

# Isolation And Evaluation of *Aspergillus Niger*: A Fungi Implicated in Infectious Diseases And its Application as Potential Biofertilizer

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## Abstract

*In the quest to improve the agronomic effectiveness of Rock Phosphate (RP), a widely available and cost-effective phosphate fertilizer, this study aimed to overcome its limited solubility by isolating fungal strains capable of solubilizing rock phosphate. Soil samples were collected from Garhmukteshwar, Hapur, for the isolation process. Among several isolates, three strains exhibited the highest rock phosphate solubilization within a seven-day timeframe, coinciding with a significant decrease in soil pH. To assess the practical implications of these findings, the bio-fertilizer activity of the identified isolates was evaluated in natural environmental conditions, specifically on *Phaseolus vulgaris* (Common bean). The experimental results demonstrated a significant positive impact on plant growth when rock phosphate was co-applied with these phosphate solubilizing fungi. This symbiotic interaction between the fungal strains and rock phosphate not only increased nutrient availability for the plants but also showcased the potential to enhance overall soil fertility. These findings contribute valuable insights to sustainable agriculture, emphasizing the potential of leveraging microbial interactions to optimize the performance of widely used fertilizers. The significance of this study goes beyond Garhmukteshwar, offering a promising approach to address the solubility challenges of rock phosphate and enhance its efficacy as a phosphate fertilizer in various agricultural settings. The identified fungal strains represent a novel avenue for the development of bio-fertilizers, contributing to more sustainable and productive agricultural practices.*

**Keywords:** *Aspergillus niger*, Fungus, Biofertiliser, Phosphate solubilizing, Isolation

## INTRODUCTION

Phosphorus (P) is a crucial macronutrient for plant growth. When utilized as a soluble fertilizer, it tends to become immobilized quickly, reducing its availability to plants [1]. This often necessitates frequent and expensive reapplication, which is environmentally undesirable. Sustainable agricultural practices advocate for the use of more cost-effective phosphorus sources [2]. Rock phosphate (RP) is commonly employed to maintain soil P levels in a plant-accessible form. However, the major obstacle to using RP as a phosphatic fertilizer is its poor solubility. Physical and chemical processes, such as particle size reduction and partial acidification, are typically employed to transform these rocks into more valuable products [3].

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Research suggests that the application of RP as a phosphate fertilizer, coupled with the activity of soil microorganisms, can be successful. To prevent the depletion of high-grade RP stock, there is a growing importance in using phosphate-solubilizing microorganisms (PSM) and RP in combination as a biofertilizer [4]. In the current study, RP fungi were isolated from crop areas in and around

Garhmukteshwar with rhizosphere soil. Raw materials for phosphoric acid were obtained from Senegal RP. Qualitative and quantitative assessments of phosphate solubilization activity were conducted in a modified Pikovskaya's (PVK) medium, and the isolates with the highest inorganic phosphate solubilizing activity were assessed in pot experiments using *Phaseolus vulgaris* (Pharasin) [5].

It is increasingly likely that the ability of PSMs to chelate metabolites such as organic acids bound to phosphates and cations, especially calcium, is what makes them so effective against *P. aeruginosa*. Citric acid, among these organic acids, has been reported to promote phosphate rock solubilization comparable to that achieved by conventionally used sulfuric acid [6]. Because of its rapid growth and organic acid production, *Aspergillus niger* is a potential PSM whose biological P solubilization is influenced by the concentration of citric acid in the medium. While most strains of *A. niger* can produce citric acid, some can have been more effective than others as well. Consequently, the selection of fungal species is important to create a process that can biologically leach P from phosphate rocks [7].

By increasing the amount of soluble P in soils, phosphate-soluble microorganisms (PSMs) act as biofertilizers by converting insoluble P into water-soluble form. One environmentally relevant treatment of infertile soils and the use of P biofertilizers. Application of PSM has been found to stimulate plant growth and increase the availability of soluble P in the presence of growing plants [8]. By their ability to synthesize phytohormones, increase the availability of trace elements (Zn and Fe), and enhance the efficiency of natural nitrogen fixation, phosphate-soluble bacteria promote plant growth. Research conducted on PSM fertilization showed higher plant growth and higher P uptake [9]. Benefits of managing and adding rhizosphere microbes include increased root and shoot count, root and root length, increased P supply to the crop, increased yield. Phosphate-solubilizing microorganisms also exert various effects on the growth and development of plants [10].

## MATERIALS AND METHODS

### Sample Collection

Soil samples were collected from *Phaseolus vulgaris* (Pharasin) community of Garh Mukteshwar, Hapur. Upon arrival at the laboratory, the soil underwent screening through a 2mm sieve to eliminate rocks and other plant materials (Figure 1). Subsequently, the soil was meticulously mixed to guarantee homogeneity. Subsequently, the samples were stored for later use in hermetically sealed polyethylene bottles. The pH and electrical properties of the obtained samples were measured and recorded.



**Figure 1.** Sample of plant collected from field.

### Isolation of Phosphate-Solubilizing Fungi

The collected soil samples were utilized for isolating phosphate fungi on Pikovskaya's Agar media (PVK). The solution was autoclaved at 121°C for 15 min and approximately 20 ml of agar solution was

added to the petriplates, where it solidified prior to incubation. To isolate, the roots were vigorously agitated to remove loosely adherent soil,

and the root samples, along with their adhering soil, were cut into pieces using sterile scissors. A 500-ml Erlenmeyer flask containing sterile distilled water was filled with 10 g of suspended soil of each plant root fragment, weighed aseptically and the mixture was thoroughly shaken before standing there for five minutes.

The test tube was filled with 9 ml of sterile physiological saline solution, and 1 ml portions from the sample supernatant were added. The segments are then gradually rolled from  $10^{-1}$  to  $10^{-6}$ . Aliquots of 0.1 ml of serially diluted suitable soil suspensions were spread onto Pikovskaya agar plates, cultured at 25–28°C for 5–7 days and treated with fungal isolates a indicating clear areas around the clusters into Pikovskaya agar medium for further purification. Pure cultures were kept at 4°C on a potato dextrose agar (PDA) slant for further experiments. (Figure 2). The diameter of the clearance zone was measured after 24 hours and 7 days, and the Solubilization Index (SI) ratio was recorded in triplicate.



**Figure 2.** Figure of pure culture with PDA.

### **Principle Analysis of PDA Media**

The principle behind Potato Dextrose Agar (PDA) lies in its composition, which includes Dextrose as a carbohydrate source, stimulating growth, and Potato infusion providing a nutrient base for the flourishing growth of most fungi. Agar is added as a stabilizer.

### **Screening of Fungi for Phosphate Solubilization**

Fungal isolates were evaluated for their phosphate mobilization activity in rhizospheric soils through both agar plates and liquid cultures.

#### **Identification and description of phosphate-solubilizing fungi**

Rock phosphate (RP) soluble fungi were extracted using PVK, or Pikovskaya dye. The medium consists of Yeast extract 0.5 g/L, Dextrose 10 g/L, Ammonium sulfate 0.5 g/L, Rock phosphate 5 g/L (providing 700 of P), Potassium chloride 0.2 g/L, Magnesium sulfate 0.1 g/L, Manganese sulfate 0.0001 g/L, Ferrous sulfate 0.0001 g/L, Agar 15 g/L, and Bromophenol blue 2.4 g/L. Microorganisms were tested for their ability to release phosphorus (P) from insoluble sources in liquid media. A modified PVK broth was used, and mycelia suspensions were inoculated. Incubation occurred for 30 days, with intermittent sample withdrawals. The quantification of solubilized P was conducted using the Vanadomolybdophosphoric yellow color method.

### **Principle Analysis of PVK and Broth Media:**

Pikovskaya agar medium was originally developed for phosphate solubilizing bacterial culture, but Pikovskaya broth is such a variation. In the broth, inorganic and organic phosphate naturally present in soil is utilized. Numerous soil fungi and bacteria act as phosphate solubilizers, contributing to the maintenance of the phosphate balance in crop plants. The medium contains phosphate in the form of calcium phosphate, while Dextrose serves as the energy source. Various salts and Yeast extract support organism growth. The growth observed in Pikovskaya's Broth (M1719) can be assessed for phosphate solubilization through subculturing as spot incubation on Pikovskaya's Agar (M520), with phosphate solubilization indicated by clearance around the growth or colony.

## RESULT

### Analysis of Phosphorus

For the analysis of phosphorus, plant roots underwent digestion, and nutrient analysis was conducted. Phosphorus content was determined using the Vanadomolybdate phosphoric yellow color method.

### Pot Studies

In preparation for inoculation, three rock phosphate (RP) solubilizing strains were cultured in Petri plates for 10 days, and spore suspensions were created by flooding the agar with sterile distilled water. The suspension was then poured into a 500 mL flask and shaken for two minutes to obtain a final count. Greenhouse pot experiments were conducted with three replications of each treatment (T2-T7) and one control (T1). Seedlings were transplanted into sterilized plastic pots, inoculated with fungal spore suspension, and supplemented with Senegal RP. The pots were kept under natural conditions for 21 days, and various plant parameters were recorded for comparative evaluation.

### Soil Analysis

Soil samples were tested for mineral elements before and after plant collection. The samples underwent air drying, sieving, and analysis for pH, nitrogen, organic carbon, phosphorus, potash, electrical conductivity, and micronutrients.

### Identification of Fungal Strains

Fungal isolates, specifically *Aspergillus* and *Penicillium*s, were identified based on their growth patterns on Potato Dextrose Agar (PDA) plates. Microscopic analysis confirmed these identifications, revealing black dense mycelial growth on the front side and dirty white on the back side. *Penicillium* spp. exhibited grey and yellow colonies, while *Aspergillus* had grey and yellow colonies. Conidia were observed as globular.

### Determination of RP Solubilization in Solid Media

Phosphate solubilization zones were observed, with *Aspergillus* demonstrating the highest Solubilization Index (SI) of 3.5, followed by *Penicillium* sp.1 and *Penicillium* sp.2. The study highlighted the importance of phosphate-soluble fungi in increasing organic soil phosphate availability for plant growth. Phosphate-solubilizing fungi (PSF) were detected in two fungal groups named AP1 and AP2.

### Isolation and Characterization of Phosphate-Solubilizing Fungi

The study used both solid and liquid Pikovskaya (PVK) solutions to isolate and characterize phosphate-soluble fungi. Among 150 fungal isolates from rhizosphere soil samples, 167 inorganic phosphates were detected (46.52%). The identified phosphate-solubilizing fungi belonged to the genera *Aspergillus* (55.69%), 23.35% *Penicillium*, and 9.58% of *Fusarium* spp. 1.10 to 3.05 p.m. The trend was solubility index (SI).

### Effects of Phosphorous Solubilizing Fungus on Rock Phosphate

In this study, the effect of phosphorus-solubilizing fungus *Pleurotus ostreatus* in rock phosphate solution and its addition to brewery wet on the growth of *Zea mays* L was investigated for phosphate utilization. On the ninth day of incubation, the highest concentration of soluble phosphorus (P) was observed in Pikovskaya's broth containing P source and rock phosphate. The *Ostreatus* extract was exposed to RP and winery sludge, which significantly increased the levels of plant traits such as chlorophyll content, nutrient accumulation, root and shoot weight, and root and shoot length.

### Effect of fungi for RP solubilization



First week observation.



Second week observation



Third week observation



Final observation

**Figure 3.** Figure showing the observation of samples in first, second, third and fourth week.

## CONCLUSION

The research highlights the significance of phosphate solubilizing microorganisms in enhancing the bioavailability of soil phosphorus, a critical factor influencing plant nutrition. These microorganisms play a crucial role in converting insoluble phosphate compounds into forms that are readily accessible to plants, thereby contributing significantly to improved plant growth, increased yield, and enhanced produce quality. The study specifically focuses on selected isolates with the capability to mobilize tricalcium phosphate (TCP) and rock phosphate (RP) in a PVK broth, revealing their efficiency, particularly in media containing TCP.

The demonstrated effectiveness of these isolates in solubilizing both TCP and RP holds promising implications for sustainable agricultural practices. The proficiency of phosphate-solubilizing fungi in mobilizing these phosphate sources suggests a potential reduction in reliance on synthetic fertilizers, addressing environmental concerns associated with their excessive use. By enhancing soil fertility through the conversion of insoluble phosphates, the study advocates for a more environmentally friendly approach to agriculture.

Moreover, the findings underscore the broader applicability of phosphate solubilizing microorganisms in promoting sustainable farming practices. Incorporating these microorganisms into agricultural systems improves nutrient uptake by plants and contributes to a balanced soil organic matter throughout the ecosystem. This research encourages the adoption of bio-fertilizers as a viable strategy to optimize soil phosphorus dynamics, promote environmentally conscious agricultural practices, and support the long-term health and productivity of agricultural lands.

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