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Characterization of Phosphate Solubilizers as Biocontrol Agents against Fungal Phytopathogens

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Abstract : *Phytopathogenic fungi cause widespread plant diseases leading to severe yield losses and deterioration of crop quality. Phosphate-solubilizing bacteria (PSB) play a vital role in sustainable agriculture by enhancing phosphorus availability and suppressing plant pathogens. The present study focuses on the isolation, characterization, and evaluation of PSB as potential biocontrol agents against fungal phytopathogens, Fusarium spp. and Alternaria spp. PSB were isolated from rhizospheric soil using Pikovskaya's agar medium and characterized based on morphological traits. Fungal phytopathogens were isolated from rotten fruits and identified using lactophenol cotton blue staining. Antifungal activity was evaluated using the agar well diffusion method. The study suggests PSB as effective eco-friendly biocontrol agents.*

Keywords: *Phosphate Solubilizing Bacteria, Biocontrol Agent, Fungal Phytopathogens, Fusarium, Alternaria*

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Introduction:

Phosphorus is one of the essential macronutrients required for plant growth and development, playing a crucial role in root development, energy transfer (ATP), and nucleic acid synthesis (Agrios, 2005). Despite its abundance in soil, a major portion of phosphorus exists in insoluble forms such as tricalcium phosphate, iron phosphate, and aluminium phosphate, which are not readily available to plants (Pikovskaya, 1948). As a result, phosphorus deficiency is a common problem in agricultural soils worldwide.

To tackle this issue, farmers often depend on chemical phosphate fertilizers, though excessive application contributes to soil decline, environmental harm, and greater production costs. (Rodriguez & Fraga, 1999). In recent years, attention has shifted towards eco-friendly alternatives such as phosphate-solubilizing microorganisms (PSMs), particularly bacteria, which enhance phosphorus availability through organic acid production, proton extrusion, and enzymatic activity (Gaur, 1990).

Besides improving soil nutrient availability, phosphate-solubilizing bacteria play an important role in stimulating plant growth and maintaining sustainable agriculture. (Sharma et al., 2013). A number of bacterial genera demonstrate strong phosphate-solubilizing capability along with antagonistic activity toward plant-infecting pathogens.

Fungal phytopathogens such as *Fusarium* spp. and *Alternaria* spp. are responsible for severe crop losses worldwide, causing diseases like wilt, leaf spot, root rot, damping-off, and fruit rot (Agrios, 2005). The use of chemical fungicides for their management carries risks including the development of resistant pathogens, toxic effects, and environmental harm (Whipps, 2001). Therefore, microorganisms possessing dual functions—nutrient mobilization and pathogen suppression—are of significant importance.

The present study aims to isolate phosphate-solubilizing bacteria from rhizospheric soil, characterize them based on morphological features, and evaluate their biocontrol potential against *Fusarium* spp. and *Alternaria* spp., contributing to sustainable and eco-friendly plant disease management.

Materials and Methods:

Isolation of Phosphate Solubilizing Bacteria: Rhizospheric soil samples were collected aseptically in sterile sample packets. The soil samples were serially diluted in sterile normal saline up to 10^{-2} dilution (Fig.1). Aliquots from 10^{-2} and 10^{-3} dilutions were spread on Pikovskaya's agar medium plates using the spread plate technique. The plates were incubated at 37°C for 48 hours. Colonies showing clear halo zones around them were selected as phosphate-solubilizing bacteria and sub-cultured on fresh Pikovskaya's agar plates to obtain pure cultures (Fig.2).



Fig. 1: PVK agar media and Serial Dilution

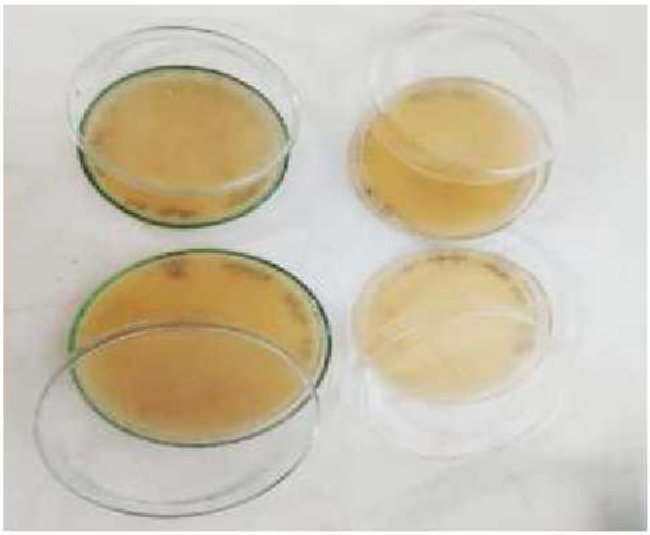


Fig. 2: Poured plates of PVK media

Characterization of Phosphate Solubilizing Bacteria: Morphological characterization: gram staining and microscopic inspection were performed on the plates to identify shape, color, margin, elevation, and texture. Further purification was carried out by streaking the isolates on Pikovskaya's agar medium. Pure cultures were inoculated into nutrient broth and incubated in a shaker incubator at 180 rpm for 6 hours for further studies.



Fig. 3: PSB's Colonies

Isolation of Phytopathogenic Fungus: Samples were collected from visibly rotten fruits such as banana, orange, and apple showing clear symptoms of fungal decay. The fruits were first washed thoroughly with autoclaved distilled water to remove surface debris. Small pieces of infected tissue were then excised

aseptically from the margin of the rot lesion using a sterile scalpel, as this region typically contains actively growing fungal hyphae. The collected tissue was transferred using a sterile inoculating loop onto sterile Petri plates containing Potato Dextrose Agar (PDA) by gently tapping the loop on the agar surface to ensure proper inoculation. The inoculated plates were incubated at 26°C for 3–5 days and observed daily for the appearance of fungal colonies (Fig.4).



Fig. 4: Phytopathogenic Fungus

Characterization of Phytopathogenic Fungus: The fungus was identified as *Alternaria* and *Fusarium* by their morphological characterization observed under microscope by performing Lactophenol cotton Blue staining (Fig.5), *Alternaira* spp. And *fusarium* spp. Were characterized based on cultural and microscopic features. Alternaria isolates grown on PDA showed dark brown to black, velvety colonies with irregular margins. Microscopically, septate pigmented hyphae and muriform, condia arranged in chains were observed. Fusarium isolates exhibited rapid growth with white to pink, cottony colonies (Fig.6). Microscopic examination revealed hyaline, septate hyphae, sickle – shaped macroconidia, oval microconidia (Fig.7).



Fig. 5: Staining slides of phytopathogenic fungus (LPCB)

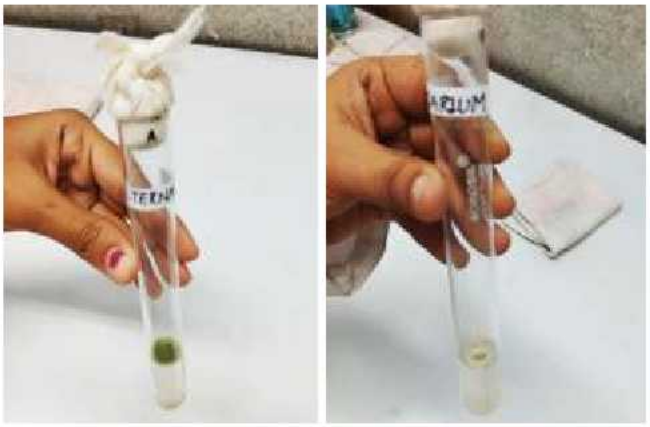


Fig. 6: Growth of Alternaria and Fusarium

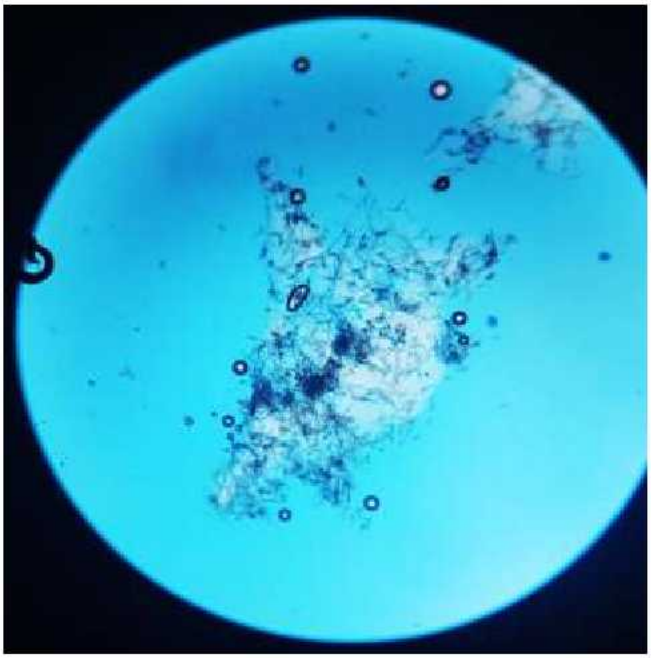


Fig. 7: Microscopic view of *Alternaria*

Characterization of Antifungal Activity Well Diffusion Test: The Agar Well Diffusion Technique is used to screen substance for antimicrobial activity.

A pure PSB culture was grown in nutrient broth, while the fungal culture was introduced into potato dextrose broth and incubated on a shaker until growth appeared. PDA plates were poured and allowed to solidify. The fungal suspension was uniformly spread over the surface of freshly prepared PDA plates using a sterile spreader to form a confluent fungal lawn. After the fungal lawn dried (5–10 minutes), sterile cork borers were used to punch wells of 5– 6 mm diameter into the agar. The agar plugs were carefully removed using a sterile needle. Each plate contained 2 wells spaced

evenly apart to prevent overlapping zones. Bacterial isolates were grown in nutrient broth and incubated at 37°C for 24hrs. After incubation, the broth cultures were put in a shaker incubator. Using a sterile micropipette, 10µL of the bacterial culture was loaded into each well. The plates were incubated at 28°C for 48 hours. Fungal growth inhibition was monitored regularly (Fig.8).



Fig. 8. Well diffusion test

Results:

Table 1. Morphological Characterization of Phosphate Solubilizing Bacteria

Isolates	Shape	Color	Margin	Elevation	Texture
S I	Irregular	White	Irregular	Slightly elevated	Opaque
S II	Irregular	Off white	irregular	Slightly elevated	Opaque

Table 2. Morphological Characterization of Fungal Isolates

Isolates	Shape	Color
P I	Cottony	Black
B I	Cottony	Off white

PSB showed inhibition zones of 2 mm against Fusarium spp. and 2.5 mm against Alternaria spp. (Fig.9).



Fig. 9: Antifungal activity of Phosphate Solubilizer

Discussion:

The present study focused on isolating phosphate solubilizing bacteria (PSB) from rhizospheric soil and evaluating their dual ability to solubilize insoluble phosphorus and inhibit fungal phytopathogens such as *Fusarium spp.* and *Alternaria spp.* The combination of P-solubilization and antifungal potential makes these isolates strong candidates for biofertilizer and biocontrol applications. In the current investigation, rhizospheric soil was selected as the sampling site because it is known to harbour a rich microbial population influenced by root exudates. The selective use of Pikovskaya's agar medium successfully enabled differentiation of PSB based on halo formation. A total of four PSB isolates were obtained, all of which were capable of producing visible clear zones around their colonies. This halo formation is widely reported as an indicator of phosphate solubilization efficiency, as supported by numerous studies such as Chen et al. (2006) and Rodríguez & Fraga (1999). The production of a halo suggests secretion of organic acids, primarily gluconic acid, which reduce pH and help solubilize tricalcium phosphate present in the medium. The isolates obtained showed morphological uniformity with smooth, elevated, entire colonies and gram-negative reaction, indicating possible belonging to genera such as Pseudomonas, a commonly reported phosphate solubilizer in rhizospheric soil. Even though biochemical identification was not performed, the morphological traits align with prior literature which reports that most rhizosphere-associated PSB are gram-negative rods with smooth, mucoid colonies. The antifungal activity assessed through the Well Diffusion Assay showed

zones of inhibition measuring 2–3 mm against both *Fusarium* and *Alternaria*. Although the inhibition zones were small, they are significant because PSB are not primarily fungicidal microbes; instead, they inhibit pathogens through indirect mechanisms such as production of siderophores, lytic enzymes, competition for nutrients, and secretion of antimicrobial secondary metabolites. Prior studies by Whipps (2001) and Mehta et al. (2014) have demonstrated that PSB often exhibit low to moderate inhibition in vitro but produce much stronger effects in natural soil systems due to synergistic interactions. The fungal pathogens isolated (*Fusarium* and *Alternaria*) were successfully identified based on colony morphology and Lactophenol Cotton Blue (LPCB) staining. The presence of characteristic septate hyphae, conidiophores, and macroconidia confirmed the identity of the pathogens. The confirmation of these phytopathogens strengthened the validity of the biocontrol assay performed.

One significant observation was the subtle colour shift around PSB colonies. Such a change is connected with acid production, as acidifying Pikovskaya's medium often affects the colour of the surrounding agar, further indicating the solubilization capacity of the isolates.

Overall, the results of this study align with earlier research, confirming that rhizospheric PSB possess significant agronomic potential. Although the inhibitory zones were modest, the combination of nutrient mobilization and pathogen suppression positions PSB as an eco-friendly substitute to chemical fertilizers and fungicides. Future work should include molecular characterization, biochemical profiling, quantification of soluble phosphorus, and greenhouse trials to validate plant growth–promoting effects in vivo.

Conclusion:

The results of present study revealed that phosphate-solubilizing bacteria isolated from rhizospheric soil possess antifungal activity against *Fusarium* spp. and *Alternaria* spp. These findings suggest that PSB can act as effective biocontrol agents and provide an eco-friendly alternative to chemical fertilizers and fungicides in sustainable agriculture.

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