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Isolation and Screening of Acid Phosphatase Producing Fungi from Textile Sizing Site

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Abstract:

Phosphorus (P) is a crucial macronutrient for plants, playing an indispensable role in metabolic functions, root development, flowering, and energy transfer. Despite its significance, P availability in soil is frequently limited due to fixation into insoluble forms, posing challenges for plant growth and productivity. Chemical phosphate fertilizers, though widely used, contribute to soil salinization, water eutrophication, and ecological imbalance. Microbial phosphate solubilization offers a sustainable alternative, with fungi showing remarkable efficiency in transforming bound phosphorus into bioavailable forms through enzymatic activities, particularly acid phosphatases. This study focuses on isolating acid phosphatase-producing fungi from soil samples collected at textile sizing sites in Bhiwandi, Maharashtra. Using serial dilution techniques, 120 fungal isolates were obtained and screened for acid phosphatase activity on Pikovskaya's agar medium, supplemented with $Ca_3(PO_4)_2$ as a substrate. Among these, 35 isolates demonstrated significant phosphate solubilization, evident from halo zone formation around fungal colonies. Microscopic analysis identified acid phosphatase-producing fungi predominantly from the genera *Aspergillus*, *Trichoderma*, *Penicillium*, and *Fusarium*. Acid phosphatase produced by fungi not only supports sustainable agriculture by enhancing soil fertility and plant growth but also holds promise for industrial applications such as biofertilizers, diagnostic kits, and bioremediation processes in waste environments. This research underscores the potential for scaling up enzyme production and purification to integrate fungal acid phosphatases into commercial systems, meeting global demands while mitigating environmental impacts.

Key Words: Acid phosphatase, Phosphate-solubilizing fungi, Bioremediation, Textile sizing site.

Introduction:

Phosphorus (P) is an essential macronutrient required for the normal growth and metabolic functions of plants and microorganisms (Souza et al., 2016). It plays a pivotal role in the development of plant cells, the formation of fine roots, flowers, fruits, and seeds, as well as in energy storage and release (Walpola and Yon, 2012; Elfiati et al., 2021). Furthermore, phosphorus is involved in the biosynthesis of cellular components such as nucleic acids, phospholipids, and proteins (Altomare et al., 1999; Souza et al., 2016). Plants absorb phosphorus in the form of orthophosphate. However, the availability of phosphorus in soil is generally low due to its fixation process, where it binds to Fe phosphate and Al-phosphate in acidic soils or Ca-phosphate in alkaline soils (Chang and Yang, 2009; Oliveira et al., 2009; Malviya et al., 2011; Tallapragada and Seshachala, 2012; Sharma et al., 2012; Hou et al., 2018). A deficiency in phosphorus can disrupt the growth of plant roots, particularly fine roots, hindering nutrient absorption and thus restricting plant growth. Plants cannot take up phosphorus in its bound form; it must first be converted into a simpler form to be accessible to plants (Elfiati et al., 2021). To address phosphorus deficiency, chemical phosphate fertilizers are added to the soil, but under natural conditions, over 90% of soil phosphorus remains unavailable to plants. Most of the phosphate fertilizers applied react with metal ions in the soil, forming insoluble phosphates (Hamdali et al., 2008). Consequently, the use of chemical fertilizers may not yield the desired outcomes and can lead to soil salinization, water eutrophication, and ecological imbalance (Qiao et al., 2019).

Numerous studies have demonstrated that soil microorganisms, such as fungi and bacteria, can convert insoluble and bound phosphates into a soluble form that plants can absorb (Rodriguez and Fraga, 1999; Chatli et al., 2008; Chang and Yang, 2009; Malviya et al., 2011; Das et al., 2013; Sharma et al., 2013). Therefore, employing microorganisms to enhance soil fertility is a promising and sustainable approach (Elfiati et al., 2021).

Microorganisms in the soil that solubilize phosphate, such as phosphate-solubilizing fungi (PSF) (Mehta et al., 2019) and bacteria (PSB) (Zheng et al., 2018), play a pivotal role in the phosphorus cycle. Although the diversity and abundance of PSB exceed those of PSF (Jin et al., 2006), fungi generally demonstrate a significantly

higher phosphate-solubilizing capacity, often several times greater than that of bacteria. Fungi possess more stable genetic characteristics compared to bacteria, whose phosphate-solubilizing ability tends to diminish or disappear during subculturing (Leyval et al., 1993; Vassilev et al., 1995; Qiao et al., 2019).

Fungi from the genera *Penicillium*, *Aspergillus*, *Fusarium*, and *Sclerotium* are renowned for their high efficiency in dissolving insoluble nutrients, including phosphorus (Whitelaw, 1999; Pradhan and Sukla, 2006; El-Azouni, 2008; Malviya et al., 2011; Elias et al., 2016; Gizaw et al., 2017). These fungi can function as biological fertilizers, but effective isolates must be identified through screening (Bashan et al., 2014; Sharma et al., 2013). Compared to bacteria, fungi have been reported to possess a superior ability to solubilize phosphorus (Nahas, 1996). Many dominant fungal groups thrive in acidic soils, such as peat soils, which can serve as a source for obtaining phosphate-solubilizing fungi isolates (Elfiati et al., 2021).

The primary mechanisms by which soil microorganisms solubilize phosphorus include: (1) solubilizing and dissolving complex phosphorus compounds, (2) releasing phosphorus during substrate breakdown, and (3) producing extracellular enzymes such as phosphatases for enzymatic degradation. These enzymes are typically categorized as alkaline phosphatases, acid phosphatases, and protein phosphatases (Rawat and Tewari, 2011; Anand and Srivastava, 2012; Souza et al., 2016). Acid phosphatase (ACPase) (orthophosphoric monoester phosphohydrolase EC 3.1.3.2) is a hydrolase that facilitates the hydrolysis of monoester phosphate, converting organic phosphate into a soluble inorganic form (Duff et al., 1994; Anand and Srivastava, 2012). Structural studies of fungal acid phosphatase have revealed it to be a monomeric protein with two domains: one large domain with α-helix and β sheets (α/β strands) and another smaller α-helix domain (Xiang et al., 2004; Souza et al., 2016).

Acid phosphatases have been identified in a variety of tissues, including seeds, roots, prostate, and bone cells. They are found in a wide range of organisms, including plants, animals, and microorganisms (Ferreira et al., 1998; Custódio et al., 2004; Guimarães et al., 2004; Al-Omair, 2010; Zhang et al., 2013; Souza et al., 2016). They have so far been used in a wide variety of operations, such as scavenging, P mobilization and acquisition, improving soil fertility, and promoting plant development (Kapri and Tewari, 2010). They have been employed as biomarkers in animal cells for radioimmunoassay to diagnose prostate cancer, chronic inflammation, and bone metastases (Muniyan et al., 2013; Quintero et al., 2013).

Acid phosphatases are used in industrial processes to improve nutritional value, decrease phosphate excretion by animals, and process feed for monogastric animals that release phosphate (Azeem et al., 2015). Acid phosphatases produced by plants and mycorrhizal fungus have also been used in the bioremediation of contaminated soils (Misra et al., 2012). According to published evidence, microorganism-derived acid phosphatases are more effective than plant-derived ones at hydrolyzing organic phosphate (Kapri and Tewari, 2010). Numerous ACPases from fungi, including *Aspergillus species*, *Humicola species*, *Mucor species*, *Penicillium species*, *Metarhizium species*, and *Trichoderma harzianum*, have been identified (Guimarães et al., 2004; Boyce and Walsh, 2007; Leitao et al., 2010) with their potential in mediating availability of phosphorous to plants from organic compounds investigated by numerous groups (Chacón et al., 2007; Yadav et al., 2011; Souza et al., 2016).

The research on acid phosphatase is motivated by the wide range of applications and the increasing need for these enzymes globally. The goal of this study was to collect soil samples, isolate fungi, examine them under a microscope, and assess the variety of native fungi that could solubilize phosphate from a textile sizing location. The goal was to thoroughly investigate and evaluate the hydrolytic capability of fungi for possible future broad applications, motivated by the demand for acid phosphatases in the feed industry, diagnostic kits, genetic biology, etc.

Materials And Methods

Selection of sample sites

Different textile sizing sites of Bhiwandi city were selected for the sample collection.

Collection of soil samples

The samples were collected from different spots in each site in zip lock polythene bags. Almost 5-10 soil samples were taken from each sizing industries. The soil sample was mixed well and processed next day.

Isolation Of fungi

Fungal colonies were isolated from soil samples by serial dilution method where SDA (Sabouraud dextrose agar) media was prepared, autoclaved and poured in sterile petri plates. Soil samples (1 gm) diluted up to 10^{-5} dilution was spread on respective solidified SDA plates with the help of sterile spreader. The inoculated petri plates were incubated at 28°C for 48 h. About 120 different fungal isolates differentiated based on physical characteristics obtained after incubation were selected for the further processes. The isolates were further inoculated on SDA plates by point inoculation and incubated at 28°C for 48 h to obtain pure fungal cultures (Khan and Kumar, 2011).

Screening of Fungal Isolates for Acid Phosphatase Production

The fungal isolates were tested for Acid Phosphatase production by phosphate hydrolysis method. From 120 fungal isolates about ninety fungal isolates were screened for Acid phosphatase production efficiency. Enzymatic activity was screened based on clear zone formation on Pikovskaya's agar medium.

The media comprised (per L): glucose 10 g, $Ca_3(PO_4)_2$ 5 g, $(NH_4)_2SO_4$ 0.5 g, KCl 0.2 g, $MgSO_4.7H_2O$ 0.1 g, $MnSO_4$ 0.002 g, $FeSO_4$ 0.002 g, yeast extract 0.5 g, agar 20 g. The entire ninety fungal discs were centrally inoculated on sterile solidified Pikovsaya's agar plates with the help of cork borer. The diameter of disc was 1cm. Plates were incubated at 28°C for 72 hours. The growth of phosphate-solubilizing fungi was indicated by the formation of clear zone surrounding the fungal colony (Elfiati et al., 2021).

Microscopy of Acid Phosphatase Producing isolates

Microscopy of all the positive isolate was done by lactophenol cotton blue staining method. In aseptic condition, a loop full of fungal cultures was placed on a clean glass slide, a drop of lactophenol cotton blue stain was mixed with the culture. A clean coverslip was placed over the culture and viewed under the microscope (10X and 45X). Morphological characteristics including colour of the colony and growth pattern studies, as well as their vegetative and reproductive structures were carefully observed under the microscope (Devi and Kumar, 2012), Figure 1.

Results and Discussion

A wide variety of microorganisms can be found in soil, making it an essential habitat. Potent isolates are frequently obtained in natural environments, and the choice of soil samples can affect the effectiveness of enzymes. Fungi were isolated utilizing serial dilution techniques. The isolated fungi were subsequently evaluated for their acid phosphatase production capabilities. A screening process involving 90 fungal isolates was conducted on Pikovsaya's agar plates, which contained calcium phosphate ($Ca_3(PO_4)_2$) as a substrate for the acid phosphatase enzyme. These plates were incubated for 72 to 96 hours. Following the incubation period, varying levels of phosphate solubilization were observed.

The phosphate solubilizing activities of these isolates were assessed based on the formation of halo zones on Pikovsayas agar media. The screening results revealed that 35 isolates exhibited enzymatic activities, evidenced by halo zone formation around the colonies, indicating potential for acid phosphatase production. Microscopic analysis identified most isolates as belonging to the genera *Aspergillus, Trichoderma, Penicillium, Fusarium* and *Rhizopus.*

Fungal species like *Aspergillus, Penicillium, Fusarium,* and *Neurospora* have been found to contain acid phosphatases. *Aspergillus* and *Rhizopus* species have been examined for their production of extracellular and cellular acid phosphatases during growth, both with and without copper ions in the medium (Tsekova et al., 2000, 2002; Aleksieva et al., 2003).

Filamentous fungus belonging to the genus *Aspergillus* are among the microorganisms that produce phosphatase. These fungi were shown to produce the most active extracellular enzyme with the best pH and temperature stability properties in a number of experiments.

Therefore, microbes belonging to genera *Aspergillus* are the most commonly utilized in the industrial production of this enzyme (Haefner et al., 2005; Bhavsar et al., 2012; Neira-Vielma et al., 2018). Solid-state fermentation is a process commonly applied for the production of extracellular enzymes (Gaind and Singh, 2015; Neira-Vielma et al., 2018).

According to research on phosphate deprivation, *Trichoderma spp*. has the ability to fix phosphates in the soil, increase soil fertility, and promote plant growth (Kapri and Tewari, 2010). Leitão et al. (2010) isolated and described acid phosphatase (ACPase) from *T. harzianum.* This enzyme was highly inhibited by sodium tungstate and showed an optimal pH and temperature of 4.8 and 55°C, respectively.

A wide variety of phosphorylated esters, including chemical compounds crucial in cellular processes like adenosine triphosphate (ATP), adenosine diphosphate (ADP), and adenosine monophosphate (AMP), were hydrolysed by this non-specific enzyme (Souza et al., 2016).

Acid phosphatases have drawn particular interest because of their wide range of biotechnological applications in bioremediation, industry, diagnostics, P mobilization and acquisition, soil fertility enhancement, and plant development (Souza et al., 2016).

In summary, textile sizing sites offer favourable environment for fungi that produce acid phosphatase, and they merit additional investigation to ascertain whether these isolates can aid in the bioremediation of waste environments associated with sizing environment. Using possible isolates that solubilize phosphate, future studies should focus on producing and purifying acid phosphatase enzymes on a pilot-scale.

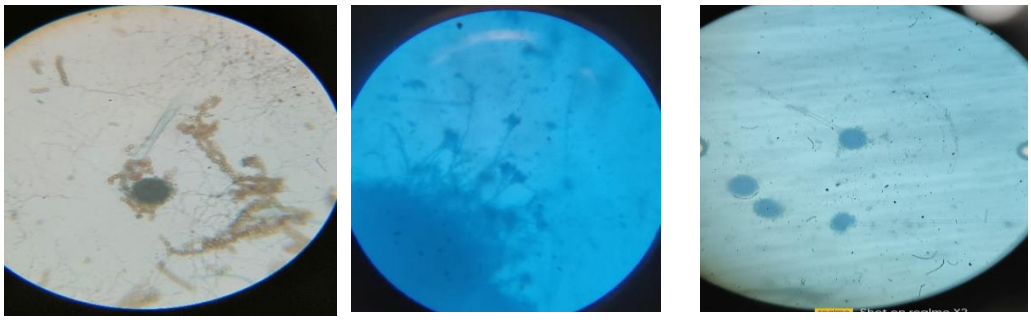

Figure 1: Microscopic Images

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