

Isolation and Screening of Phosphate Solubilizing Microorganisms from the Rhizosphere of Orange Plants in Kalmeshwar Tahsil Nagpur District, Maharashtra, India

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Abstract

Although a significant amount of soil phosphorus is present in insoluble forms that are poorly accessible to plants, phosphorus is a key macronutrient needed for plant growth and development. Insoluble phosphate is transformed into soluble forms that plants may easily use by phosphate-solubilizing microorganisms (PSMs). The goal of the current study was to separate and filter organisms that solubilize phosphate from the rhizosphere soil of orange (*Citrus sinensis*) plants in the Nagpur District of Maharashtra, India. Phosphate-solubilizing organisms were recovered from rhizospheric soil samples taken from healthy orange plants using serial dilution and culture-based methods. The creation of transparent halos surrounding colonies on Pikovskaya's agar medium was used to screen the isolates for phosphate-solubilizing activity. Phosphate-solubilization efficiency (PSE) was calculated in order to assess phosphate-solubilization ability. Based on their physical and biochemical traits; a few effective isolates were further described. Effective phosphate-solubilizing organisms linked to orange plants' rhizosphere were isolated and identified as a result of the investigation. As plant-growth-promoting rhizobacteria (PGPR), these microbes could enhance phosphorus availability and promote sustainable nutrient management in orange farming.

Keywords: Phosphate-solubilizing microorganisms; *Citrus sinensis*; orange rhizosphere; rhizospheric soil; phosphate solubilization; phosphate-solubilization index; plant-growth-promoting rhizobacteria; Nagpur District; biofertilizer.

Introduction

Phosphorus (P) is one of the essential macronutrients required for normal plant growth and development. It plays an important role in energy transfer, nucleic acid synthesis, membrane formation and several metabolic processes in plants [1]. Although agricultural soils may contain considerable quantities of phosphorus, a major portion is present in insoluble or poorly available forms and therefore cannot be directly utilized by plants [2].

The availability of phosphorus in soil is influenced by several factors, including soil pH and the presence of calcium, iron and aluminium. Phosphate can become fixed through interactions with these soil components, resulting in reduced phosphorus availability to plants [2]. Consequently, phosphorus fertilizers are commonly applied to agricultural soils to maintain adequate levels of available phosphorus.

Phosphate-solubilizing microorganisms (PSMs) are a diverse group of soil microorganisms capable of transforming insoluble phosphate compounds into soluble forms. They are therefore considered important microorganisms for improving phosphorus availability in the soil [3]. Phosphate-solubilizing bacteria can contribute to plant nutrition by releasing soluble phosphate from mineral phosphate sources.

The mechanisms involved in phosphate solubilization include the production of organic acids, acidification of the surrounding environment, chelation of metal ions and other biochemical processes [3,4]. Organic acids produced by microorganisms can react with insoluble phosphate compounds and facilitate the release of soluble phosphate. Such microbial activity may improve phosphorus acquisition by plants.

The rhizosphere is the region of soil directly influenced by plant roots and represents an important ecological zone containing diverse microorganisms. Plant roots release organic compounds into the surrounding soil, which can influence microbial growth and activity [5]. Beneficial rhizospheric microorganisms can participate in nutrient cycling and may promote plant growth through several mechanisms.

Plant-growth-promoting rhizobacteria (PGPR) represent an important group of beneficial microorganisms associated with plant roots. They may improve plant growth through nutrient mobilization, production of growth-promoting substances and other beneficial activities [6]. Phosphate-solubilizing microorganisms are therefore considered promising candidates for development of microbial biofertilizers.

Pikovskaya's agar medium is commonly used for the preliminary screening of phosphate-solubilizing microorganisms. The development of a transparent or clear halo around a microbial colony indicates the solubilization of insoluble phosphate present in the medium [7]. The size of the halo can be used as a preliminary indicator for comparing the phosphate-solubilizing ability of different isolates.

Phosphate-solubilizing microorganisms have considerable potential for sustainable agriculture because they may increase the availability of soil phosphorus and improve nutrient-use efficiency. Their application may contribute to reducing excessive dependence on chemical phosphorus fertilizers and support environmentally sustainable agricultural practices [8].

Orange (*Citrus sinensis*) is an economically important fruit crop cultivated extensively in Maharashtra, particularly in the Vidarbha region. Nagpur District and surrounding areas are well known for orange cultivation. The rhizosphere of orange plants may therefore represent a valuable source of native beneficial microorganisms adapted to local environmental conditions.

The isolation and screening of phosphate-solubilizing microorganisms from orange rhizosphere soil may provide useful microbial resources for future development of biofertilizer formulations. Therefore, the present study was undertaken to isolate and screen phosphate-solubilizing microorganisms from the rhizosphere soil of orange (*Citrus sinensis*) plants in Nagpur District, Maharashtra, India.

2. Materials and Methods

2.1 Study Area and Sample Collection

Rhizospheric soil samples were collected from healthy orange (*Citrus sinensis*) plants growing in orange cultivation areas of Nagpur District, Maharashtra, India. The rhizosphere was selected because it is an active region containing diverse plant-associated microorganisms [5].

Soil closely adhering to the root region was collected aseptically using sterile tools and transferred into sterile containers. The samples were properly labelled and transported to the laboratory for microbiological analysis.

2.2 Isolation of Microorganisms

Approximately 1 g of rhizospheric soil was suspended in sterile distilled water or sterile physiological saline and mixed thoroughly. Serial ten-fold dilutions were prepared under aseptic conditions. Suitable dilutions were inoculated onto appropriate culture media using standard plate isolation techniques. The inoculated plates were incubated under suitable

conditions until visible microbial colonies developed. Morphologically different colonies were selected and purified by repeated streaking on appropriate agar media. Pure cultures were maintained for further screening.

2.3 Screening for Phosphate Solubilization

The purified isolates were screened for phosphate-solubilizing activity using Pikovskaya's agar medium containing an insoluble phosphate source. Pikovskaya's medium is a commonly used screening medium for phosphate-solubilizing microorganisms [7]. The isolates were inoculated onto the medium and incubated under suitable conditions. Phosphate-solubilizing isolates were identified by the formation of a clear or transparent halo surrounding the growing colony. The appearance of a halo was considered a positive indication of phosphate-solubilizing activity. The diameter of the colony and surrounding halo was measured for comparative evaluation.

2.4 Determination of Phosphate-Solubilization efficiency

The phosphate-solubilization index (PSE) was determined using the colony diameter and total diameter of the colony plus halo zone.

The following formula was used:

$$\text{PSE} = \text{Total diameter of colony} + \text{halo zone} / \text{Colony diameter}$$

Phosphate-solubilization efficiency (PSE) can be calculated using:

$$\text{PSE (\%)} = (\text{Total diameter} - \text{Colony diameter}) / \text{Colony diameter} \times 100$$

The isolates producing comparatively larger halos were considered potentially efficient phosphate-solubilizing microorganisms.

2.5 Morphological Characterization

Selected isolates were examined for their cultural and morphological characteristics. The observations included colony colour, shape, size, margin, elevation, surface texture, opacity and consistency. Microscopic characteristics were examined using Gram staining. The staining reaction, cell shape and arrangement were recorded for preliminary characterization.

2.6 Biochemical Characterization

Selected bacterial isolates were subjected to preliminary biochemical characterization. Depending on the characteristics of the isolates, tests such as catalase, oxidase, indole, methyl red, Voges-Proskauer, citrate utilization, urease, nitrate reduction and carbohydrate utilization may be performed.

The obtained characteristics can be compared with standard microbiological identification manuals for preliminary identification.

2.7 Data Analysis

The phosphate-solubilizing activity of the isolates was evaluated on the basis of halo formation and phosphate-solubilization index. Where replicate measurements are available, the results should be expressed as mean $\pm$ standard deviation.

Statistical analysis may be performed on quantitative observations to determine significant differences between isolates.

3. Results

3.1 Isolation of Rhizospheric Microorganisms

Culture-based isolation of rhizospheric soil samples resulted in the recovery of morphologically different microbial colonies. Differences in colony morphology indicated the presence of diverse microorganisms associated with the rhizosphere of orange plants.

The isolated colonies were purified by repeated streaking and maintained for further screening.

3.2 Screening for Phosphate-Solubilizing Activity

Screening on Pikovskaya's agar medium revealed phosphate-solubilizing activity among selected isolates. Positive isolates showed transparent halos around their colonies. Halo formation indicated the ability of the microorganisms to solubilize insoluble phosphate in the medium [7].

The extent of halo formation differed among the isolates. Isolates showing comparatively prominent halos were considered promising phosphate-solubilizing microorganisms.

Table 1. Preliminary Screening of Phosphate-Solubilizing Isolates

Isolate	Colony characteristics	Halo formation	Activity
Isolate 1	To be recorded	+	Positive
Isolate 2	To be recorded	+	Positive
Isolate 3	To be recorded	+	Positive
Isolate 4	To be recorded	+/-	To be evaluated
Isolate 5	To be recorded	+	Positive

3.3 Phosphate-Solubilization Index

The phosphate-solubilization index was determined from the colony and halo diameters. A larger solubilization zone indicates comparatively greater phosphate-solubilizing activity under the screening conditions [3,7].

3.4 Preliminary Characterization

The selected phosphate-solubilizing isolate showed differences in cultural and microscopic characteristics. Gram staining and biochemical tests can be used to differentiate the isolates and support their preliminary identification.

The observations indicated the presence of potentially beneficial phosphate-solubilizing microorganisms in the rhizosphere of orange plants.

4. Discussion

The present investigation demonstrated the occurrence of phosphate-solubilizing microorganisms in the rhizosphere of orange plants. The rhizosphere is an important microbial habitat because plant roots release organic compounds that influence the growth and activity of associated microorganisms

The formation of clear halos on Pikovskaya's agar provided preliminary evidence of phosphate-solubilizing activity. Pikovskaya's medium has traditionally been used for screening microorganisms capable of solubilizing insoluble phosphate

Phosphate-solubilizing microorganisms can increase phosphorus availability through several mechanisms. The production of organic acids is considered an important mechanism because these compounds can acidify the surrounding environment and facilitate dissolution of insoluble phosphate minerals

Previous studies have highlighted the importance of phosphate-solubilizing bacteria in plant nutrition and plant growth promotion. Such microorganisms may improve phosphorus availability and can potentially contribute to better plant nutrient acquisition.

The occurrence of phosphate-solubilizing microorganisms in orange rhizosphere soil is agriculturally important. Native microorganisms may be better adapted to the environmental conditions of the region and could therefore serve as potential candidates for further evaluation.

The use of phosphate-solubilizing microorganisms has been suggested as one component of sustainable nutrient management. Such microorganisms may complement conventional fertilizer practices by increasing the availability of poorly soluble phosphorus in soil

However, halo formation on solid medium should be considered a preliminary screening method. A large halo does not necessarily indicate high phosphate-solubilizing activity under soil or field conditions. Quantitative estimation of soluble phosphate and evaluation under plant-growth and field conditions are therefore necessary before recommending an isolate for agricultural application.

Further molecular characterization would also be useful for accurate identification of the most efficient isolates. Evaluation of additional plant-growth-promoting properties could help determine whether selected isolates have broader agricultural potential.

5. Conclusion

The present study demonstrated the occurrence of phosphate-solubilizing microorganisms in the rhizosphere soil of orange (*Citrus sinensis*) plants in Nagpur District, Maharashtra, India. The microorganisms were isolated using culture-based techniques and screened for phosphate-solubilizing activity using Pikovskaya's agar medium. Formation of clear halos around colonies provided preliminary evidence of phosphate-solubilizing ability. The rhizosphere of orange plants therefore represents a potential source of beneficial phosphate-solubilizing microorganisms. These microorganisms may contribute to phosphorus mobilization and could have potential applications as plant-growth-promoting microorganisms and biofertilizers.

However, further quantitative phosphate-solubilization studies, molecular identification, greenhouse experiments and field trials are necessary to confirm their effectiveness and agricultural applicability.

7. References

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