Journal of Advanced Zoology

ISSN: 0253-7214
*Volume **43** Issue **S-1 Year 2022** Page 112:117*

Isolation and Characterization of Phosphate Solubilizing Fungi from Rhizospheric Soil of Paddy Crop Fields and their Functional Capacity

Pooja Gond[1*], Dr. Shweta sao[2]

[1,2]Department of Biotechnology, Dr. C.V. Raman University, Bilaspur, Chhattisgarh (India).
Email: pooja4802@gmail.com[1], drshwetasao02@gmail.com[2]

**Corresponding author's E-mail: pooja4802@gmail.com*

Article History

Received: 06 June 2022
Revised: 05 Sept 2022
Accepted: 13 Dec 2022

Abstract

The study's purpose was to determine the most effective fungal isolates and growing conditions for sparing phosphorus release from soil. Based on their capacity to dissolve tricalcium phosphate, two fungal isolates were chosen from a total of 12. Aspergillus niger and Penicillium oxalicum were chosen because of their capacity to successfully produce a halo zone encircling colony on petri plates. The optimal activity of selected fungal isolates was discovered with a solubilization index (PSI) of 2.4, incubation durations of 7 days, and temperatures of 30°C. pH 7.0, pH 6.0, and pH 8.0 were the optimal conditions for phosphate solubilization in Aspergillus niger and Penicillium oxalicum..

Keywords: *Phosphate solubilization, tri-calcium phosphate, phosphate solubilizing fungi*

1. Introduction

Phosphorus (P) is an essential biochemical component of various processes in plants, including photosynthesis, respiration, energy storage and transmission, cell division, and cell growth, according to Sargervanshi et al. (2012). Fungi may be used as safe, economical, and ecologically acceptable catalysts to help transform insoluble -P into soluble -P due to the limited availability of chemical -P fertilizers in alkaline settings (Coutinho et al., 2012). The introduction of Aspergillus increased maize productivity and P absorption. When organic P molecules undergo mineralization, the end product is P, which plants may then utilize as a nutrient. This process of turning organic phosphorus into soluble forms of phosphorus can be carried out by a variety of soil microbes or rhizosphere microflora (Rodriguez et al., 2006).

The enzymes soil bacteria produce, particularly phosphatases and phytases Maougal et al., (2014), influence the mineralization process. The enzymes necessary for organic P-mineralization are produced by a diverse range of bacteria, fungi, and other organisms, including Actinomycetes, Pseudomonas, and Bacillus species, according to Richardson and Simpson (2011). The process of releasing phosphate from organic phosphorus compounds into the soil solution, known as phosphate mineralization, is catalysed by the enzyme phosphatases, which are released into the soil solution by extracellular microorganisms. The optimum temperature between 27+3°C, the optimum pH occurring at soil 6.5 pH, moisture, aeration, crop type, presence of growing plants, and fertilizer phosphate addition all influence organic phosphate mineralization. The bio-geochemical cycle of phosphorus in natural settings depends on microorganisms. The importance of phosphate-solubilizing bacteria is widely known since phosphate solubilization is a crucial stage in plant growth (Velazquez and Rodriguez-Barrueco, 2007). Microbial activity depends on a number of factors, including pH, temperatures, and incubation durations. Finally, relationships between the potential for phosphate solubilization and associated elements including incubation periods, temperatures, and pH were identified.

Moreover, PSF exhibit adaptability across diverse soil types and can effectively function under varied environmental conditions. This versatility makes them suitable candidates for promoting sustainable crop production in both conventional and organic farming systems. Additionally, incorporating PSF into biofertilizer formulations has shown promising results in enhancing nutrient availability, plant growth, and crop yield, rendering them a valuable asset in sustainable agriculture practices.

In conclusion, phosphate solubilizing fungi hold immense potential in addressing phosphorus limitations in agricultural soils. Their ability to convert insoluble phosphates into easily accessible forms not only improves plant nutrient availability but also contributes to sustainable farming practices. The utilization of PSF as a natural alternative to chemical fertilizers presents an environmentally friendly approach for sustainable soil management and food production. The continued exploration of this fascinating group of fungi holds promise in ensuring global food security while preserving our precious ecosystems.

2. Materials And Methods

Collection of soil sample: - Rhizosphere soil sample from the rice plant has been collected from Amane (Bilaspur). A sample of soil was taken from the top 0–15 cm of the soil surface and also from the rhizosphere of plant root. The soil samples were stored in a refrigerator at 5 °C under aseptic conditions before being transported to the lab in sterile plastic bags. A 2-mm sieve was used to separate air-dried dirt.

Fungal strains were isolated from different soil samples on PDA media [Potato (infusion form)-200gm, Dextrose- 20gm, agar-agar- 15gm10 g for 1l] of each soil sample 10– fold dilution was made to drop 1ml of (10-1-10-6) on plates containing PDA agar medium. The plates were spread and incubated at 28°C for 7 days. After incubation period, growing fungal isolates were purified by repeated culturing and maintained on slants at 4°C to be ready for identification.

Fungal identification-: PDA was used, according to Diba et al., (2007), to promote growth and produce sufficient conidia. Using mycological identification keys and taxonomic descriptions from Cheesbrough M. (2000), the characteristics of fresh cultures were examined in order to identify the isolated fungus down to the genus level. The colonies' surface look, texture, and colour on both the top and bottom sides were used to identify them, along with other microscopic properties. Conidia, conidiophores, spore arrangement, and vegetative structures were also identified using microscopy. The discovered fungus was stored on Potato Dextrose Agar (PDA) tilted at (-4°C) for further investigation. A slide culture was made in order to detect the spores and mycelia of pure fungal isolates. According to Stevens (1974), fungal isolates' spore and mycelia shapes were examined, recognised, and identified by lacto phenol cotton blue staining under a microscope and after being grown on a slide.

Qualitative method: On selective medium (Pikovskaysa's agar medium (PVK)) (Pikovskaysa, 1948) (g/l): 0.5g (NH4)2 SO4, 0.5g MgSO4, fungal isolates have been studied.7H2O, 0.03g FeSO4, 0.3g NaCl, and 0.3g KCl.7H2O, 0.02g MnSO4, 10.0g Ca3(PO4), and H2O.2, 15.0g of agar, and 10.0g of glucose. Pikovskaya's medium was transferred into sterilised Petri dishes once it had solidified on the plate, and under aseptic circumstances, a point inoculation of fungal strains was placed in the middle of the plates. The plates were incubated at 28 °C for 7 days. The following equation was used to determine the solubilization index (SI) (Edi - premono, 1996).

Solubilization Index (SI)=(Colony Diameter+Halo Zone Diameter)/(Colony Diameter)

Quantitative method: - Tri-calcium phosphate was solubilized using fungi isolates with high ratios of the clear zone and maximal phosphate solubilization index (PSI), and the colony size was determined using a quantitative approach. A 1ml of fungus spore solution (1.0×106 spores/ml) was given to each flask. For 7 days at 28 °C, the flasks were incubated in a rotary shaker revolving at 200 rpm. The cultures have been collected on Whatman paper No. 42, which was used for filtering. The supernatants were subjected to phosphate availability analyses by Olsen et al. (1954), who also assessed the activity of alkaline and acid phosphatases.

$$olubilization\ Index\ (SI) = \frac{Colony\ Diameter + Halo\ Zone\ Diameter}{Colony\ Diameter}$$

Optimizing culture conditions for phosphate solubilization -: The sterile PVK broth media has a range of pH values, temperatures, and incubation periods. The uninoculated autoclaved media with phosphate substrate was incubated under equivalent conditions to serve as controls for all the parameters that were examined. A set of triplicate culture flasks was made for each fungal isolate, and each flask contained 50 mL of sterile PVK broth media with a pH of 7.0. Each flask was incubated at 28°C for 3, 5, 7, 9, and 11 days, respectively, to manufacture phosphatase enzymes after being injected with 1 ml of (106 spores) of the two most virulent fungal isolates. For a comparable set of culture flasks, temperature ranges of 25, 27, 30, 35, and 40°C were employed.

3. Results and Discussion

Isolation of Fungi

During the isolation and purification procedures, 12 fungal isolates were found. The morphological characteristics of each fungal isolate were used to identify it. Five species of *Aspergillus*, two of *Fusarium*, three of *Penicillium*, one of *Curvularia* and *Alterneria* make up this group of fungus species.

Screening of phosphate solubilizing fungi

Phosphate solubilization was evaluated in 12 fungal isolates, including *Aspergillus niger, Aspergillus flavus, Aspergillus nidulans, Aspergillus terreus, Aspergillus fumigatus, Fusarium oxysporum, F. solani, Pencillium oxalicum, P. crysogenum, P. frequentans, Curvularia lunata,* and *Alterneria alternata*. A plate technique based on a zone clearing on PVK medium at 28 °C for three days was noticed. Only two of the 12 isolated fungus on PVK agar medium had a zone of P solubilization. Around fungal colonies, a halo zone was seen (Fig. 1). It came out that the two isolates with the highest clear zone / colony diameter ratio were the most effective P solubilizing fungus (PSF). On the third day of incubation, the zone of P solubilization became visible. Phosphate solubilizing capacity was rising up to 7 days after inoculation, according to ongoing observation of the halo zone creation. The zones' respective diameters were 3.6 and 3.9 cm. (Table-1)

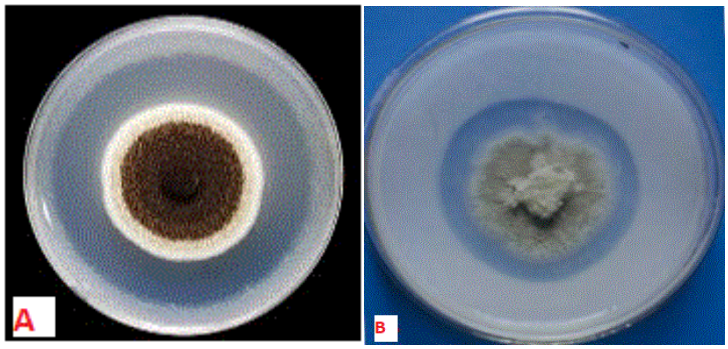


Fig 1: Clear halo zone around the colonies (A) *Aspergillus niger* (B) *Pencillium oxalicum*

Table 1: Estimation of a fungal isolate's phosphate solubilization index.

Fungal Isolates	Colony diameter (cm)	Halo zone (cm)	Solubilizing index
Aspergillus niger	2.5	3.6	2.4
Pencillium oxalicum	2.9	3.9	2.3

Quantitative Assay

Aspergillus niger, one of the two strains chosen, was the most effective solubilizer. Because of this, *Aspergillus niger* and *Pencillium oxalicum* were chosen for additional qualitative and quantitative analysis. *Pencillium oxalicum* came in second with 36.3 µg/ml of soluble-Phosphorous, followed by *Aspergillus niger* with 36.9 µg/ml., 22.85 µg/ml and 21.9µg/ml of alkaline phosphatase enzyme activity, respectively. The enzyme activity for acid phosphatase were 15.1 µg/ml and 11.7 µg/ml, respectively. (Table-2).

Table2: Fungal isolates' alkaline and acid phosphatase activity.

Fungal Isolates	Soluble Phosphate (µg/ml)	Alkaline Phosphatase Activity (µg/ml)	Acid phosphatase Avtivity (µg/ml)
Aspergillus niger	36.9	22.85	15.1
Pencillium oxalicum	36.3	21.9	11.7

Effect of incubation periods on P solubilization

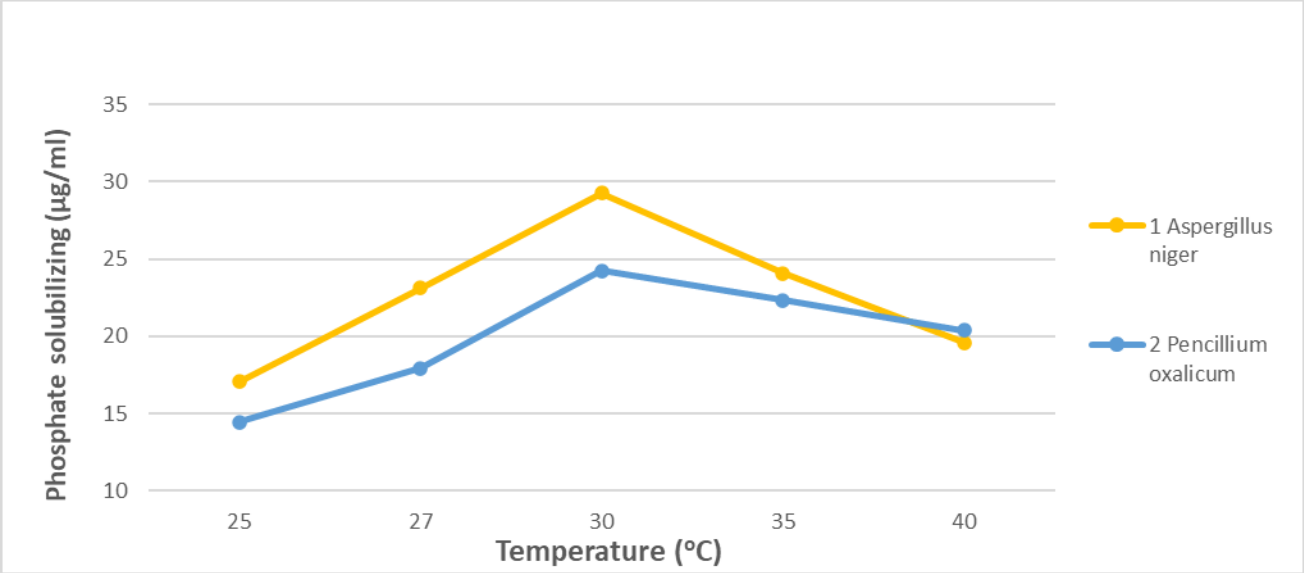
Phosphate solubilization began to rise on the 5th day of the incubation period and peaked after seven days. *Aspergillus niger and Pencillium oxalicum* both had maximum phosphate availability at 7 and 9 days, respectively (28.26 and 21.61 µg/ml and 27.08 and 21.28 µg/ml, respectively). From 9 days to the conclusion of the incubation period, phosphate solubilization decreased. (Table-3)

Table 3: Effect of various incubation times on the amount of released phosphate (µg/ml) by *Aspergillus niger* and *Pencillium oxalicum*

Phosphate solubilizing (µg/ml)					
Fungal isolates	Incubation periods(days)				
	3	5	7	9	11
Aspergillus niger	16.6	20.56	28.26	27.08	26.23
Pencillium oxalicum	12.43	18.56	21.61	21.28	16.26

Effect of temperature on phosphate solubilization fungi

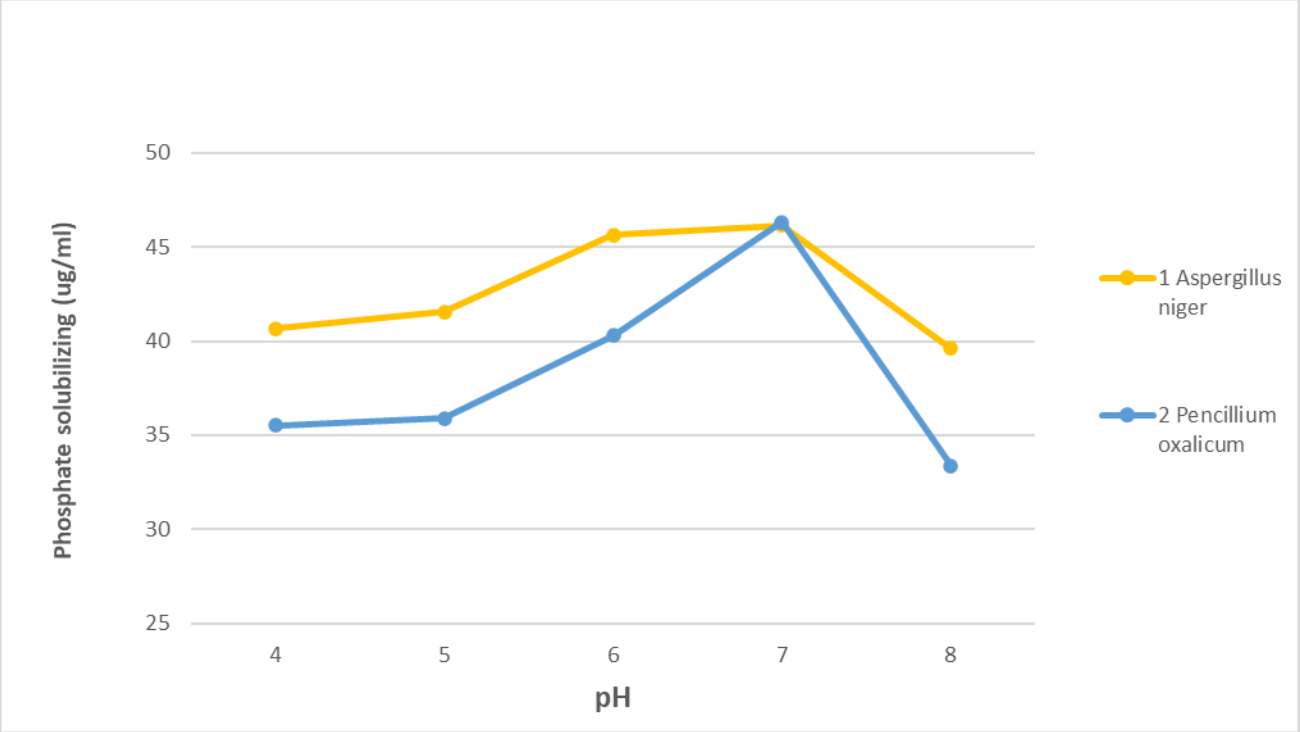
Aspergillus niger and *Pencillium oxalicum* both solubilized the most phosphate at 30°C (58.53 and 48.50 µg/ml, respectively), which has an effect on the solubilization of phosphate. Phosphate solubilization decreased at temperatures above or below 30°C. It has been demonstrated that *Aspergillus niger* and *Pencillium oxalicum* can both thrive at temperatures higher than 40 °C, however the latter generated less easily available Phosphorous. At 30°C (29.26 and 24.25 µg/ml, respectively), *Aspergillus niger* and *Pencillium oxalicum* both solubilized the most phosphate, followed by 35°C (24.08 and 22.31 µg/ml), 27°C (23.13 and 17.93 µg/ml), 40°C (19.61 and 20.40 µg/ml), and 25°C (17.08 and 14.45µg/ml). (Graph-1)



Graph 1: Effect of temperature on the solubilization of phosphate (ug/ml) produced by *Aspergillus niger and Pencillium oxalicum*

Effect of pH on Phosphate solubilizing Fungi

In comparison to un-inoculated treatments, the maximum phosphate solubilization by *Aspergillus niger* and *Pencillium oxalicum* was seen at pH 7 (46.16 and 46.33 µg/ml), followed by pH 6 (45.66 and 40.28 µg/ml). (Graph 2)



Graph 2: Effect of varying pH on induced phosphate solubilization (ug/ml) by *Aspergillus niger and Pencillium oxalicum*

4. Conclusion

Based on morophological characteristics, the study identified 12 different species of fungi, including five Aspergillus species, two Fusarium species, three Penicillium species, and one Curvularia and Alterneria species. A plate approach was used to evaluate the fungi's ability to dissolve phosphate. Out of the 12 isolates, only two demonstrated a positive phosphate solubilization zone on PVK agar medium. The more effective P solubilizing fungi (PSF) were thought to be the two isolates with the largest clear zone/coloy diameter ratio. The quantitative assay showed that Aspergillus niger was the most efficient solubilizer, with a soluble phosphate activity of 36.9 µg/ml. Aspergillus niger and Pencillium oxalicum achieved a maximum phosphate availability of 28.26 and 21.61 µg/ml after 7 days and 27.08 and 21.28 µg/ml after 9 days. The PS decreased starting at day 9 and continued until the incubation period was over. The effects of pH and temperature on phosphate solubilization was also examined. Aspergillus niger and Pencillium oxalicum solubilized the most phosphate at 30°C, while temperature above or below 30°C reduced the solubilization of phosphate. Both Aspergillus niger and Pencillium oxalicum were capable of growing at temperatures greater than 40°C, but produced less readily accessible phosphate. In conclusion, the study highlights the importance of fungi in phosphate solubilization and their potential applications in various environments.

References:

1. Bhatacharya, S.; Das, A.; Bhardwaj, S. and Rajan, S.S. (2015): Phosphate solubilizing potential of Aspergillus niger MPPF-8 isolated from Multhupettai Mangrove. J. Sci. industr. Res., 74: 503.
2. Cheesbrough M. (2000) District Laboratory Practice in Tropical Countries Part 2. Cambridge, UK: Cambridge University Press; 2000.
3. Coutinho, F.P.; Felix, W.P. and Yano–Melo, M. (2012): Solubilization of phosphates in vitro by Aspergillus sp .and Penicillium sp. Ecol. Eng., 42: 85.
4. Diba K., Kordbacheh P., Mirhendi S. H., Rezaie S., Mahmoudi M. (2007) Identification of Aspergillus sp. using morphological characteristics. Pakistan Journal of Medical Sciences.;23(6):867–872.
5. Elias,F., Woyessa, D., Mulet,D. (2016) phosphate solubilizing potential of rhizosphere fungi isolated from plants in Jimma Zone, Southwest Ethiopia. Int. J. Microbiol. 2016:5472601. Doi: 10.1155/2016/5472601
6. Olsen, S.R.; Cole, C.V.; Watanabe, F.S. and Dean, L.A. (1954): Estimation of available phosphorus in soils by extraction with sodium bio-carbonate. US Department of Agriculture circular No. 939.USDA, Washing ton. D.C.
7. Pikovskaya, R.I. (1948): Mobilization of phosphorus in soil connection with the vital activity of some microbial species. Microbiol., 17:362.
8. Richardson, A.E. and Simpson, R.J. (2011): Soil microorganisms mediating phosphorus availability. Plant Physiol., 156: 989.
9. Rodriguez, H.; Fraga, R.; Gonzalez, T. and Bashan, Y. (2006): Genetics of phosphate solubilization and its potential applications for improving plant growth-promoting bacteria. Plant Soil, 287: 15.

10. Ryan,J., Estefan,G., and Rashid, A.(2001). Soil and Plant Analysis Laboratory Manual, second Edn. Aleppo: ICARDA.
11. Stevens R. B. (1974) Mycology Guide Book. Lawrence, Kan, USA: Mycological Society of America.
12. Velazquez, E. and Rodriguez-Barrueco, C. (2007): First international meeting on microbial phosphate solubilization (Developments in Plant and Soil Scince vol 102). Springer, The Netherlands.
13. Edi-Premono, M. (1996): Effect of phosphate - solubilizing Pseudomonas Putida on the growth of maize rhizosphere. Indo. J. Crop. Sci., 11: 13.