion

The present investigation forms the first to report P-solubilizing activity of *Mycobacterium cosmeticum* under varied cultural conditions. Thus it can be utilized as a bioinoculant to eradicate the use of chemical fertilizers and also to enhance soil P-availability to plants in a sustainable way.

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