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# ISOLATION AND CHARACTERIZATION OF PHOSPHATE-SOLUBILIZING FUNGI FROM SALINE REGION FOR INCREASED CROP PRODUCTIVITY

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## ABSTRACT

The study designed to isolate and characterize PSF from the saline rhizosphere of *Avicennia marina*, which is dominant mangrove species, to evaluate their potential as biofertilizer in saline soil of Kutch region. Eight fungal isolates from mangrove soils in Kandla, Gujarat, were identified as *Alternaria alternata*, *Aspergillus fumigatus, Aspergillus versicolor*, and *Penicillium oxalicum* species using 18S rRNA gene sequencing. Phosphate solubilization was measured using NBRIP and PVK medium. The development of plant growth assets particularly siderophore and indole acetic acid synthesis were investigated. The quantitative study of *A. fumigatus* (PSF-4) revealed the highest IAA synthesis (59.0 µg/ml), the strongest siderophore activity, and the highest phosphate solubilization ability (700 µg/ml in NBRIP and 680 µg/ml in PVK). Furthermore, *A. versicolour* (PSF-6) demonstrated considerable potential due to its growth-promoting and persistent solubilization characteristics. PSF-4 and PSF-6 exhibited the highest levels of plant growth-promoting characteristic which includes siderophore synthesis and indole-3-acetic acid (IAA), suggesting their dual activity. This result confirmed that native fungal isolates from soils related with mangroves have the potential to be effective bioinoculants for sustainable agriculture, especially in saline conditions.

**KEYWORDS:** *Avicennia Marina*, Biofertilizer, Phosphate-Solubilizing Fungi (PSF), Phylogenetic Analysis, Saline Soil, Sustainable Agriculture

**ABBREVIATIONS:** PSF- Phosphate-solubilizing fungi, BLAST - Basic local alignment search tool, TCP- Tricalcium phosphate, IAA- Indole-3-acetic acid, PVK- Pikovskaya's medium, PDB- Potato dextrose broth, NBRIP- National botanical research institute's phosphate medium, PCR- Polymerase chain reaction, OD- Optical density RNA - Ribonucleic acid, ITS- Internal transcribed spacer

## 1. INTRODUCTION

Phosphorus constitutes a vital macronutrient for development of plant, supporting several biological functions including energy transmission, photosynthesis and nucleic acids [RNA/DNA] formation [1]. However, phosphorus is not always easily available to plants because it occurs in soil in forms that plant cannot use [2]. Most phosphate in agricultural soils occurs in insoluble mineral forms, such as tricalcium and hydroxyapatite phosphate, as it exists in inaccessible forms for plants to use [3]. This causes phosphate deficits, which have an influence on plant growth and yields, particularly in areas where availability of phosphorus to plant is naturally limited. To solve this issue, farmers typically use chemical fertilizers to feed plants with accessible phosphorus, however only a tiny portion of phosphorus applied was taken up via the plants as a large proportion of phosphorus either washes away or bind to soil particles. This causes environment concerns such as nutrient runoff and eutrophication, as well as enhanced fertilizer expenses [4]. Phosphate solubilizing microorganisms are the one of the alternate methods for enhancing phosphorus availability that are increasing popularity as a long-term treatment for phosphorus insufficiency.

The process by which microbes convert insoluble phosphorus particles in soils which forms plants may easily absorb which is known as Phosphate solubilization [5]. The particular ability of phosphate solubilizing fungus to mobilize insoluble phosphorus via synthesis of organic acid which includes the oxalic, gluconic, and citric acid [6]. The pH of soil around plants which is lowered by these acids because they breakdown the mineral phosphorus components and release phosphate ions which plants can absorb [7]. PSF can increase phosphorus availability by hydrolyzing organic phosphorus molecules with the aid of enzymes like phosphatase and phytase in addition to organic acids. This method promotes the soil microbial diversity, nitrogen cycling, and improved plant absorption of phosphorus [8].

Phosphate solubilizing fungi are important because they help to give plants phosphorus that they can easily use [9]. Unlike chemical fertilizers PSF slowly reduce phosphorus from soil which allowing accessible for plants to grow [10]. Furthermore, PSF are typically able to support plants in additional growth promoting mechanisms which includes the synthesis and release of phytohormones like indole acetic acid which increase root growth and other nutrient absorption [11]. Many PSFs also create siderophore that is iron- chelating compounds which help plant for absorb iron from soil enhancing plant

development and health [12]. These synergistic benefits make PSF an excellent option for chemical fertilizers, particularly in phosphorus-deficient soils, as well as for sustainable farming approaches [13].

Phosphate-solubilizing fungi are found everywhere in nature, with many different kinds of species, including *Aspergillus, Fusarium, Mucor, Penicillium, and Trichoderma*, confirming high phosphate solubility ability [14]. Different fungal species employ varying approaches to increase phosphorus availability; certain fungal species synthesize various organic acids, whereas others may use physiological mechanisms, such as the secretion of exopolysaccharides, to modify soil conditions to promote phosphorus solubilization [15]. PSF diversity ensures that their performance is different in varying soils and environmental conditions. Fungi from salt environments, e.g., mangrove forests, might have special adaptations that make them capable of solubilizing phosphorus in high salinity [16]. The adaptability ensures that PSF is effective not only in the common agricultural soil but also in adverse environments where chemical fertilizers are less effective or less sustainable [17].

Their extensive use has enormously enhanced agricultural productivity by providing necessary nutrients like nitrogen, phosphorus, and potassium. Nonetheless, their continuous and extensive use has brought some ecological and agronomic challenges, including deterioration of soil quality, imbalance of nutrients, contamination of water body due to run off , and disturbance of indigenous soil microbial communities [18]. Additionally, production and use of chemical fertilizers are energy-demanding process that emit greenhouse gases and drive climate change [19]. Such environmental and sustainability issues have motivated increasing attention toward the discovery of bio-based substitutes that can potentially mitigate reliance on synthetic inputs.

Phosphate-solubilizing fungi (PSF) are considered potential bio-fertilizers because of their capacity to enhance accessibility of phosphorus for plant uptake [20]. Although Phosphorus is important for development of plants. However, many soils contain it in forms that plants are unable to absorb [21]. PSF helps by converting insoluble phosphorus forms into plant-usable forms, improving nutrient uptake while encouraging healthy plant development [22]. PSF can enhance soil quality by maintaining beneficial microbial populations and releasing phosphorus, which makes it more ecologically friendly than chemical fertilizers [23].

However, most studies on PSF has focused on normal agricultural soils, with not much attention given to their existence and usefulness in more problematic or unique situations. PSF research under such conditions could uncover new strains with improved phosphorus solubility and adaptability to harsh environments. The research aims to isolate and analyze PSF from such environments to precisely understand their potential as biofertilizers for enhancing soil fertility and promoting sustainable farming practices.

## 2. MATERIALS AND METHOD

### 2.1 Collection of sample

Three separate samples were taken from the specified area as part of a sampling operation from the Kandla region of Kutch, Gujarat (23°01'58.6" N, 70°09'32.9" E). Samples of rhizospheric soil were taken from a 15-cm patch, and specimens of *Avicennia marina* roots were collected and stored in a sterile plastic container with careful labeling for identification.

### 2.2 Isolation of Fungi

Root tissues from healthy matured Avicennia marina crops were used for endophyte isolation. The samples were transferred in sterile polythene bags and surface-sterilized with help of modified technique of Kjer et al. [24]. Rhizospheric soil was serially diluted in sterile distilled water. 0.2 ml of supernatant from $10^{-3}$, $10^{-4}$ and $10^{-5}$ dilutions were spread on potato dextrose media and incubated at 30 ± 2 °C for 5 to 7 days.

### 2.3 Molecular Characterization

Isolates which displayed better phosphate solubilizing ability were subjected to molecular identification. Eight fungal strains, namely PSF-1 to PSF-8, were identified and subjected to DNA amplification and partial sequencing of the 18S rRNA gene using ITS 1 and ITS 4 primers. Collected sequence were analyzed in contrast to those found in the GenBank database, the BLAST algorithm. Sequences showing a high similarity index of >98% were selected for further analysis. The sequence was submitted into GenBank (NCBI) and their Accession number was generated.

### 2.4 Phylogenetic Analysis

Phylogenetic analysis of the fungal 18S rRNA gene sequences from the selected six fungal endophytes was conducted using MEGA XII software [25]. The analysis involved the UPGMA method with 1,000 bootstrap replicates to assess the robustness of the inferred clades.

### 2.5 Qualitative Screening for Phosphate Solubilization

Pikovskaya's agar through 0.5% TCP as the insoluble source of phosphorus was used for qualitatively evaluating the phosphate solubilization by fungal isolates [26]. After being added in the middle of the plate, a 6 millimeter disc of a 5 day old fungal cultures were incubated for seven days at 28 ± 2°C. A clear halo surrounding colony indicates the solubilization of phosphate. To calculate the solubilizing index using below formula:

$$\text{Solubilization Index (SI)} = \frac{\text{Colony Diameter + Zone Diameter}}{\text{Colony Diameter}}$$

### 2.6 Quantitative Estimation of Solubilized Phosphate

The phosphate solubilization was directly determined with Pikovskaya's broth and NBRIP, which were enhanced with 0.5% TCP. A 1 ml inoculum of fungal isolates ($OD_{520}$ = 0.3) was added to 50 ml of broth and cultured for 7 days at 150 rpm at 28 ± 2°C. Using the molybdenum blue method [27], the supernatant was centrifuged for ten minutes at ten thousand rpm. The absorbance was measured at 820 nm, and the phosphate concentration was calculated using a $KH_2PO_4$ reference curve.

### 2.7 Siderophore Production

Spots of fungal strains were placed on Chrome-Azurol S (CAS) media and allowed to incubate at 28°C for four days. The process of siderophore formation was monitored by observing the growth of an orange halo around the colonies.

### 2.8 Synthesis of IAA

The isolates of fungi were cultured for 5–7 days at 28 ± 2 °C (120 rpm) in PDB containing 0.1% L-tryptophan. The supernatant was combined with Salkowski's reagent (1:1) and allowed to sit in the dark for 30 minutes in centrifugation at 10,000 rpm for 10 minutes. Pink colouration indicated generation of IAA, which was measured at 530 nm using a pure IAA standard curve (Sigma-Aldrich) and expressed in µg/ml.

## 3. RESULTS AND DISCUSSION

### 3.1. Identification of Fungal species

This study investigation about 11 different fungi were isolated among with 3 were obtained from roots sample of *A.marina* and 8 were isolated from soil. All the species was morphological assessment was conducted using colony appearance and microscopically through lactophenol cotton blue staining. Also, upon screening these isolates for phosphorous solubilisation, selected isolates were subjected to molecular identification by 18SrRNA gene sequencing. The sequence acquired from the isolates were deposited in the NCBI gene bank and their Genbank accession number is mentioned in Table 1.

| Sr. No. | Isolate ID no. | Closest identified relative | Genbank Accession no. |
|---|---|---|---|
| **1** | PSF-1 | *Alternaria alternata* | PV546249 |
| **2** | PSF-2 | *Aspergillus fumigatus* | PV546251 |
| **3** | PSF-3 | *Alternaria sp.* | PV546252 |
| **4** | PSF-4 | *Aspergillus niger* | PV546253 |
| **5** | PSF-5 | *Aspergillus versicolor* | PV546272 |
| **6** | PSF-6 | *Penicillium chrysogenum* | PV546275 |
| **7** | PSF-7 | *Penicillium oxalicum* | PV546276 |
| **8** | PSF-8 | *Penicillium sp.* | PV546279 |

### 3.2. Phylogenetic characterization of Isolates

Phylogenetic analysis based on 18S rRNA sequences revealed that PSF- 1, PSF- 2, AND PSF -3 fungal strains are closely align with the *Alternaria alternata* species complex, forming a strong clade with 100% bootstrap support alongside reference strains like MF072541, MZ314132, and OM630609. This confirms their classification as *A. alternata*, a known rhizospheric fungus involved in nutrient mobilization and moderate phosphorus solubilization via secretion of organic acids, particularly citrate and gluconate. While PSF-1 and PSF-2 likely share these metabolic traits, PSF-3 showed slightly reduced P-solubilization, suggesting strain-level functional differences. Other studies using ITS, GAPDH, RPB2, TEF1 and Alt a 1 gene location revealed genetically distinct *Alternaria* species without clear regional clustering [28].

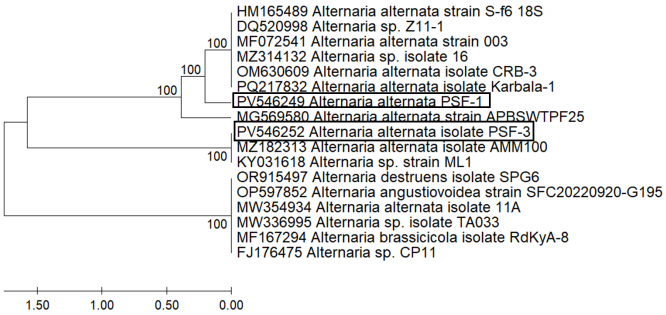


**Figure 1:** Phylogenetic Analysis of Alternaria isolates

Figure 2 shows that isolates PSF-4, PSF-5, and PSF-6 formed well-supported clades with *Aspergillus* species. PSF-4 clustered closely with A. *fumigatus* strains (KR023997, OR578448) with >99% bootstrap support, confirming its identity and known abilities to solubilize phosphate and synthesize IAA, iron chelating compounds, and other secondary metabolites. PSF-5 and PSF-6 grouped with A. *versicolor* strains (JN938969, KP872530), also with strong support (up to 100%). These consistent clade positions validate molecular identification. Phylogenetic analysis of *Aspergillus* using nuclear and mitochondrial genes (ITS, IGS, benA, rpb2, rns, cox1) further confirms species-level resolution across clades like *Candidi, Fumigati, Flavi,* and *Nigri* [29].

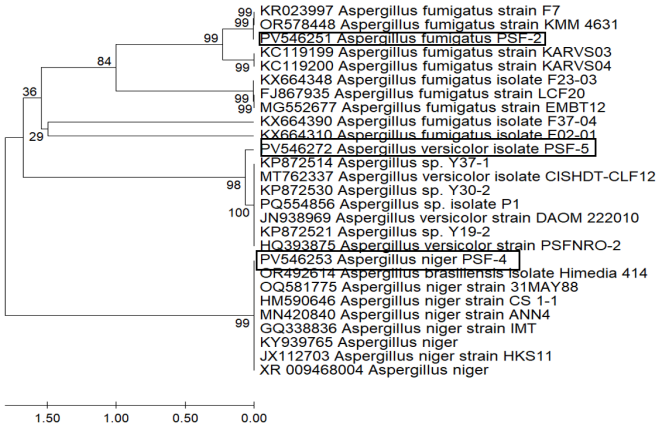


**Figure 2:** Phylogenetic Analysis of Aspergillus isolates

Figure 3 shows that isolates PSF-7 and PSF-8 clustered within the *Penicillium oxalicum* complex, closely aligning with sequences XR007588132 and KF152942, supported by >95% bootstrap values. This confirms their identity as P. *oxalicum*, known for effective inorganic phosphate solubilization and oxalic acid production, aiding nutrient mobilization. Although phylogenetic placement indicates genetic potential, actual solubilization and metabolite output varied between the isolates, highlighting the need for both molecular and functional evaluation. A comparative study using ITS and benA genes also showed strong species-level resolution between P. *simile* and P. *raistrickii*, with multiple genetic markers improving phylogenetic clarity in the *Penicillium* genus [30].

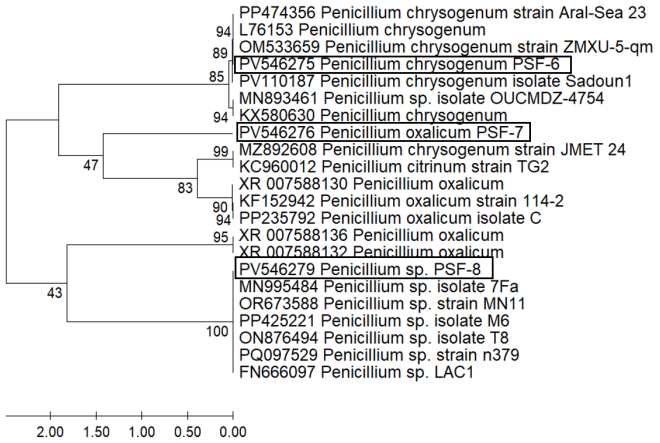


**Figure 3:** Phylogenetic Analysis of Penicillium isolates

### 3.3. Quantitative Analysis of Phosphate Solubility using Fungal Isolates

Phosphate solubilization was assessed using NBRIP and Pikovskaya's (PVK) media, revealing varied efficiencies among the eight fungal isolates. PSF-4 (*Aspergillus fumigatus*) showed the highest solubilization (700 µg/ml in NBRIP, 680 µg/ml in PVK), attributed to its synthesis of low molecular weight acids such as citrate, gluconate and oxalate that enhance P availability. Other isolates showed moderate to low solubilization: PSF-1 (110/90), PSF-2 (490/420), PSF-3 (90/80), PSF-5 (310/280), PSF-6 (580/550), PSF-7 (300/260), and PSF-8 (200/120 µg/ml). These findings support PSF-4's potential as a bioinoculant for saline soils. Previous studies reported P-solubilization capacities of up to 728.77 µg/ml and emphasized the role of such fungi as effective bio fertilizers [31].

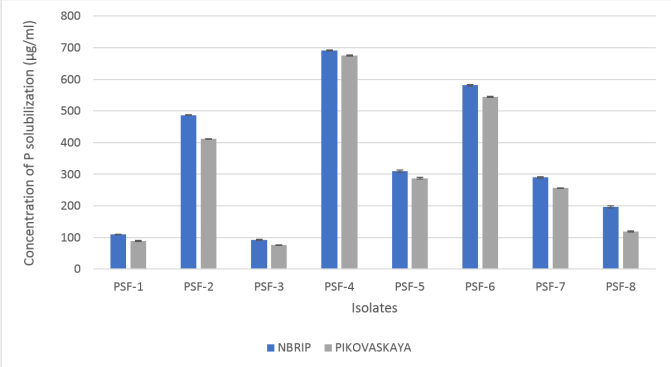


**Figure 4:** Quantitative Estimation of Phosphate Solubilization

### 3.4. Characterization of Auxin and Siderophore Production in PSF Isolates

IAA and siderophore production (Table 2) varied significantly among the fungal isolates, reflecting their plant growth-promoting potential. PSF-4 showed the highest IAA production (59.0 ± 0.9 µg/ml) and strong siderophore activity (++++)—highlighting its role in enhancing root growth and iron uptake. PSF-6 also exhibited high IAA levels (54.1 ± 0.1 µg/ml) and strong siderophore synthesis, confirming its biofertilizer potential. PSF-5 and PSF-7 showed moderate IAA (30.2 and 25.2 µg/ml) and siderophore activity (++), suitable for moderate growth enhancement. PSF-1 and PSF-2 had low IAA levels (9.7 and 11.2 µg/ml), with only PSF-1 showing siderophore activity. PSF-3 and PSF-8 lacked both traits, indicating limited plant growth-promotion potential. Similarly, A. *flavus* JKJ7 was reported to produce high siderophores (83.7%) and IAA (68.51 mg/L), supporting its use in sustainable agriculture [32].

| Isolate ID | IAA | Siderophore production |
|---|---|---|
| PSF- 1 | 9.7 ± 0.2 | + |
| PSF- 2 | 11.2 ± 0.9 | + |
| PSF- 3 | - | - |
| PSF- 4 | 59 ± 0.9 | ++++ |
| PSF- 5 | 30.2 ± 0.4 | ++ |
| PSF- 6 | 54.1 ± 0.1 | ++++ |
| PSF- 7 | 25.2 ± 0.3 | ++ |
| PSF- 8 | - | - |

**Table 2:** IAA and Siderophore Profiles of PSF Fungal Isolates

## 4. CONCLUSION

The study emphasizes the potential of indigenous PSF strain obtained from the rhizosphere of *Avicennia* as viable biofertilizers that help improve nutrient availability and plant growth in adverse environments. Molecular identification showed the presence of effective strains, particularly *Aspergillus fumigatus* (PSF-4) and A. versicolor (PSF-6) that demonstrated high phosphorus solubilisation abilities and beneficial plant growth-promoting functions, including IAA and siderophore synthesis. While species such as *Alternaria alternata* and *Penicillium oxalicum* displayed limited potential, their ability to adapt to saline conditions implies potential synergistic applications in microbial consortiums. However, more research is required to determine their in vivo effectiveness, long-term effects on soil health, and incorporation into sustainable biofertilizer formulations. The study has proven the ability of mangrove derived fungi in saline regions as a potential candidate for biofertilizer.

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