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

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. 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.

Keywords: Rhizobacteria, Rhizosphere, Solubilization, Biochemical.

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* (**Odoh, 2017)**. 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 **(Bhattacharyya *et. al.*, 2012).** The potentiality of PGPR offers an attractive way to replace the use of chemical fertilizers, pesticides and other supplements to increase agriculture yield. 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.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 culture on seed germination.

Materials and methods:

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.

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.

Morphological and biochemical characterization of isolates

Colony morphology were observed by Gram staining technique under 40X objective of the microscope **(Cappuccino and Sherman, 1996)**. 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, 1996)** and **(Aneja, 2003)**.

Molecular characterization of the isolated strains

Molecular characterization of the isolated strains was based on 16S r-DNA Sequence methods **(Altschul *et al.*, 1997).**

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.

Result and Discussion:

The aim of current study to isolate the PGPR bacteria from soil samples and isolated culture

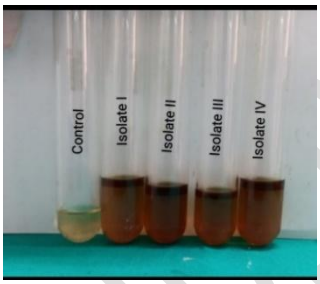
identification was based on biochemical characterization.

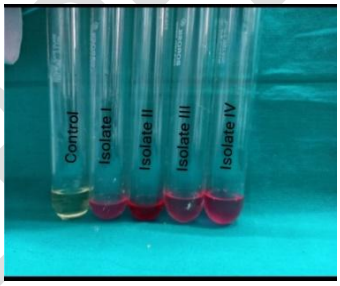
Screening and isolation of PGPR

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.

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 Figure 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.





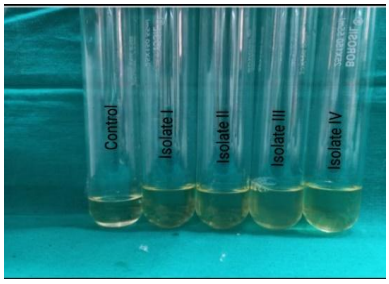
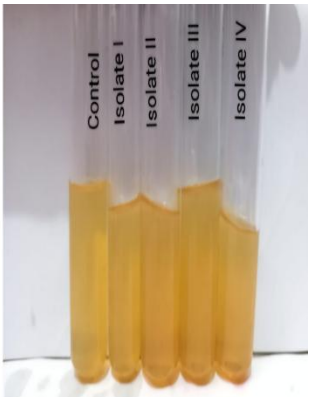


Figure1A: Indole test of isolates; **Figure1B:** Methyl test of isolates**; Figure1C:** VP test of isolates



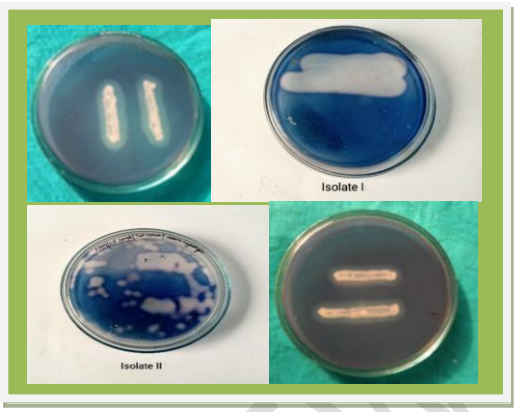
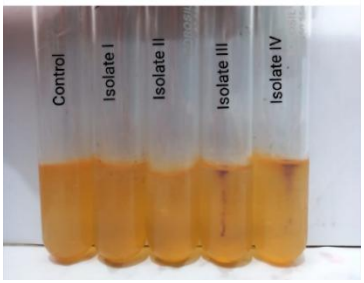
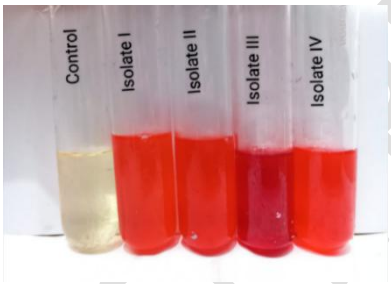


Figure1D: Citrate test; **Figure1E:** Gelatine hydrolysis test; **Figure1F:** Starch hydrolysis test





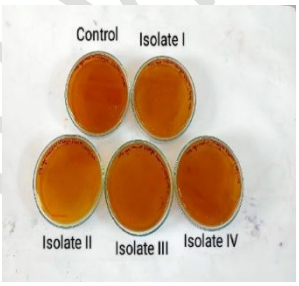
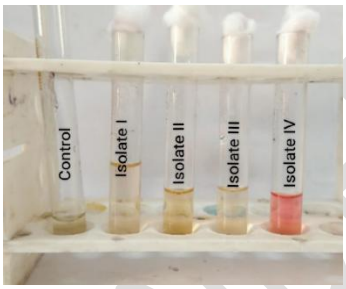
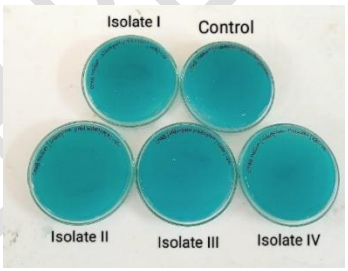


Figure1G: Showing H_2S test; **Figure1H:** showing No_2 test; **Figure1I:** Showing Nfb test





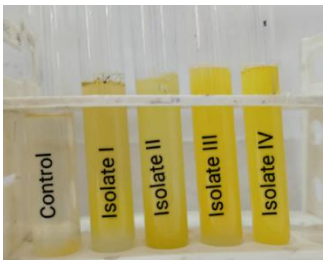


Figure1J: Showing IAA test **Figure1K:** Siderophore test **Figure1L:** Ammonium test

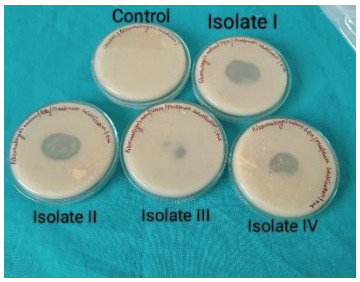


Figure1M: Showing PSB test of isolate

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.

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 show 30% more green gram seed germination

Figure 2: Observation of percentage seed germination in presence of rhizobacteria

Conclusion

Summary and Conclusion of the current work was isolated bacteria from rhizospheric region of *Raphanus sativus* (Radish) root was Plant Growth Promoting Rhizobacteria (*Bacillus subtilis*), which was showing phosphate solubilizing activity. Isolated rhizobacteria were showed 30% more moong seed (green gram) germination.

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