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PHOSPHATE SOLUBILIZATION POTENTIAL OF RHIZOSPHERE FUNGI ISOLATED FROM AGRICULTURAL FIELDS OF MARATHWADA REGION

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ABSTRACT Phosphate solubilizing fungi play a noteworthy role in increasing the bioavailability of soil phosphates for plants. The present study was aimed at isolating and characterizing phosphate solubilizing fungi from rhizosphere soil. Fungi isolates was examined on Pikovskaya's agar medium containing recalcitrant P sources, including $Ca_3(PO_4)_2$ and microscopic appearance by lactophenol cotton blue staining technique. All fungal isolates were evaluated for their P-solubilizing ability. Total 40 isolate were isolated and only 11 fungal isolates were showed P-solubilizing ability. Among these PQ9, PQ24 and PQ33 which was identified as *Aspergillus fumigatus. Aspergillus niger, Aspergillus flavus, Penicillium spp.* and *Trichoderma spp.* by morphological and biochemical test. These isolates showed maximum zone of solubilization with 34, 31 to 30 mm selective agar medium after 48 hrs of incubation respectively. The potent phosphate solubilising fungi were identified 18S rRNA analysis. The fungal species isolated from the Rhizosphere soil can be use in soils that are deficient in Phosphorous or where insoluble Phosphorous is abundant. The present study concluded that the use of Phosphorous solubilising fungi in the Phosphate deficient soil will help to enhance the growth and yield of crop. Therefore, these species can be candidate and exploited after further evaluation as bio fertilizers for crop productivity.

KEYWORDS : Phosphate solubilization. Rhizosphere soil, fungi and Marathwada region.

INTRODUCTION

Phosphorus (P) is one of the most important macro elements for plant growth and development[1-3]. Phosphorus contributes remarkably to photosynthesis, energy and sugar production, nucleic acid synthesis, and promotes N_2fixation in legumes[4]. In plants, phosphorous increases the strength of cereal straw, promotes flower formation and fruit production, stimulates root development and essential for seed formation.[5] It also plays a role in root development, stalk and stem strength, flower and seed formation, maturity and production, crop quality and resistance to plant diseases[6]. Mobility of phosphate ions in the soil is very low due to their high retention in soil and the recovery rate of P fertilizer by plants is only about 10-30% [7]. The remaining 70-90% is accumulated in soil or in the form of immobile that is bound by Al or Fe in acid soils, or Ca and Mg in alkaline soils[8]. Phosphorus is highly insoluble and unavailable to plants. It must be converted into soluble form. Phosphate solubilizing microorganisms can play an important role in dissolving both of fertilizer phosphorus and bound phosphorus in the soil that is environmentally friendly and sustainable. Soil is an uppermost layer of earth's crust and is a mixture of organic matter, minerals and organisms that together support life. Soil plays a vital role in maintaining the balance of earth's ecosystem[7]. Soil is one of the important and valuable resources of the nature. It is composed of particles of broken rock that have been altered by chemical and mechanical processes that include weathering and erosion. All living things are directly and indirectly dependent on soil for day to day needs and 95 % of the human food is derived from the earth. Soil has complex function which is beneficial to human and other living organism. It acts as a filter, buffer storage, transformation system and thus protects the global ecosystem against the adverse effects of environmental pollutants[8]. Rhizosphere is a dynamic changing environment that differs from bulk soil both in physical and chemical properties.Phosphorus solubilizing activity is determined by the ability of microbes to release metabolites such as organic acids, which through their hydroxyl and carboxyl groups chelate the cation bound to phosphate, the latter being converted to soluble forms. Phosphate solubilization takes place through various microbial processes/ mechanisms including organic acid production and proton extrusion. A wide range of microbial P solubilization mechanisms exist in nature and much of the global cycling of insoluble organic and inorganic soil phosphates is attributed to bacteria and fungi[9].

Several groups ofmicroorganisms including fungi, bacteria and actinomycetes are knownas efficient fixed P solubilizes[10]. Fungi are the important componentsof soil microbes typically constituting more of the soil biomass thanbacteria, depending on soil depth and nutrient conditions. Fungi havebeen reported to have greater ability to solubilize insoluble phosphatethan bacteria[11]. A wide range of soil fungi are reported to solubilizeinsoluble phosphorous such as *Aspergillus niger* and *Penicillium* sp. which are the most common fungi capable of phosphate solubilization[12]. Exploration of phosphate solubilizing microorganisms hasbeen conducted by many researchers from soils, mangrove andrhizosphere[13-14] respectively.

The beneficial plant-microbes interactions in the rhizosphereare determinants of plant health and soil fertility[15]. Among the rhizosphere microbes, the important generaof P-solubilizing bacteria include *Rhizobium, Bacillus*, and *Pseudomonas*[16-17]. *Penicillium* and *Aspergillus* spp. are the dominant P-solubilizing filamentous fungi found in rhizosphere[18]. Filamentous fungi are highly importantin RP solubilization. They are widely used as producers oforganic acid. *Aspergillus niger* and some *Penicillium* species have been tested for solubilization of RP and other biotechnologicalimportance such as biocontrol, biodegradation, and phosphate mobilization[19-20]. In Marathwada region, now a day, large number of fertilizers are used instead of manures due to this the crop productivity increases speedily but the quality of the soil and the microbial diversity is decreasing day by day. However, information on thediversity of phosphate solubilizing fungi inhabiting variousrhizospheres in this region is limited. The present study wastherefore designed to isolate and characterize the phosphatesolubilizing fungi isolated from various rhizospheric soils.

MATERIALS AND METHODS

The Study Area

The study was conducted in Marathwada region of Maharashtra, India occupied Marathwada has total area of 64590 km^2. The study areas are located in between 230-300 km north of Mumbai, the capital city of Maharashtra. Marathwada region lies between 17^0 35' north latitude to 20^0 41' north latitude and 70^0 40' east longitude to $78^0$16' east longitude. (Fig.1).

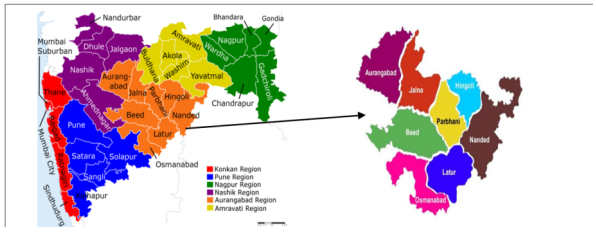

Fig.1: Study Area (Marathwada region of Maharashtra, India)

Collection of Samples: A total of 10 rhizosphere soil samples were collected from wheat rhizosphere from 10 locations of Marathwada. The study primarily focused on testing of soil samples collected from four representative sites namely Aurangabad, Jalna, Beed and Parbhani district of Marathwada. After collection, a portion of each sample was immediately transferred to laboratory and stored at 4^0C for microbial analysis.

Screening and of Phosphate Solubilizing Fungi: Collected rhizosphere soil samples were used for the isolation of phosphate solubilizing fungi on Pikovskaya's (PKV) agar medium, containing the following (g/L): 0.5 g $(NH_4)_2SO_4$, 0.1g $MgSO_4 \cdot 7H_2O$, 0.02g NaCl, 0.02g KCl, 0.003g $FeSO_4 \cdot 7H2O$, 0.003 g $MnSO_4 \cdot H2O$, 5g $Ca_3(PO_4)_2$, 10.0g glucose, 0.5g yeast extract, 15.0g agar, and 1000mL distilled water[21] (Pikovskaya, 1948).The medium was autoclaved at 121^{0}C for 15minutes; about 20mL of the sterilized molten agar medium was poured into each petri dish and supplemented with 25 μg/mL chloramphenicol to inhibit bacterial growth and allowed to solidify before inoculation. The appearance of a transparent halo zone around the fungal colony indicated the phosphate solubilizing activity of the fungus and the diameter of the zone was measured in millimeters.

Identification and Characterization of Phosphate Solubilizing: Fungi. PDA was used to accelerate the growth rate andthe production of enough conidia as reported by[22] Diba *et al*., 2007. The characteristics of fresh cultures were compared with mycological identification keys and taxonomic description[23] to identify the isolated fungi to the genus level. Identification was based on colony characteristics and microscopic features, among the colonial characteristics such as surface appearance, texture, and colour of the colonies both fromupper and lower side. In addition, conidia, conidiophores, arrangement of spores, and vegetative structures were determined with microscopy. The identified fungi were maintained on Potato Dextrose Agar (PDA) slant at (4^{0}C) for further investigation. Slide culture was prepared in order to identify spores and mycelia of pure fungal isolates. Accordingly, themorphology of spores and mycelia of fungal isolates was examined and identified by lacto phenol cotton blue staining using microscope and identified after growing them on slide according to Stevens[24]

Qualitative analysis of Phosphate solubilisation

All the suspected colonies were screened for phosphate solubilization on Pikovskayas medium. Isolates showingphosphate solubilizing ability were spotinoculated at the centre Pikovskaya's plate and incubated at 37^{0}C. Diameter of clearance zone was measured successivelyafter 24 hours, up to 7 days. Then Phosphate Solubilization Efficiency (PSE) is the ratio of total diameter. i.e. clearance zone including bacterial growth and the colony diameter.

$$PSI = \frac{\text{Colony diameter + Halozone diameter}}{\text{Colony diameter}}$$

All the observations were recorded intriplicate. Strains developing clear zonesaround their colonies could easily identifyas PSF.

Quantitative analysis of Phosphate solubilisation

Pikovskaya's broth medium (100 ml) with Tricalcium phosphate (0.3g/100ml) was prepared and sterilized; 1ml of each isolate was inoculated into the broth medium. Then the inoculated sample were incubated for 5 days on rotatory shaker370C after incubation, culture broth was centrifuged at 10,000rpm for 30min.Uninoculated broth served as control. The available Phosphorous was determined using colorimetrically at 410nm with standard KH_2PO_4.

RESULTS AND DISCUSSION

In this study, a total of 40 fungal isolates were obtainedfrom 10 rhizosphere soil samples from different location of Marathwada District. Out of the isolated fungi, a total of 11 phosphate solubilizing fungal cultures having potential of phosphate solubilization were isolated (Table1).

Table: 1. List of obtained ZSB isolates from wheat rhizosphere at different area of Latur district

Sr.No	Location	No. of isolates	Name of the isolates
1.	Aurangabad	12	PQ1
2.			PQ2
3.			PQ3
4.			PQ4
5.			PQ5
6.			PQ6
7.			PQ7
8.			PQ8
9.			PQ9
10.			PQ10
11.			PQ11
12.			PQ12
13.	Jalna	8	PQ13
14.			PQ14
15.			PQ15
16.			PQ16
17.			PQ17
18.			PQ18
19.			PQ19
20.			PQ20
21.	Beed	11	PQ21
22.			PQ22
23.			PQ23
24.			PQ24
25.			PQ25
26.			PQ26
27.			PQ27
28.			PQ28
29.			PQ29
30.			PQ30
31.			PQ31
32.	Parbhani	9	PQ32
33.			PQ33
34.			PQ34
35.			PQ35
36.			PQ36
37.			PQ37
38.			PQ38
39.			PQ39
40.			PQ40
	Total	**40**	

Table 2: Phosphate solubilization of potent fungal isolates

Sr.No	Culture number	Zone diameter in mm
1.	PQ3	21mm
2.	PQ7	24 mm
3.	PQ11	25 mm
4.	PQ13	30 mm
5.	PQ14	22mm
6.	PQ19	34 mm
7.	PQ21	22 mm
8.	PQ24	26 mm
9.	PQ36	32 mm
10.	PQ37	23 mm
11.	PQ40	21mm

Table 3: Colony morphology and microscopic characteristics of the fungal isolates.

Sr. No	Culture number	Colony morphology	Microscopic observations	Name of isolates
1.	PQ3, PQ11, PQ21	Coloniesgrew rapidly on PDA and initiallywhite floccose mycelium spreadingrapidly and quickly become blackcolor colonies with production ofblack spores Reverse is white to pale yellow.	Conidia were small, black, brownishblack, green in colour. Septatehyphae with rough brown andsmooth colorless conidiophores with distinctive conidial heads(flask-shaped)	*Aspergillus* species
2.	PQ7, PQ24	Colonies were initially white and turned yellowish green to light green (Figure 1(a)).	Septate, hyaline, acute angle branching, tree- or fan-like branching. The organism is characterized by green echinulate conidia	*Aspergillus fumigatus*
3.	PQ13, PQ40, PQ14	Colonies on potato dextrose agar at 25°C are olive to lime green with a cream reverse. Rapid growth. Texture is woolly to cottony to somewhat granular. Sclerotia, when present, are dark brown	Septate distinct, bearing acluster of branches,phialides born on cylinder branchesand arranged in brush-like headSingle celled spherical conidiaremaining together in one chain with the youngest at the base of chain.	*Aspergillus flavus*

4.	PQ19, PQ37	Colonies are initially white, change to a brownish red color and later to green or bluish green color. The colony surface appears flat and powdery.	Septate, hyaline, acute angle branching, tree- or fan-like branching.	*Penicillium* spp.
5.	PQ36	Viride appears to be a bit granular on PDA, with green conidia distributed throughout. An irregular yellow zone without conidia was present around the inoculum. Some white pustules were also found growing on the green mat of conidia.	Irregularlybranched, not VerticillateSingle celled, not remaining together in one chain, grouped in small clusters held together by slime	*Trichoderma spp.*

In the present study, three PSF isolates namely, PQ13 (30mm), PQ19 (34) and PQ36 (32) produced maximum zone of solubilization. It was observed that PQ19 is potent phosphate solubilizing bacteria which showed 34 mm zone of solubilization than PQ36 and PO13. Phosphorus deficiencies are wide spread on soil throughout the world and one of the limiting factors for crop productivity. Phosphorus fertilizers represent major cost for agricultural production. Many bacteria, fungi and a few actinomycetes are potential solubilizes of bound phosphates in soil thus playing an important role makingit available to plants in the soluble form Nitrogen, phosphorous and potassium are the main soil nutrients for normal germination, growth and maturity of plants. The availability of nitrogen depends on the varying degree of soil microbial decomposition[25] (Gairola and Soni, 2010). Application of phosphorus is necessary for maintaining a balance between the other plant nutrients and ensuring the normal growth of the crop. Phosphorous act as a limiting or co-limiting factor of ecosystem productivity[26-27]. and low P availability can constrain N_2 fixation[28-29]. Chhabra *et al.,* (1996) have shown that available manganese decreased with soil pH and available copperincreased with clay and organic carbon content[30].

A distinct variation was observed in fungal and spore morphology among the phosphate solubilizing *Aspergillus* and *Penicillium* and *Trichoderma* spp isolates. On the basis of fungal and spore morphology, three distinct groups of Aspergillus isolates were formed and these were: 8 of the *Aspergillus* isolates produced white floccose mycelium; 7 with velvety green mycelium and 14 with white cottony loose woven thread like mycelium forming black-brown spores in due course. Moreover, Penicillium isolates formed three separate groups and these were: 3 of the Penicillium isolates produced velvet, dark green spreading mycelium; 5 withdark green to turquoise colony and 9 with yellow mycelial colony forming dark grey, blue-green to yellow-green spores in due course.

All fungal species evaluated for their phosphate solubilization ability on Pikovskaya (PVK) selective media. Among all 11fungal isolates were positive for phosphate solubilization. *Aspergillus fumigates, Aspergillus niger, Aspergillus flavus, Penicillium* spp. and *Trichoderma* spp.

CONCLUSION

The present study revealed distinct variability among *Aspergillus fumigates, Aspergillus niger, Aspergillus flavus, Penicillium* spp. and *Trichoderma* spp. isolates recovered from the rhizosphere soils of various physiographic regions of Marathwada region with respect to morphological characterization, phosphate solubilizing ability and genetic makeup. Based on phosphate solubilizing ability, the highly efficient *Aspergillus flavus*, *Penicillium* spp. *Trichoderma* spp. strains may be further exploited in biofertilizer production.

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