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Research Article

BIOCONTROL ACTIVITY OF PHOSPHATESOLUBILIZING FUNGIISOLATED FROM RHIZOSPHERE SOIL OF MARATHWADA, INDIA

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ABSTRACT

The phosphate solubility and biocontrol activity of fungi isolated from rhizosphere soil of Marathwada were studied in the present study. Fungi were isolated from rhizosphere soil and all the 11 fungal isolates showed good phosphate solubilizing ability. The potent phosphate solubilising isolates *Aspergillus niger* (PQ9), *Trichoderma*spp (PQ36) and *Penicillium* spp (PQ19) showed significant zone of solubilization with 34, 31 to 30 mm respectively after 48 hours of incubation. All potent phosphate solubilising fungal isolates were tested for *in vitro* antagonistic activity against bacterial plant pathogen *Fusarium oxysporum, Fusarium solani, Rhizoctonia solani, Rhizopus oryzae* and *Macrophomina phaseolina*. The study therefore proposed that these fungal species have strong phosphate solubilizing properties and the isolated fungal strains in present investigation indicates broad spectrum activity against tested fungal phytopathogen. Therefore, the fungal species can be used for excellent crop productivity as a biofertilizer and as biocontrol agent.

Keywords: Fungal species, Phosphate solubilization, Biocontrol activity, Rhizosphere soil.

1. INTRODUCTION

Phosphorus is one of the most important nutrients for plants, second only to nitrogen in terms of demand. It makes up about 0.2 percent of the dry weight of plants and is important for their production and growth. In the living plant, it is needed for photosynthesis, respiration, energy storage and transfer, cell division, cell enlargement, and a variety of other processes. A sufficient supply of phosphorus in the early stages of plant growth promotes physiological functions such as early root formation and is essential for laying down the primordia for plant reproductive parts [1, 2].

Because significant deposits of cheaper and lower-grade rock phosphate (RP) are locally available in many countries around the world, the use of natural phosphate-bearing materials such as rock phosphate (RP) as fertiliser for P-deficient soils has gotten a lot of attention in recent years [3].The phosphate solubilizing microorganism has the ability to release metabolites such as organic acids, which chelate into the soil due to their hydroxyl and carboxyl groups and are then converted to soluble forms. Organic acid synthesis and proton extrusion are two microbial reactions that are used to solubilize phosphate. In the cycling of insoluble organic and inorganic soil phosphates, different microbial phosphate solubilization pathways exist in nature [4].

Fungi are essential components of soil microbes, and depending on soil depth and nutrient requirements, they usually make up more of the soil biomass than bacteria. Fungi have been found to be more capable than bacteria at solubilizing insoluble phosphate [5]. Soil fungi such as *Aspergillus niger* and *Penicillium* sp., which are the most common fungi capable of phosphate solubilization, have been documented to solubilize insoluble phosphorus [6]. Microbial solubilization of rock phosphate and its use in agriculture are receiving more attention as part of a sustainable agricultural system. Acidification, chelation, exchange reactions, and the synthesis of organic acids are all ways in which soil fungi and bacteria can convert inorganic phosphate into soluble forms [7]. It has been documented that using phosphate solubilizing fungi in the field increases crop yield. Chemical fertilisers are hazardous to human health and have an adverse effect on soil microbial populations by destroying the physical structure of the soil, resulting in a lack of oxygen in the plant root region, as well as being expensive and raising the cost of output. It has been documented that using phosphate solubilizing fungi in the field, increases crop

yield. Chemical fertilisers are hazardous to human health and have an adverse effect on soil microbial populations by destroying the physical structure of the soil, resulting in a lack of oxygen in the plant root region, as well as being expensive and raising the cost of output.

Fungi have been discovered to be more capable of solubilizing insoluble phosphate than bacteria [8]. Soil fungi such as *Aspergillus niger* and *Penicillium*, which are the most common fungi capable of solubilizing phosphate, have been known to solubilize insoluble phosphorous species [9].

The dominant filamentous phosphate solubilizing fungi in the rhizosphere soil are *Aspergillus* spp. and *Penicillium [10]* (Pandey *et al.,* 2008). The aim of this research was to investigate the phosphate solubilization efficiency of isolated fungi and to assess their biocontrol activity against plant pathogens, as well as their ability to promote plant growth in nutrient-deficient soils.

2. MATERIAL AND METHODS

2.1. Sample Collection

Rhizosphere soil samples were collected from the different locations of Marathwada region, India. Samples were collected in a plastic bucket and then thoroughly mixed on a piece of clean cloth and the lumps were broken using wooden pestle and mortar and were air dried [11]. The air-dried samples were sieved in 10 mesh diameters, stored in glass bottles and labeled for analysis. After collection, a portion of each sample was immediately transferred to laboratory and stored at 4°C for microbial analysis.

2.2. Primary Screening of Phosphate Solubilizing Fungi

Soil samples were proceeded for microbial analysis viz. isolation of phosphate solubilizing fungi on Pikovskaya's (PKV) agar medium supplemented with 25μg/mL chloramphenicol to inhibit bacterial growth [12]. The observation of transparent halo zone around the fungal colony indicated the phosphate solubilizing activity of the fungus and the diameter of the zone was measured in mm.

2.3. Characterization of Phosphate Solubilizing Fungi

After screening of phosphate solubilizing, all the fungal isolates were transferred on Potato Dextrose Agar to accelerate the growth rate and the production of enough conidia [10] (Pandey *et al.,* 2008). To identify the isolated fungi to the genus level, isolates were compared with mycological identification keys and taxonomic description [13] such as surface appearance, texture, and colour of the colonies both from upper and lower side. In addition, conidia, conidiophores, arrangement of spores, and vegetative structures were determined with microscopy [14]. The identified fungi were maintained on Potato Dextrose Agar (PDA) slant at (4°C) for further investigation.

2.4. Qualitative analysis of Phosphate solubilisation

Isolates showing phosphate solubilizing ability were spotinoculated at the centre Pikovskaya's plate and incubated at 37°C. Diameter of clearance zone was measured after 24 hours, up to 7 days. Then Phosphate Solubilization Efficiency (PSE) is the ratio of total diameter.

2.5. Quantitative analysis of Phosphate solubilisation

For Quantitative analysis of Phosphate, Pikovskaya's broth medium with Tricalcium phosphate (0.3g/100ml) was prepared and sterilized; 1ml of eachisolate was inoculated into the broth medium. Then the inoculated sample were incubated for 5 days on rotatory shaker37°C after incubation, culture broth was centrifuged at 10,000rpm for 30min.Uninoculated broth served as control. The available Phosphorous was determined using calorimetrically at 410nm with standard KH_2PO_4. Solubilization index was evaluated according to the ratio of the total diameter (colony + halo zone) and the colony diameter [15].

PSI = (Colony diameter + Halozone Diameter)/Colony diameter

2.6. Biocontrol activity of fungi isolates against phytopathogen

All potent phosphate solubilising fungal isolates were tested for *in vitro* antagonistic activity against bacterial plant pathogen *Fusarium oxysporum, Fusarium solani, Rhizoctonia solani, Rhizopus oryzae* and *Macrophomina phaseolina*. The overnight grown culture of bacterial plant pathogen was spread uniformly on PKV plates. After drying, 10µl of 24 hr. old nutrient broth culture of test organism was spotted on these plates and the plates were incubated at 30°C for 2 to 4hr. The zone of inhibition of bacterial pathogen around the colonies of the *Pseudomonas fluorescence* was measured.

3. RESULTS AND DISCUSSION

At different sites, the percentage contribution of different fungal species to the total fungal population varied. The genus *Aspergillus flavus* contributed the most to all of the samples due to its large number of species. The percentage contribution of fungal species resulted in significant differences. *Penicillium* sp ranked first of all P-solubilizers, contributing the highest percentage of 9.15 percent to the total fungal population. *Aspergillus flavus* contributed 3.26 percent of the total, *Penicillium* sp contributed 2.61 percent, and *Trichoderma* spp contributed 5.22 percent. Together, these species account for 24.81 percent of the total fungal population.

The excellent 11 PSF isolates namely, PQ3, PQ7, PQ9, PQ13, PQ14, PQ19, PQ21, PQ24, PQ36 PQ37 and PQ40, produced maximum zone of solubilization. It was observed that PQ9 is potent phosphorus solubilizing bacteria which showed 34 mm zone of solubilization than others isolate. Phosphorus deficiency is a natural occurrence on soil around the world, and it is one of the crop production limiting factors. Fertilizers containing phosphorus account for a significant portion of agricultural production costs. Many bacteria, fungi, and a few actinomycetes are potential solubilizers of bound phosphates in soil, and thus play an important role in making soluble phosphate accessible to plants. The key soil nutrients required for normal plant germination, development, and maturity are nitrogen, phosphorus, and potassium.

In the present study, the results of phosphate solubilization capacity of all the potent fungal isolates was studied among them the three i.e. *Aspergillus niger, Penicillium spp., Trichoderma spp* were found to be excellent phosphate solubilizer. The phosphate solubilization capacity of *Aspergillus niger* (PQ9) on day three recorded maximum efficacy in solubilization (3.15 ppm of Phosphate) followed by PQ19 and PQ36 isolates.

The phosphate solubilization capacity of *Aspergillus niger* (PQ9) on day six recorded maximum efficacy in solubilization (5.32 ppm of Phosphate) and on nine recorded maximum efficacy in solubilization (6.14 ppm of Phosphate) followed by PQ19, PQ36 strain. The phosphate solubilization efficacy of PQ9 strain increased 32.53% on third day, 23.18% phosphate solubilization on sixth day and 20.16% phosphate solubilization on ninth day of incubation. Microbial solubilization of insoluble phosphate, especially low grade and it's use in agriculture is receiving greater attention. This process not only compensates for higher cost of manufacturing fertilizers in industry but also mobilizes the fertilizers added to the soil [16].

Table 1: Phosphate solubilization of potent fungal isolates

Sr. No	Culture number	Zone diameter in mm	Name of Isolates
1.	PQ3	21mm	*Aspergillus spp*
2.	PQ7	30 mm	*Aspergillus fumigatus*
3.	PQ9	34 mm	*Aspergillus niger*
4.	PQ13	28 mm	*Aspergillus flavus*
5.	PQ14	22mm	*Aspergillus flavus*
6.	PQ19	30 mm	*Penicillium* sp.
7.	PQ21	22 mm	*Aspergillus spp*
8.	PQ24	26 mm	*Aspergillus fumigatus*
9.	PQ36	32 mm	*Trichoderma spp*
10.	PQ37	23 mm	*Penicillium* sp.
11.	PQ40	21mm	*Aspergillus flavus*

Table 2: Efficacy of phosphatase activity of isolated fungal strains

Isolates	*Phosphatase activity µmol min^{-1} (Day)			Increased % of phosphatase activity (Day)		
	3^{rd}	6^{th}	9^{th}	3^{rd}(%)	6^{th}(%)	9^{th}(%)
Aspergillus niger	0.180±0.04	0.228±0.06	0.239±0.06	6.51	7.04	1.27
Penicillium spp	0.195±0.05	0.245±0.08	0.264±0.04	15.38	15.02	11.86
Trichoderma spp	0.209±0.05	0.237±0.07	0.267±0.06	23.67	11.27	13.14

Photo Plate: Phosphate solubilizing fungi

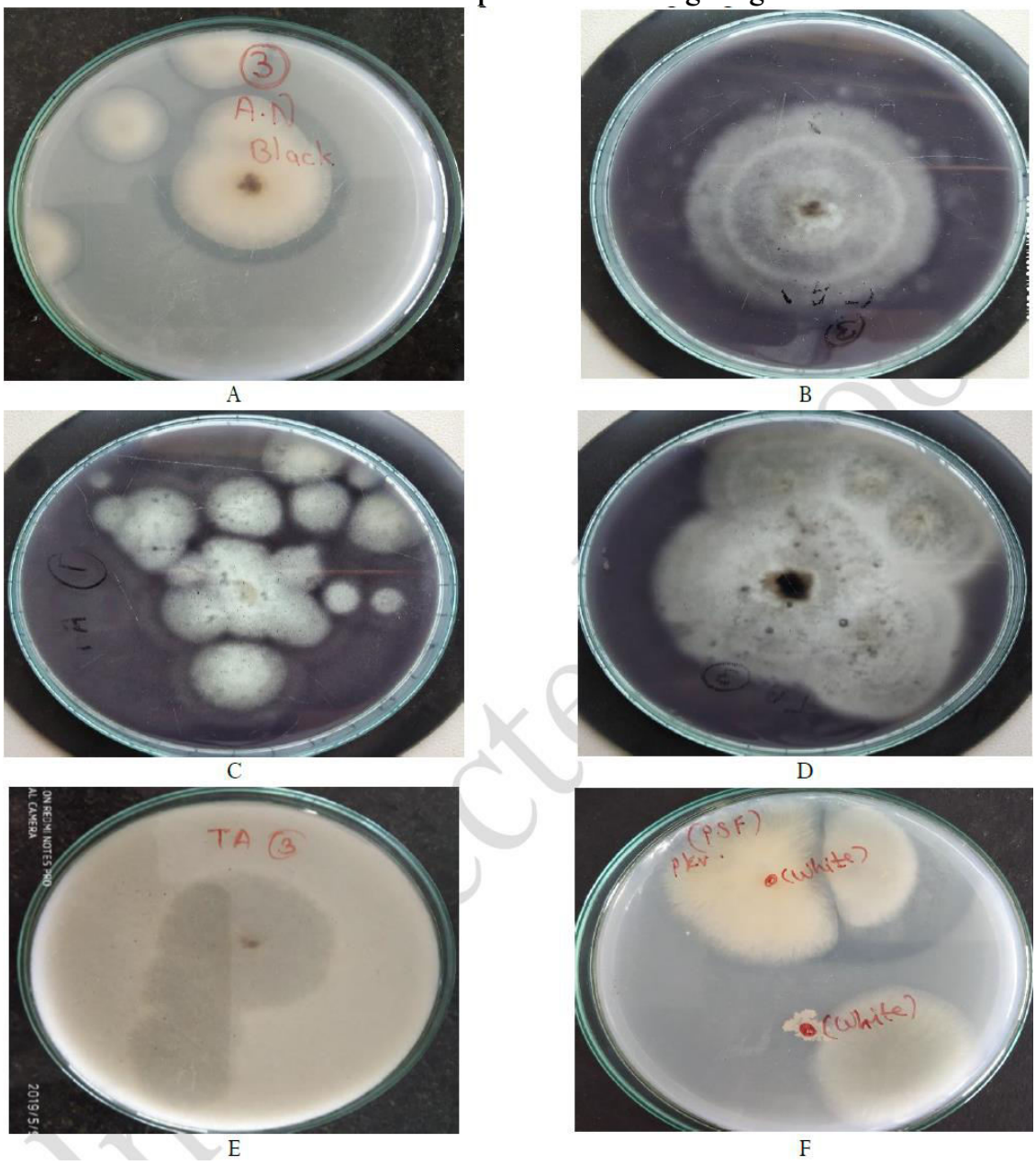

Photo plate 1: *In vitro* characterization of microorganisms for phosphate solubilization in PVK medium.

3.1. Biocontrol activity of PSF against fungal phytopathogen

PGPR are specific strains of root-colonizing bacteria which can elicit increased rates of plant growth, suppress soil pathogens and induce systemic resistance (ISR) against diseases and insect pests. The effect of PGPR on insect pests could be in direct via ISR and/or direct as an entomopathogen. The indirect effect of PGPR against insects initiated through recognition of the microbes by the host plant, elicitation of specific hormonal signal pathways that might lead to the biosynthesis of defense-related chemical compounds, enzymes, protein, secondary metabolites, and volatile organic compounds (VOCs) against insect herbivores [17-20].

The existence of certain bioactive compounds formed by *Aspergillus niger, Trichoderma* spp., and *Penicillium* spp. may have a novel structure that explains its broad-spectrum microbial activity against fungal phyto-pathogens including *Fusarium oxysporum, Fusarium solani, R. solani, R. oryzae,* and *Macrophomina phaseolina*. The isolated and purified compound from *Aspergillus niger,*

Trichoderma spp, Penicillium spp strains in present investigation indicates that compound may have unique structure therefore it shows broad spectrum activity acting against tested fungal phytopathogen. The complete structure elucidation by advance instrumentation techniques is essential for explaining its uniqueness.

Table 3: Biocontrol activity of phosphate solubilising fungi against phytopathogen (Zone of inhibition in mm)

Isolates	*Fusarium oxysporum*	*F. solani*	*R. solani*	*R. oryzae*	*Macrophomina phaseolina*
Aspergillus niger (PQ9)	23	16	18	16	23
Trichoderma spp (PQ36)	24	18	22	18	22
Penicillium spp (PQ19)	17	18	20	16	17

4. CONCLUSION

According to the findings of this report, rhizosphere soils contain a diverse group of phosphate-solubilizing fungi isolates, including *Aspergillus niger, Trichoderma* spp., and *Penicillium* spp. These may be used for inoculum development and their inoculation effect on plant growth, as well as a biocontrol agent, but further research is needed in green houses and field trials.

Conflict of interest

None declared

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