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Isolation and Characterization of Plant Growth Promoting Rhizobacteria form *Raphanus sativus* (Radish)

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Authors' contributions

This work was carried out in collaboration between both authors. Both authors read and approved the final manuscript.

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ABSTRACT

Rhizobacteria are present in rhizosphere region of plant root system, which enhance the plant growth by various way like biological nitrogen fixation, siderophore production, phosphate solubilization and phytohormone production. The soil samples were collected from rhizosphere region of *Raphanus sativus*, after enrichment rhizobacteria was isolated by serial dilution method, diluted sample were spread on respective solid agar media plates. Isolated rhizobacteria was identify by biochemical and molecular characterization methods. The isolated PGPRs was *Bacillus subtilis* which was showed phosphate solubilization activity and they were enhanced 30% more green gram seed germination. The method of current research is screening, isolation and biochemical characterization of rhizobacteria form rhizosphere region of *Raphanus sativus*. Phosphate solubilizing bacteria, solubilized the unavailable phosphate and provide to plant. The main purpose of this research paper is to widen the understanding of the role of phosphate solubilizing bacteria in crop production as biofertilizers.

Keywords: Rhizobacteria; rhizosphere; solubilization; biochemical; phosphate.

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1. INTRODUCTION

The groups of microorganism which is colonized in rhizospheric region of plant roots called as plant growth-promoting rhizobacteria (PGPR), they enhanced plant growth by nutrient immobilization. Some common examples of PGPR genera exhibiting plant growth promoting activity are *Pseudomonas, Azospirillum, Azotobacter, Bacillus, Burkholdaria, Enterobacter, Rhizobium, Erwinia, Mycobacterium, Mesorhizobium, Flavobacterium, etc* [1,2]. PGPRs have been shown to directly enhance plant growth by several mechanisms, including the fixation of atmospheric nitrogen transferred to the plant, the production of siderophores that chelate iron and make it available to the plant root, the solubilization of minerals like phosphorus, and the synthesis of phytohormones [3,4]. The potentiality of PGPR offers an attractive way to replace the use of chemical fertilizers, pesticides and other supplements to increase agriculture yield [5,6]. The different growth-promoting characteristics of the rhizobacteria identified in the rhizosphere of *Raphanus sativus* are discussed in the current work, as well as their morphological characterization, biochemical characterization and molecular characterization of the isolated strains.

Purpose of current research to isolate and identify the potential phosphate solubilizing rhizobacteria, which enhances the growth and yield of crops. Most soils possess considerable amounts of phosphate, phosphorus is a very reactive element and does not exist as elemental form in the soil, and large proportion is bound to soil particles. A group of rhizobacteria capable of hydrolyzing organic and inorganic insoluble phosphate compounds to soluble phosphate form that can easily be assimilated by plants. The major objective of current study:

- To screening and isolation of plant growth promoting rhizobacteria from rhizosphere of *Raphanus sativus* (Radish) root.
- Biochemical and molecular characterization of PGPR.
- Effect of rhizobacterial isolates on seed germination.

2. REVIEW OF LITERATURE

Plant growth promoting rhizobactera consist of a group of rhizobacteria that colonize in rhizosphere which can increases the root growth and influence the plant physiology. These rhizobacteria have a crucial role in sustaining soil fertility and plant health [7,8]. Plant growth promoting rhizobactera increase plant growth through either direct mechanism by providing readily available nutrient for plants such as nitrogen, phosphorus and plant hormones or thought indirect mechanism by synthesizing hydrogen cyanide (HCN), antibiotics, and siderophores [9]. The mechanism of solubilization of insoluble phosphate, rhizobacteria having ability to secrete organic acids and phosphatase enzymes which solubilized the insoluble phosphate into soluble form, which deacrases the dependency of high cost phosphatic fertilizers in agriculture practice. Hashem et al. [10], reported the activity of *B. subtilis* in the rhizosphere, *Bacillus* species are a significant type of rhizobacteria which can form spores that can survive in the soil for long period of time under adverse conditions and also it is a root colonizer increase crop productivity under conditions of biotic and abiotic stress. Agustiyani et al. [11], they characterized the PGPR isolated from rhizospheric soils of various plant and checked its effect on growth of radish (*Raphanus sativus* L.), total 15 PGPR isolates were isolated from various plant roots and in vitro screening was done for different plant growth promotion activities. PGPR on Radish growth in the green house showed that all isolates had effects on increasing growth and tuber formation compared to control.

3. MATERIALS AND METHODS

3.1 Collection of Rhizospheric Soil Samples

Rhizospheric soil sample was collected form Radish plant agriculture field Prayagraj, Uttar Pardesh, India. The roots and adherent topsoil were put in sterile sample collecting bags and delivered to the laboratory for isolation.

3.2 Screening and Isolation of PGPR

Screening of PGPR by serial dilution method, diluted suspension were spread on Pikovskaya agar media, Jensen media, Azospirillum media (BTB media), King's B media. Spread plant were incubated at 32^{o}C for 72 hrs. Gown colony were screening on the basis of colour change and clear hydrolytic zone around the colony.

3.3 Morphological and Biochemical Characterization of Isolates

Colony morphology were observed by Gram staining technique under 40X objective of the

microscope [12]. The biochemical identification like Indole Test, Methyl Red Test, Voges-Proskauer (VP) Test, Citrate Utilization Test, Gelatin Hydrolysis Test, Starch Hydrolysis Test, Hydrogen Sulphide Test, NO_2 Reducing Test, Nitrogen Fixation Test, IAA Production, Siderophore Production, Ammonium Production and Phosphate Solubilization was done as per the procedure given by Cappuccino and Sherman, [12] and Aneja, [13].

3.4 Molecular Characterization of the Isolated Strains

Molecular characterization of the isolated strains was based on 16S r-DNA Sequence methods [14].

3.5 Application of the Isolated Rhizobactera Along with Phosphate Rich Organic Manure

200 ml of full grown rhizobacterial culture mixed with 800 gm phosphate rich organic manure, and which was incubate at room temperature to maintain the moisture 25% for 30 days, now that were used in pot soil before sowing of green gram seed.

4. RESULTS AND DISCUSSION

The aim of current study to isolate the PGPR bacteria from soil samples and isolated culture identification was based on biochemical characterization.

4.1 Screening and Isolation of PGPR

Screening and isolation of PGPR bacteria based on various function viz. halo zone around the bacterial colony and colour change around the colony. Total 4 bacterial isolates (Isolates - I, II III and IV) were selected. Isolates were grown on only Pikovskaya agar media and they showed halo zone around the bacterial colony. No any isolates was grown and shown positive character on Jensen media, BTB media and King's B media.

4.2 Morphological and Biochemical Characterization of Isolates

The morphology of the selected isolates were tested by gram's staining method, isolates I, II, III and IV was Gram positive and rod shaped bacteria. According to Table 1 and Fig. 1, isolates was positive for Indole production, Acids produced, Citrate utilization, Gelatin hydrolysis, Starch hydrolysis, Hydrogen sulphide, NO_2 reduction, Ammonium production and Phosphate solubilisation. While, isolates was negative for Voges-Proskauer (VP) test, Nitrogen fixation test, Siderophore production and in case of IAA production all isolates are negative only isolates IV was positive.

4.3 Molecular Characterization of the Isolated Strains

During molecular characterization of the isolated strains genomic DNA was extracted and 16S-rDNA fragment was amplify by PCR methods. Amplifying PCR products was sequenced and sequence were aligned and examined its closest neighbors. The Microbe was found to be *Bacillus subtilis* as the *Bacillus subtilis* strain AS1 16S ribosomal RNA gene, partial sequence was found to have highest percent identity.

Table 1. Biochemical test of the isolates

Biochemical test	Isolate-I	Isolate-II	Isolate-III	Isolate-IV
Phosphate solubilisation	+Ve	+Ve	+Ve	+Ve
Indole production	+Ve	+Ve	+Ve	+Ve
Acid produced	+Ve	+Ve	+Ve	+Ve
Citrate utilization	+Ve	+Ve	+Ve	+Ve
Gelatin hydrolysis	+Ve	+Ve	+Ve	+Ve
Starch hydrolysis	+Ve	+Ve	+Ve	+Ve
Hydrogen sulphide	+Ve	+Ve	+Ve	+Ve
NO_2 reduction	+Ve	+Ve	+Ve	+Ve
Ammonium production	+Ve	+Ve	+Ve	+Ve
Voges-Proskauer (VP) test	+Ve	+Ve	+Ve	+Ve
Nitrogen fixation test,	+Ve	+Ve	+Ve	+Ve
Siderophore production	+Ve	+Ve	+Ve	+Ve
IAA production	-Ve	-Ve	-Ve	+Ve

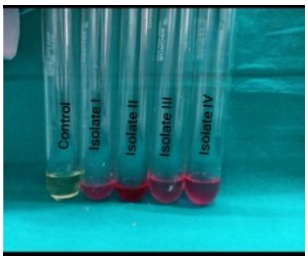

Fig. 1A. Indole test of isolates

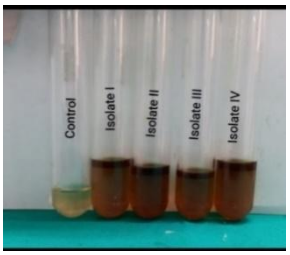

Fig. 1B. Methyl test of isolates

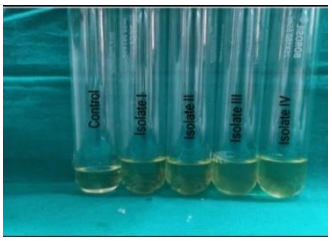

Fig. 1C. VP test of isolates

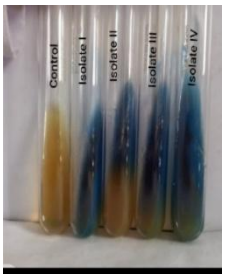

Fig. 1D. Citrate test

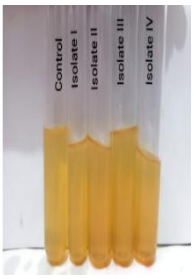

Fig. 1E. Gelatine hydrolysis test

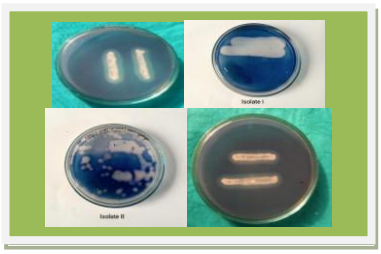

Fig. 1F. Starch hydrolysis test

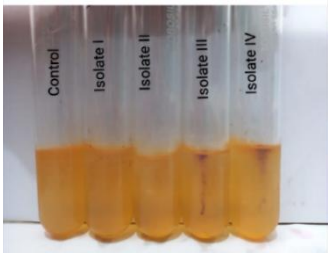

Fig. 1G. Showing H_2S test

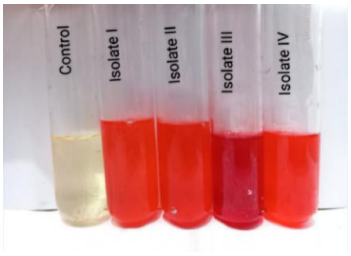

Fig. 1H. showing No_2 test

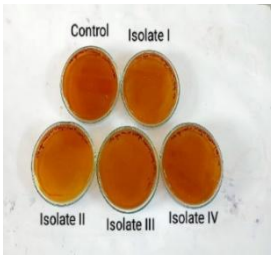

Fig. 1I. Showing Nfb test

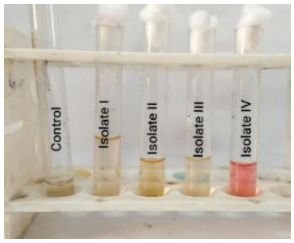

Fig. 1J. Showing IAA test

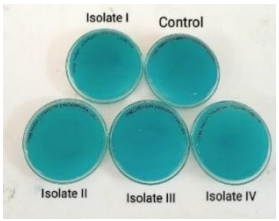

Fig. 1K. Siderophore test

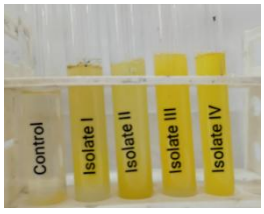

Fig. 1L. Ammonium test

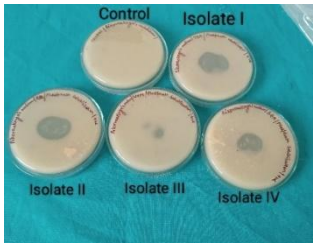

Fig. 1M. Showing PSB test of isolate

Fig. 2. Observation of percentage seed germination in presence of rhizobacteria

4.4 Application of the Isolated Rhizobactera Along with Phosphate Rich Organic Manure

Rhizobacterial enriched phosphate rich organic manure were used in pot soil before sowing of seeds. Rhizobacterial treated pot showed 30% more green gram seed germination.

5. CONCLUSION

Conclusion of the current work, isolated bacteria from rhizospheric region of *Raphanus sativus* (Radish) root was *Bacillus subtilis*. Isolated rhizobacteria was a plant growth promoting rhizobacteria and showed phosphate solubilizing activity which was showed to increases green gram seed germination up to 30% more.

CONFERENCE DISCLAIMER

Some part of this manuscript was previously presented in the conference: 6th International Conference on Strategies and Challenges in Agricultural and Life Science for Food Security and Sustainable Environment (SCALFE-2023) on April 28-30, 2023 in Himachal Pradesh University, Summer Hill, Shimla, HP, India. Web Link of the proceeding: https://www.shobhituniversity.ac.in/pdf/Souvenir-Abstract%20Book-Shimla-HPU-SCALFE-2023.pdf

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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