



Interactive Effect of Efficient P Solubilising Fungi in Solution Culture

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Abstract

The synergistic effect of P solubilisers viz. *Aspergillus niger*, *Aspergillus flavus* and *Trichoderma sp.* in dual and triple culture was assessed in liquid PVK and NBRIP broth. Highest TCP solubilisation was observed by triple inoculation of *Aspergillus niger*, *Aspergillus flavus* and *Trichoderma sp.* (565 µg/ml) in NBRIP broth, followed by dual culture of *Aspergillus niger* and *Trichoderma sp.* (443 µg/ml) in NBRIP broth. In PVK broth, the efficacy of these P solubilisers was less than that in NBRIP broth. In PVK broth, triple culture of *Aspergillus niger*, *Aspergillus flavus* and *Trichoderma sp.* also represented maximum TCP solubilisation (436 µg/ml) followed by dual culture of *Aspergillus niger* and *Trichoderma sp.* (310 µg/ml). The highest pH reduction was observed in triple inoculation rather than double inoculations of fungal cultures in both PVK and NBRIP broth. The decrease in pH of broth was also maximum in NBRIP broth as compared to that in PVK broth.

Keywords: P Solubilisers, PVK broth, NBRIP broth, Synergism, TCP Solubilisation

Introduction

The microbial growth is mainly dependent on the presence of available Phosphorus (P) in soil. The oxidation and reduction of P compounds occur in cyclic manner thus maintaining balance in nature (Ohtake et al., 1996). The output of plants is mainly dependent on available P in soil (Daniels et al., 2009; Chatli et al., 2017). But the major amount of P is present in insoluble form (Goldstein, 1965) and it has to be solubilized (Chatli et al., 2008). The use of chemical phosphate fertilizers is a costly proceeding and there is an urgent need for development of some ecofriendly alternative sources. There are various

mechanisms for P solubilisation but the principle mechanism is the production of organic acids (Naik et al., 2013) and chelating agents (Makkar et al., 2019). Also, acid phosphatases play major role in the mineralization of insoluble Phosphorus in soil. Phosphate solubilising microbes (PSM) have been used singly and in mixed cultures for improving the soil fertility. The mutual effect of PSM can serve as the most efficient key for enhancing soil fertility by dissolving inorganic P. Thus the present study was undertaken with the objective:

- To evaluate the phosphate dissolution abilities of coinoculating PSM in Pikovskaya (PVK) and National Botanical Research Institute (NBRIP) broth using tricalcium phosphate (TCP) as insoluble source of P.

Materials and Methods

The fungal cultures (*Aspergillus niger*, *Aspergillus flavus* and *Trichoderma sp.*) were isolated on Potato Dextrose Agar (PDA). The isolated fungal cultures were identified by following standard AOAC methods (Sidhu et al., 2007).

Quantitative estimation of P solubilisation in culture broth

30×10^5 fungal spores per ml in dual and triple culture respectively were inoculated in 100 ml PVK and NBRIP broth, respectively for 6 days under shake at 250 rpm. Uninoculated broth served as control. The solubilised P was determined in clear filtrate using Ascorbic acid method (Watanabe and Olsen, 1965). Then the intensity of blue color 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 was also estimated.

Results and Discussion

Table 1: Synergistic effect of Phosphate Solubilising Fungi (PSFs) on TCP solubilisation ($\mu\text{g/ml}$) after 6 days

PSFs	PVKbroth		NBRIP broth	
	TCP Solubilised ($\mu\text{g/ml}$)	pH	TCP Solubilised ($\mu\text{g/ml}$)	pH
<i>Aspergillus niger</i> and <i>Trichoderma sp.</i>	310	3.55	443	3.50
<i>Aspergillus niger</i> , <i>Aspergillus flavus</i> and <i>Trichoderma sp.</i>	436	3.50	565	3.48

Conclusions

These results show remarkable synergism in triple culture in comparison to dual and single cultures under *in vitro* conditions. The phosphate solubilisers can prove to be beneficial for the soils of Punjab when

Synergistic effect of interacting microbes in liquid medium

Based on the efficiency of Phosphate Solubilising Fungi (PSFs) to solubilise TCP in PVK and NBRIP broth, the maximum P solubilisation was observed in triple cultures of *Aspergillus niger*, *Aspergillus flavus* and *Trichoderma sp.* in NBRIP broth ($565 \mu\text{g/ml}$) and in PVK broth ($436 \mu\text{g/ml}$) followed by dual inoculation of *Aspergillus niger*, *Trichoderma sp.* in NBRIP broth ($443 \mu\text{g/ml}$) and in PVK broth ($310 \mu\text{g/ml}$) (Table 1).

The efficacy of these PSF was less when used singly (Kaur et al., 2019). A positive correlation was found between P-solubilisation with the decrease in pH of filtrate. Similar results were observed by Goswami et al., 2018. The maximum decrease in pH of broth was observed in triple culture inoculation of *Aspergillus niger*, *Aspergillus flavus* and *Trichoderma sp.* (3.50 in NBRIP broth and 3.55 in PVK broth) (Table 1). Similar results were reported by Chatli et al (2006) who also observed the interactive effect of PSMs in liquid culture. The efficacy of these PSMs may be due to more production of organic acids when used in triple culture than in dual culture.

The synergistic effect of PSM was reported to be highest due to maximum interaction of these microbes with each other resulting in their increased efficiency to solubilise P than used singly.

inoculated synergistically. This interaction between P solubilisers can be used in improving soil fertility. These organisms can be used on commercial scales by various Government and Non-Government organizations for improving the economy of country too.

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