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Research Journal of Agricultural Sciences
An International Journal

P- ISSN: 0976-1675
E- ISSN: 2249-4538

Volume: 12
Issue: 06

Res. Jr. of Agril. Sci. (2021) 12: 2059–2062

CARAS

Res. Jr. of Agril. Sci. (Nov-Dec 2021) 12(6): 2059–2062
www.rjas.org

Full Length Research Article

Screening of Plant Growth-promoting Activities of Phosphate Solubilizing Fungi from Rhizosphere of Marathwada, India

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Received: 07 Aug 2021 | Revised accepted: 24 Oct 2021 | Published online: 20 Nov 2021

ABSTRACT

Based on soil depth and nutritional requirements, fungi are the basic components of soil microbes that usually make up more of the soil biomass than bacteria. Phosphate solubilizing fungi were isolated from the soil of the Marathwada district of Maharashtra in the current study and all fungal isolates were tested for their capacity to solubilize phosphate. Total 40 isolate were isolated and only 11 fungal isolates were showed P-solubilizing ability. Among these *Aspergillus niger* (PQ9), *Trichoderma* spp (PQ36), *Penicillium* spp (PQ19) fungal isolates showed significant zone of solubilization with 34, 31 to 30 mm selective agar medium after 48 hours of incubation. The potent phosphate solubilizing fungi were identified 18S rRNA analysis. The study therefore proposed that these fungal species have strong phosphate solubilizing properties and can be used for excellent crop productivity as a biofertilizer.

***Key words*:** Fungal biodiversity, Phosphate solubilization, Rhizosphere soil

Several soil, mangrove and rhizosphere researchers have performed exploration of phosphate solubilizing microorganisms [1-2]. The phosphate solubilizing microorganism having property allows to release metabolites such as organic acids, which chelate into the soil by their hydroxyl and carboxyl groups, and which are converted into soluble types. Different microbial reactions, including organic acid synthesis and proton extrusion, are used to solubilize phosphate. In nature, there are distinct microbial phosphate solubilization pathways involved in the cycling of insoluble organic and inorganic soil phosphates [3].

Fungi have been reported to have greater ability to solubilize insoluble phosphate than bacteria [4]. A large variety of soil fungi, such as *Aspergillus niger* and *Penicillium*, are documented to solubilize insoluble phosphorous species, which are the most common fungi capable of solubilizing phosphate [5]. Many soil researchers have performed research into phosphate solubilizing microorganisms [6-7], mangrove [8] and rhizosphere [9-10]. From such exploration's different forms of phosphate solubilizing microorganisms have been successfully described. A wide variety of rhizosphere bacteria and fungi have been known as phosphate in recent decades, including *Penicillium*, *Azotobacter chroococcum*, *Bacillus subtilis*, *Bacillus cereus*, *Bacillus megaterium*, *Arthrobacterium ilicis*, *Escherichia coli*, *Pantoea agglomerans*, *Pseudomonas putida*, *Pseudomonas aeruginosa*, *Enterobacter aerogenes*, *Microbacterium laevaniformans* and *Micrococcus luteus* [11].

Aspergillus spp. and *Penicillium* the dominant filamentous phosphate solubilizing fungi in the rhizosphere are [12]. As sources of organic acid and other biotechnological uses, such as biocontrol, biodegradation and mobilisation of phosphate, they are widely used [13-14]. 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 the diversity of phosphate solubilizing fungi inhabiting various rhizospheres in this region is limited. The aim for the present study was to study the biodiversity of phosphate solubilization efficiency of isolated fungi from the Marathwada region with the ability to promote plant growth particularly for nutrient deficient soils.

MATERIALS AND METHODS

Sample collection

A total of 160 rhizosphere soil samples were collected from various locations namely Aurangabad, Beed, Hingoli, Jalna, Latur, Nanded, Osmanabad and Parbhani

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districts of Marathwada, Maharashtra (India). The study primarily focused on testing of soil samples collected from every tehsil of Marathwada district of Marathwada.

Physicochemical analysis of soil samples

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 [15]. 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. Physicochemical parameters like pH, Electrical Conductivity (EC), Total Organic Carbon, Available Nitrogen (N), Available Phosphorus (P_2O_5) and available Potassium (K_2O) of soil samples were analyzed as per the methods recommended by APHA [16].

Primary screening of phosphate solubilizing fungi

Soil samples were procced for microbial analysis viz. isolation of phosphate solubilizing fungi on Pikovskaya's (PKV) agar medium supplemented with 25μg/mL chloramphenicol to inhibit bacterial growth [17]. 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.

Characterization of phosphate solubilizing fungi

After Screening of Phosphate Solubilizing all fungal isolate were transferred on Potato Dextrose Agar to accelerate the growth rate and the production of enough conidia [14]. To identify the isolated fungi to the genus level, isolates were compared with mycological identification keys and taxonomic description [17]. 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 [18]. The identified fungi were maintained on Potato Dextrose Agar (PDA) slant at (4°C) for further investigation.

Qualitative analysis of phosphate solubilization

Isolates showing phosphate solubilizing ability were spot inoculated 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.

Quantitative analysis of phosphate solubilization

For Quantitative analysis of Phosphate, Pikovskaya's broth medium 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 shaker 37°C after incubation, culture broth was centrifuged at 10,000 rpm for 30min. Uninoculated broth served as control. The available Phosphorous was determined using colorimetrically at 410nm with standard KH_2PO_4.

Assessment of the biodiversity of isolates

The diversity of the isolates was made from the microscopic, cultural/morphological description of the fungal isolates. This description allowed highlighting the similarities and the differences between isolates.

Molecular identification of fungal isolates

The molecular techniques are being very much employed for classification and characterization of various fungal species [19]. For species level identification, genomic DNA of the potent phosphate solubilizing fungi was extracted by a standard protocol and analyzed on 0.8% agarose electrophoresis. Further, PCR was carried out to amplify the 18S rRNA gene of the extracted genomic DNA of fungi using Gene Amp PCR with the forward and reverse primers. The genus of the strain was determined based on the sequence of 601bp of 18S rRNA gene. The obtained 18S rRNA gene sequences were assembled and exploited for phylogenetic analysis. The 18S rRNA gene sequence related taxa were acquired from the GenBank database at the National Center for Biotechnology Information. The report (sent online) includes NCBI-BLASTn (http://blast.ncbi.nlm.nih.gov) result for fungi showing closest VALID neighbour of the organism in the TYPE database along with percent similarity.

RESULTS AND DISCUSSION

In the present study, the physicochemical analysis of the soil rhizosphere were carried out, the examination of the soil samples show that the values of pH range from 7.7 to 8.7, while ideal pH range from 7.5 to 7.8, which shows that the soil of Marathwada is slightly alkaline due to excessive evaporation of water in dry areas, which bring salts to the surface. pH can affect the availability of nutrients in the soil [20] and plant growth is limited in alkaline medium (Table 1). It may be due to formation of these soils from basaltic parent material rich in basic cations. Similar results were reported by Padole and Mahajan [21].

Table 1 Number of soil samples collected from different area of Marathwada region (Properties of soil in Range)

Location	No. of samples	pH	E.C m/s.	P (k/hec)	N (Kgs. /Ha)	O.C (%)	K (k/hec)
Aurangabad	27	7.2-8.2	15.9-18.4	6.50-20.45	80.40-250.30	0.15-0.95	100-320
Beed	24	7.1-7.8	16.1-18.7	5.78-20.58	75.60-237.56	0.18-0.89	110-310
Hingoli	15	7.1-8.5	16.-18.4	5.20-19.21	78.30-180.90	0.20-0.82	98-290
Jalna	27	7.0-7.8	16.1-17.8	5.50-20.90	90.70-240.20	0.17-0.86	105-355
Latur	15	7.3-8.2	16.-18.3	7.90-16.20	100.50-219.50	0.15-0.73	100-280
Nanded	16	7.0 -8.5	15.7-18.1	6.11-18.30	90.50-170.70	0.25-0.83	110-260
Osmanabad	15	7.1-8.6	15.8-17.5	5.25-17.80	95.50-210.50	0.17-0.79	100-340
Parbhani	21	7.1-8.8	16.2-18.7	7.50-18.30	100.50-200.30	0.25-0.86	95-355
Total samples	160	7.0-8.8	15.7-18.7	5.20-20.90	75.60-250.30	0.15-0.95	95-355

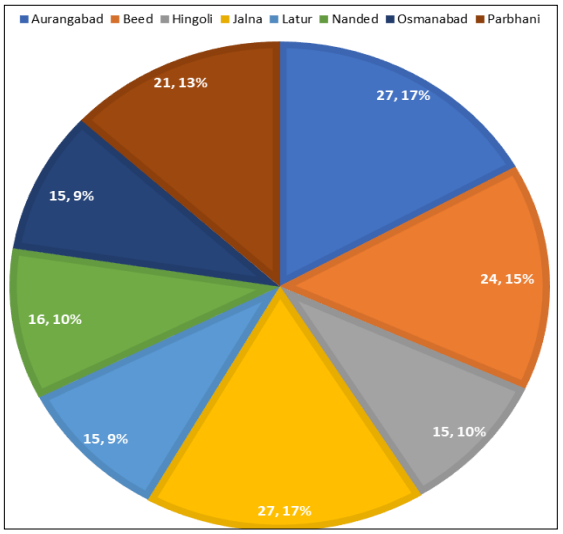

Fig 1 No. of soil samples collected from different area of Marathwada

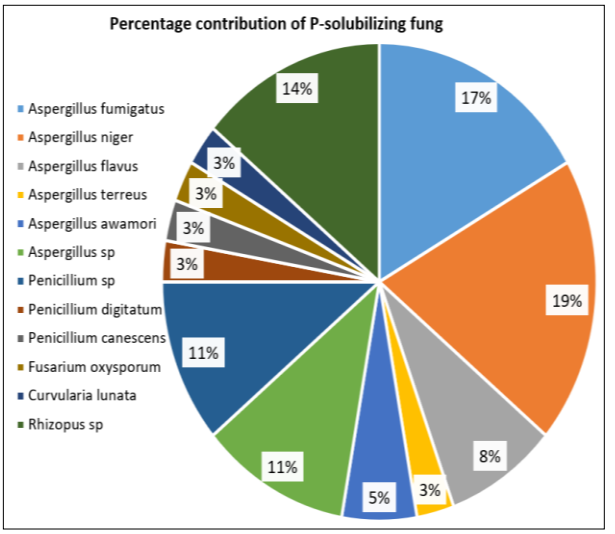

Fig 2 Percentage contribution of P-solubilizing fungi

Table 2 Colony morphology and microscopic characteristics of the fungal isolates

Culture number	Colony morphology	Microscopic observations	Name of isolates
PQ3, PQ11, PQ21	Colonies on PDA, initially white floccose mycelium. Quickly become black color colonies with production of black spores	Conidia were small, black, brownish black, green in colour. Septatehyphae with rough brown andsmooth colorless conidiophores	*Aspergillus* species
PQ7, PQ24	Colonies were initially white and turned yellowish green to light green	Septate, hyaline, acute angle branching, tree- or fan-like branching	*Aspergillus fumigatus*
PQ13, PQ40, PQ14	Colonies on potato dextrose agar was lime green color. Texture was woolly to cottony to somewhat granular	Septate distinct, bearing a cluster of branches, phialides born on cylinder branchesand arranged in brush-like head	*Aspergillus flavus*
PQ19, PQ37	Colonies are initially white, change to a brownish red color and later to green or bluish green color	Septate, hyaline, acute angle branching, tree- or fan-like branching	*Penicillium* spp.
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	Irregularly branched, not verticillate, Single celled, not remaining together in one chain	*Trichoderma spp.*

Percentage contribution of P-solubilizing fungi

In the total fungal population percentage contribution of different fungal species showed variations at different sites. In all the samples, contribution of the genus *Aspergillus flavus was* more as they represented by large number of species. Percentage contribution of fungal species spelled considerable variations. Among the P-solubilizers, in the total fungal population *Penicillium sp* contributed the maximum of 9.15% and ranked first among all. Contribution of *Aspergillus flavus* was significant with 3.26%, *Penicillium sp* contributed 2.61%, *Trichoderma spp* contributed 5.22%. These species together contribute 24.81% in the total fungal population (Fig 2).

In the present study, total 347 fungal isolates were isolated among them 40 isolates found potent phosphate solubilizer. 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. Deficiencies in phosphorus are common around the world on soil and one of the limiting factors for crop production. Phosphorus fertilizers account for large agricultural production costs. Many bacteria, fungi and a few actinomycetes are potential solubilizes of bound phosphates in soil thus playing an important role making it available to plants in the soluble form Nitrogen, phosphorous and potassium are the main soil nutrients for normal germination, growth and maturity of plants.

Based on the phosphate solubilizing ability, 11 highly efficient PSF isolates were further analyzed for their biodiversity study. On the basis of analysis, *Aspergillus niger* isolates were recovered from Parbhani and Latur district Marathwada region.

In the present study, genomic DNA was extracted by a standard protocol and analyzed on 0.8% agarose electrophoresis. Further, PCR was carried out to amplify the 18S rRNA gene of the extracted genomic DNA of UK-2 fungi using Gene Amp PCR with the forward and reverse primers (Forward Primer 5'-GAGTTTGATCCTGGCTCAG-3' and Reverse primer 5'-GAAAGGA GGTGATCCAGCC-3'). The genus of the strain was determined based on the sequence of 356 bp of 18S rRNA gene. The obtained 18S rRNA gene sequences were assembled. All fungal species *Aspergillus fumigatus. Aspergillus niger*, *Aspergillus flavus*, *Aspergillus terreus*,

Aspergillus awamori, Aspergillus sp., Penicillium sp, Penicillium digitatum, Penicillium canescens, Fusarium oxysporum, Curvularia lunata, Rhizopus sp and *Trichoderma* spp evaluated for their phosphate solubilization ability on Pikovskaya (PVK) selective media and some of them found excellent phosphorus solubilizing properties. For their plant growth promotion activities such as antagonism, development of phytohormones, siderophores and their biopesticide function, the effective isolates from the sample have been evaluated. Various mathematical instruments were used and the detailed findings of all the experiments were presented.

CONCLUSION

The present study on biodiversity of the fungal species from the rhizosphere soils of various locations of Marathwada region with respect to morphological characterization, phosphate solubilizing ability. The study revealed that the fungal isolates namely *Aspergillus fumigates, Aspergillus niger, Aspergillus flavus, Penicillium* spp. and *Trichoderma* spp. These may be used for inoculum production and their inoculation effect on the plant growth must be studied in vitro, green house and field trials.

LITERATURE CITED

1. Gupta M, Kiran S, Gulati A, Singh B, Tewari R. 2012. Isolation and identification of phosphate solubilizing bacteria able to enhance the growth andaloin-A biosynthesis of *Aloe barbadensis* miller. *Microbiol. Research* 167: 358-363.
2. Oliveira CA, Alves VMC, Marriel IE, Gomes EA, Scotti MR, Carneiro NP, Guimaraes CT, Schaffert RE, Sa NMH. 2009. Phosphate solubilizing microorganisms isolated from rhizosphere of maize cultivated in an oxisol of the Brazilian Cerrado Biome. *Soil Biology and Biochemistry* 41: 1782-1787.
3. Khan AA, Jilani G, Akhtar MS, Naqvi SMS, Rasheed M. 2009. Phosphorus solubilizing bacteria: occurrence, mechanisms and their role in crop production. *Journal of Agriculture and Biological Sciences* 1(1): 48-58.
4. Nahas E. 1996. Factors determining rock phosphate solubilization by microorganisms isolated from soil. *World Journal of Microbiology and Biotechnology* 12: 567-572.
5. Whitelaw MA, Harden JT, Helyar RT. 1999. Phosphate solubilization in solution culture by the soil fungus *Penicillum radicum*. *Soil Biology and Biochemistry* 31: 655-665.
6. Chen YP, Rekha PD, Arun AB, Shen FT, Lai WA, Young CC. 2006. Phosphate solubilizing bacteria from subtropical soil and their tricalcium phosphate solubilizing abilities. *Appl. Soil Ecology* 34: 33-41.
7. Widawati S, Suliasih D. 2006. Augmentation of potential phosphate solubilizing bacteria (PSB) stimulate growth of green mustard (*Brasica caventis* Oed) in marginal soil. *Journal of Biodiversity* 7: 10-14.
8. Vazquez P, Holguin G, Puente ME, Lopez CA, Bashan Y. 2000. Phosphate solubilizing microorganisms associated with the rhizosphere of mangroves in a semiarid coastal lagoon. *Biology and Fertility of Soils* 30(5/6): 460-468.
9. Chung H, Park M, Madhaiyan M, Seshadri S, Song J. 2005. Isolation and characterization of phosphate solubilizing bacteria from the rhizosphere of crop plants of Korea. *Soil Biol and Biochemistry* 37: 1970-1974.
10. Poonguzhali S, Madhaiyan MT. 2008. Isolation and identification of phosphate solubilizing bacteria from Chinese cabbage and their effect on growth and phosphorus utilization of plants. *Journal of Microbiology and Biotechnology* 18(4): 773-777.
11. Kumar A, Choudhary CS, Paswan D, Kumar B. 2014. Sustainable way for enhancing phosphorus efficiency in agricultural soils through phosphate solubilizing microbes. *Asian Journal of Soil Science* 9: 300-310.
12. Wakelin SA, Warren RA, Harvey PR and Ryder MH. 2004. Phosphate solubilization by Penicillium spp. closely associated with wheat roots. *Biology and Fertility of Soils* 40(1): 36-43.
13. Richa G, Khosla B, Reddy M. 2007. Improvement of maize plant growth by phosphate solubilizing fungi in rock phosphate amended soils. *World Journal of Agricultural Sciences* 3: 481-484.
14. Pandey A, Das, N. Kumar B, Rinu K, Trivedi P. 2008. Phosphate solubilization by *Penicillium* spp. isolated from soil samples of Indian Himalayan region. *World Journal of Microbiology and Biotechnology* 24(1): 97-102.
15. Tandon HLS. 1993. *Method of Analysis of Soils, Plants, Water and Fertilizers*. Fertilizer Development and Consultation Organization 204-204A Bhanot Corner, 1-2 Pamposh Enclave, New Delhi -110048, India. pp 1-48.
16. APHA. 2002. *American Public Health Association*. Standard methods the examination of water and wastewaters, 18th Edition, Washington, D.C. 2002.
17. Pikovskaya. 1948. Mobilization of phosphorus in soil connection with the vital activity of some microbial species. *Microbiologia* 17: 362-370.
18. Diba K, Kordbacheh P, Mirhendi SH, Rezaie S, Mahmoudi M. 2007. Identification of *Aspergillus* sp. using morphological characteristics. *Pakistan Journal of Medical Sciences* 23(6): 867-872.
19. Jensen MA, Webster JA, Straus N. 1993. Rapid identification of bacteria on the basis of polymerase chain reaction-amplified ribosomal DNA spacer polymorphisms. *Applied and Environmental Microbiology* 59(4): 945-952.
20. Patil S, Patil S. 2015. Physico-chemical characterization of water from forest covered Gavase wetland of Kolhapur District, Maharashtra (India). *International Journal of Current Research* 7: 24025-24033.
21. Padole VR, Mahajan SB. 2003. Status and release behaviour of potassium in some swell-shrink soils of Vidarbha, Maharashtra. *Journal of Maharashtra Agricultural Universities* 28: 3-7.