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Screening for phosphate solubilising bacteria from Agricultural Land

[1]Nandhini R, [1]Muthulakshmi T, [1]Aswini AK, [1]Navaraj M, [2*]Susithra K and [2]Lokesh P

[1]*PG Student, PG & Research Department of Microbiology, V.H.N.Senthikumara Nadar College (Autonomous), Virudhunagar, Tamilnadu*

[2]*Assistant Professor, PG & Research Department of Microbiology, V.H.N.Senthikumara Nadar College (Autonomous), Virudhunagar, Tamilnadu*

[*]*Corresponding author email: susithra.k@vhnsnc.edu.in*

Abstract

The majority of agronomic soils have substantial stocks of total phosphorus, but phosphorus deficit is caused by phosphorus fixation and precipitation, which severely limits crop development. Soil samples were collected from rhizosphere of the plant in depth of 0 - 15 cm. Soil samples were serially diluted and plated Pikovskaya's medium and incubated for 24 to 48 hours. Phosphate solubilization bacteria showed the development of clear zones surrounding the colonies on the plates. The phosphate-solubilizing bacteria were characterized using biochemical assays and colony morphology and growth patterns. The obtained phosphate-solubilizing bacteria were characterized by biochemical assays and confirmed to be as Pseudomonas fluorescens. The aim of this study is to isolate phosphate solubilising bacteria and study the mechanism of it.

Keywords: Phosphorus; phosphate solubilizing bacteria, Pseudomonas fluorescens

Introduction

Plant development and production depend on phosphorous. It is crucial for a variety of physiological processes in plants, including cell division, photosynthesis, the growth of healthy root systems, and the utilization of phosphate. When there is a phosphorus shortage, the leaves becoming brown with weak stem, little leaves, and sluggish growth. Animal manures were widely used in agriculture in the past to supply phosphorus for plant growth. Phosphorus that is organically bonded enters the soil through animal excretions, deceased animals, and the decomposition of natural flora. The impact of microflora on soil fertility was not well known at the time (Kannaiyan *et al*., 2004). The "phosphatase" enzyme, which is

found in a wide range of soil microorganisms, allows plants and microbes to absorb phosphorus from organic molecules. Only soluble forms of phosphate can be absorbed by plants. Numerous bacteria found in the soil are responsible for converting insoluble phosphate into soluble form. A significant portion of soil Microbes have the ability to break down insoluble inorganic phosphates found in soil and transform them into accessible to plants (Yosef *et al*., 1999). Adsorption to the soil surface and precipitation reactions with soil cations, especially calcium, iron, and aluminum, sequester phosphorus (P). Consequently, a lot of P fertilizer has been used to boost plant growth, which is probably going to have a detrimental effect on both the economy and the environment. Plants and microbes can create organic acids and phosphatase enzymes that can solubilize insoluble phosphate compounds. For instance, it has been demonstrated that PSB increases the solubilization of insoluble P compounds by releasing phosphatase enzymes and organic acids (Sharma *et al*., 2005). Phosphorus is obtained by plants as phosphate anion from soil solution. In plants, phosphorous strengthens cereal straw, encourages the growth of flowers and fruit, speeds up the development of roots, and is necessary for the generation of seeds. In addition to increasing their resilience to illnesses and unfavourable circumstances, adequate P fertilization may enhance the quality of fruits, vegetables, and cereal harvests. It hastens plant maturity, stimulates early root growth, and aids in the formation of meristematic tissues. Phosphate ion's negative charge causes them to be rapidly absorbed during the weathering of clays or debris particles, creating insoluble forms of calcium, iron, or aluminum phosphates that mangroves cannot use. These substances can be dissolved by bacteria and fungi (Bisen *et al*., 1996). Certain bacterial species may solubilize inorganic phosphorus and mineralize organic phosphorus, respectively. The ability of bacteria to produce metabolites, such as organic acids, which chelate the cation attached to phosphate through their hydroxyl and carboxyl groups and transform it into soluble forms, is what determines phosphorus solubilizing activity. Proton extrusion and the synthesis of organic acids are two examples of the microbial processes and mechanisms that cause phosphate solubilization.

Materials and Methods

***Collection of soil samples*:** The soil samples were collected from rhizosphere of agriculture land in sterile container.

***Isolation of phosphate solubilizing bacteria from soil sample*:** Pikovskaya's agar was prepared in petriplates and marked with respective dilutions. With the help of sterile pipettes,

0.1ml of the diluted samples was added to the respective plates. The plates were kept on the rotator and with the help of sterilzed L-rod, the sample was evenly spread. The plates were incubated at 37°C for 24 hrs.

***Isolation of phosphate solubilizing bacteria*:** Pikovskaya's medium was prepared and sterilized. The isolated colonies were streaked on the plate and incubated at 37°C for 48 hours. The zone surrounding the microbial growth indicates the phosphate solubilization. The isolated colonies on these plates were maintained on nutrient agar slants at 4°C for further analysis. The isolated bacterial colonies were further characterized for their morphological and biochemical characters.

***Microorganism identification*:** Using a sterile loop, the probable colonies were injected in a nutrient broth and cultured for 24 hours at 37°C. After that, the organisms were identified using a preliminary test. Gram staining and biochemical assays are among the tests that are employed.

***Gram staining*:** Gram staining is a technique used to identify gram positive and gram negative bacteria based on their cell walls. This four-step procedure uses certain dyes to make a bacterial cell stand out from its surroundings.

Biochemical test

These biochemical tests were conducted to confirm the genus of microorganisms. The tests include indole, methyl red, Voges-Proskauer, citrate, urease, as well as oxidative and fermentative testing, and the triple sugar iron test. The purpose of these tests was to verify if microorganisms were present. The tests include the triple sugar iron test, Starch hydrolysis, citrate, methyl red, Voges-Proskauer, and indole.

***Indole test*:** Members of the organisms can be distinguished from one another by their ability to produce indole. Kovac's reagent, which becomes cherry red when indole is present, can be used to detect its presence. If there is no change in color, the organism is indole negative because the tryptophan in the medium was not digested.

***Methyl red test*:** Gram-negative bacteria in the Enterobacteriaceae family can be distinguished using the methyl red test; a brilliant color denotes a positive result, whilst yellow or orange denotes a negative one. Certain microbes use citrate as a carbon source

when lactose or glucose are not available, and this process depends on the presence of citrate permease. A blue coloration of the medium indicates a positive outcome, whereas a green coloration or no color change indicates a negative outcome.

***Citrate test*:** The ability of bacteria to use citrate as a carbon source is assessed by a citrate test, which is commonly carried out using Simmons citrate agar. A positive result is demonstrated by a color shift from green to blue on the slant as a result of bacterial growth, whereas a negative result shows no color change and only slight agar growth.

***Voges-Proskauer test*:** Alpha-naphthol and potassium hydroxide are added to the Voges-Proskauer broth, glucose-phosphate broth that has been bacterially infected, in order to conduct the test. A positive outcome is indicated by a cherry red tint, and a negative outcome is indicated by a yellow-brown color.

***Starch hydrolysis test*:** The starch is broken down by extracellular enzymes produced by the organism. The iodine causes the zone around the growth to initially turn yellow before becoming lighter and eventually clear it the positive result.
The extracellular enzymes were not produced by the organism. Since the medium is still blue, the starch has not hydrolyzed it is the negative result.

***Trible sugar iron test*:** The Triple Sugar Iron (TSI) test basically distinguishes bacteria based on their carbohydrate fermentation patterns and hydrogen sulfide production. A positive result means that the bacteria can ferment one or more of the sugars (glucose, lactose, and sucrose) present in the medium, which is shown by a color change from red to yellow in the agar. They may also produce hydrogen sulfide gas, which is shown by a black precipitate in the tube's butt.

Results

***Collection of soil samples and plant seed*:** The soil samples were collected from rhizosphere soil of agriculture land in Seeniapuram, Tamil Nadu, India.

Figure 1: Soil sample collection

***Isolation of bacteria from rhizosphere soil sample*:** After a 24-hour incubation period, they examined the colonies from the Pikovaskaya's agar plate.

Figure 1: Spread plate technique

***Identification of phosphate solubilizing bacteria*:** Pikovskaya's medium was prepared and sterilized. The isolated colony was straight streak on the plate and incubated at 37°C for 48 hours. The zone surrounding the microbial growth indicates the phosphate solubilization.

Figure 2: Phosphate solubilizing bacteria

***Gram staining*:** The organism was identified as gram-negative bacteria and rod shaped.

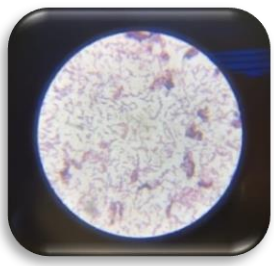

Figure 3: Gram staining

Biochemical test

The organism was further confirmed by biochemical tests as *Pseudomonas* sp.

Figure 4: Biochemical test

Table 1: Biochemical test results

Biochemical test	Result
Indole	Negative
Citrate	Positive
Methyl red	Negative
Voges Proskauer	Negative
Triple sugar iron	Alkaline/alkaline No gas production
Starch hyrolysis	Negative

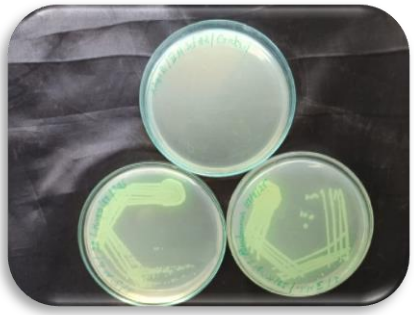

Figure 5: *Pseudomonas* sp. streak plate

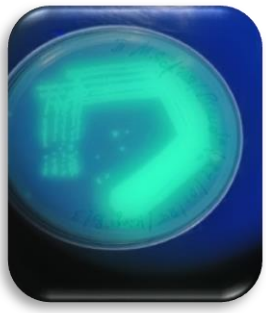

Figure 6: Under UV light

Future Aspects

Medicinal plant seeds are going to be sown in pot. 50ml and 100ml of broth are inoculate in different pot at the same time. After 10 days plant growth is observed in 50ml and 100ml Pikovaskaya's broth inoculum pot. Future research can concentrate on the widespread use of PSB in the production of medicinal plants, examining its practicability and financial sustainability.

Discussion

In the present study, we isolated PSB (*Pseudomonas* sp.) from rhizospheric soil and evaluated their potential to solubilize phosphates. The results showed that the isolated PSB strains were able to solubilize phosphates and promote plant growth. Our findings are consistent with previous studies, which have demonstrated the efficacy of PSB in solubilizing phosphates and promoting plant growth.

For instance, a study by Alam *et al*. isolated PSB from rhizospheric soil and evaluated their potential to solubilize phosphates. The results showed that the isolated PSB strains were able to solubilize phosphates and promote plant growth (Alam *et al.*, 2017). Similarly, a study by Khan *et al*. evaluated the effect of PSB on plant growth and yield. The results showed that PSB inoculation significantly improved plant growth and yield (Khan *et al.*, 2018). Another study by Singh *et al.* isolated PSB from agricultural soil and evaluated their potential to solubilize phosphates. The results showed that the isolated PSB strains were able to solubilize phosphates and promote plant growth (Singh R *et al*., 2019). A study by Sharma *et al*. evaluated the effect of PSB on plant growth and yield under different environmental conditions. The results showed that PSB inoculation significantly improved plant growth and yield under different environmental conditions (Sharma *et al*., 2020).

Conclusion

One phosphate solubilizing organism was isolated. A zone was evident surrounding the colony on the PVK medium. On the basis of morphological characteristics like rod shaped and gram negative characteristic, isolate was confirmed as bacteria. On comparison of our result with literature, the isolate was suspected to belong with *Pseudomonas fluoresces*. Further in vivo study using any medicinal plant's is required to check its efficiency of Phosphate solubilizing.

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