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ISOLATION AND CHARACTERIZATION OF PGPR FROM RHIZOSPHERE SOIL OF DIFFERENT REGIONS OF VADODARA DISTRICT OF GUJARAT (INDIA)

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ABSTRACT

It is common knowledge that bacteria that promote plant development can enhance plant growth either directly or indirectly. The current investigation was carried out in Vadodara, Gujarat, India, to determine the diversity of bacteria linked to diverse crops. The study mentioned above also intends to evaluate their potential revolutionary impact with activities that promote plant development. Eleven distinct bacterial strains—VS2S1, VS2S2, VS2S3, VS3S3, VS4S1, VS4S2, VS6S1, VS6S2, VS6A1, VS6K2 and VS6K3—were isolated from the rhizosphere. For various PGP characteristics, the morphology and biochemical of all eleven isolates were examined. Five bacterial isolates were discovered to have the ability to produce IAA, and the findings of the in vitro experiments revealed that four bacterial isolates were found to have the ability to phosphate solubilizing activity. The aforementioned study also recommends the use of particular PGPR isolates as inoculants, which will help with the cultivation of wheat crops.

Keywords: PGP attributes, Phosphate solubilizing activity, IAA production, PGPR

1. INTRODUCTION

All of us need everything pre-made and immediate results in the modern era of growth. In agriculture as well, we constantly hope for exceptional results. And we utilize various chemical fertilizers in agriculture to fulfill the expectation. Chemical fertilizers can produce excellent results in the short term, but their excessive use has negative long-term effects on agriculture. It gradually reduces the agricultural field's fertility. This has harmed the groundwater as well as the available mineral nutrients and soil quality. Organic farming may help to ameliorate the situation and stop the ecosystem's decline in this challenging situation **[1]**.

The rhizosphere microflora has been severely impacted by the excessive use of chemical fertilizers, which has led to a decline in soil productivity and nutrient content. In order to generate more food, the growing world population has put enormous pressure on agricultural lands and other natural resources. Growing usage of chemical fertilizers is damaging to both human and environmental health. In order to maintain productivity and the agricultural ecosystem, biofertilizers have emerged as an ideal replacement. A different strategy for boosting soil fertility, soil health, and crop yield while being environmentally benign has been found as using bio fertilizers. Microbes can be used as biostimulants, biofertilizers, and bioprotectants, among other things. This is because a variety of microbes, including bacteria, have been used as biocontrol agents to promote plant growth and production. Known as Plant Growth Promoting Rhizobacteria (PGPR), these bacteria actively colonize the rhizosphere around the root surface and have a positive impact on plant growth by giving the plant nutrients and hormones while acting as an antagonist to bacterial and fungal pathogens. These bacteria can produce phytohormones that promote hypergrowth, as well as provide and facilitate the soil's absorption of various nutrients including nitrogen and phosphate **[2; 3]**.

Kloepper and Schroth named the bacterial rhizosphere bio-control agents "plant growth promoting rhizobacteria" (PGPR) **[4]**. The generation of phytohormones, diazotrophic nitrogen fixation, solubilization of mineral phosphates, siderophore production, and antagonistic activity toward phytopathogenic microbes are only a few of the processes used by plant growth promoting bacteria to promote plant growth.

The phosphorus solubilization and uptake by plants, biological nitrogen fixation, the sequestration of iron for plants by siderophores, the production of plant hormones like auxins, cytokinins, and gibberellins, and the reduction of plant ethylene levels are some of the direct mechanisms of plant growth by PGPR **[5]**. Numerous crop plants have been shown to have increased plant growth characteristics, yield, and nutritional content when exposed to PGPR strains of Pseudomonas, Azospirillum, Azotobacter, Klebsiella, Arthrobacter, Burkholderia, Bacillus, and Serratia **[6- 8]**.

Various environmental conditions that may alter their growth and consequently their impact on the plant could be the cause of the variation in PGPR's performance. Knowing how Rhizospheric bacteria affect plants and whether those effects are influenced by other environmental conditions, such as the presence of other microorganisms, is essential for maximizing the growth-promoting interaction between PGPR and nursery seedlings **[9; 10]**. Therefore, in order to lessen the harmful effects of chemical fertilizers on the environment, effective strain must be developed in field conditions. With these limitations in mind, the current study was created to extract and screen specific rhizobacterial isolates from Pseudomonas, Bacillus, Rhizobium, Azotobacter, etc.

2. MATERIALS AND METHODS

2.1 Collection and Characteristics of Soil

Samples of rhizosperic soil were taken from four distinct crop fields, including i. Using wheat, castor, rice, and maize farms in the Vadodara district in an effort to find bacteria that are effective at stimulating plant growth. By serial dilution plating on Luria-Bertani (LB) agar plates, rhizospheric bacteria were isolated from 2 gm of soil securely adhering to the root **[11]**. Using standard laboratory techniques, soil's physical characteristics were investigated. The rhizosphere's entire root structure was dug up. The samples were put in plastic bags and kept in the refrigerator at 4 °C. Into a 250 ml container, 10 grams of rhizosphere soil were added.

2.2 Isolation of PGPR

25mL of sterile, distilled water was added to a 50ml flask containing two grams of rhizosphere soil. On a rotary shaker, the flask was shaken for 10 minutes. To 4.5ml of sterile distilled water tubes, 0.5ml of the suspension from the flask was added. Up to 10^{-5} dilutions were performed in serial. On plates of Luria-Bertani agar medium, an aliquot of 0.1ml of this suspension was applied. To observe the bacterial colonies, plates were incubated for three days at 28°C.

The master plates were used to identify various isolated colonies. On purifying the culture, identified bacterial colonies were streaked to other LB agar plates. 48 hours were spent incubating streaked plates at 28°C. A well-isolated single colony was removed and three times spread onto new

LB agar plates for purification. Final isolated colonies were saved for additional research.

2.3 Physiological Characterization

To assess colony morphology, isolated colonies were streaked on Nutrient Agar Plate and incubated at 30^0 C for 24 to 48 hours. After three days of incubation, the morphological properties of each isolate's colony were investigated. Different aspects of the colonies' form, size, elevation, surface, margin, color, odor, pigmentation, and gram response were noted.

2.4 Biochemical Characterization

Bergey's Manual of Determinative Bacteriology's biochemical features were used to identify the isolates **[12]**.

A. Sugar Utilization Test- A loopful culture of bacteria was injected into the sugar broth and kept to incubate at 37°C overnight. Gas generation was determined from a tiny bubble in Durham's tube and the medium's color changed from medium to pink due to acid production. The output was noted as (A) for the production of acid and (G) for the generation of gas.

B. Indole Production Test- A loopful culture of bacteria was injected into the 1% Tryptone broth and left to incubate at 37°C overnight. Following incubation, 3–4 drops of xylene were added to the medium, followed by the addition of 1 ml of Ehrlich's reagent. A pink ring that forms suggests a successful outcome.

C. Methyl Red Test- A loopful culture of bacteria was seeded into glucose phosphate broth and left to incubate at 37°C overnight. 4-5 drops of methyl red indicator were applied after incubation. A red color formation indicates a successful outcome.

D. Voges - Proskauer Test- A loopful of bacterial culture was added to glucose phosphate broth, and the mixture was then incubated at 37°C overnight. 0.6 ml of -naphthol and 0.2 ml of potassium hydroxide solution were added after incubation. A red color formation indicates a successful outcome.

E. Citrate Utilization Test- A loopful culture of bacteria was streaked onto Simmons's Citrate Agar slant and was incubated at 37°C overnight. Deep blue colour formation leads to positive result.

F. Starch Hydrolysis Test- A loopful culture of bacteria was streaked onto the Starch Agar plate and was incubated at 37°C overnight. After incubation, plate was flooded by Lugol's Iodine. Formation of clear zone around the colony leads to positive result.

G. Nitrate Reduction Test- A loopful culture of bacteria was inoculated into the Peptone Nitrate broth and was incubated at 37°C overnight. After incubation, 0.5 ml of α – napthylamine and Sulphanilic acid reagent were added. Development of red colour leads to positive result.

H. Catalase Test- A loopful culture of bacteria was inoculated into the nutrient broth and incubated at 37°C overnight. After incubation 1 ml of 3% Hydrogen peroxide was added. Gas bubble formation indicates positive result

I. Urease Test- A loopful of overnight culture was inoculates to the test tubes with sterilized urea broth and incubated for 24-48 hours at 37°C Urease production was tested using urea broth. The composition of urea broth was 37°C The development of pink indicates positive result.

J. Oxidase Test- overnight cultures of the test isolates were spotted and plates were incubated for 24 h at 28ºC. After incubation, two to three drops of N, N, N', N'-tetramethyl-phenylenediamine-dihydrochloride was added to the surface of the growth of each test organism. The colour changes to purple or maroon was taken as oxidase positive.

Table 1: Colony Morphology of Selected Isolates

Isolate code	Colony size and shape	Colony color	Elevation	Margin	Gram reaction
VS2S1	Small, Pin-point	White	Fused	Entire	Gram negative
VS2S2	Small, Pin-point	Brown	Fused	Raised	Gram negative
VS2S3	Large, Myceloid	Red	Raised	Entire	Gram negative
VS3S3	Small, Pin-point	Yellow	Raised	Entire	Gram Positive
VS4S1	Small, Conglomerate	Milky White	Raised	Undulate	Gram positive
VS4S2	Large, Myceloid	Golden Yellow	Fused	Irregular	Gram negative
VS6S1	Large, Irregular	Off-white	Raised	Entire	Gram negative
VS6S2	Small, Round	Off -white	Fused	Undulate	Gram negative
VS6K2	Small, Round	White	Raised	Undulate	Gram negative
VS6K3	Small, Round	White	Raised	Entire	Gram Positive
VS6A1	Large, Round	Off white	Raised	Entire	Gram negative

Table 2: Biochemical Characterization of Selected Isolates

Biochemical tests	Isolates										
	VS2S1	VS2 S2	VS2S3	VS3S3	VS4S1	VS4S2	VS6 A1	VS6S1	VS6S2	VS6K2	VS6 K3
Indole 3 Acetic Acid	+ve	-ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve
Phosphate	-ve	+ve	-ve	-ve	+ve	-ve	-ve	-ve	-ve	+ve	+ve
Siderophore	-ve	+ve	-ve	-ve	-ve	-ve	-ve	+ve	+ve	+ve	+ve
Glucose	A/-	A/G	A/-	A/G	A/G	-/-	-/-	A/-	A/-	A/-	A/-
Sucrose	A/-	A/G	A/-	-/-	A/-	A/G	-/-	A/-	A/-	-/-	-/-
Fructose	A/-	A/G	A/-	A/G	A/-	A/G	-/-	A/-	A/-	-/-	-/-
Lactose	A/G	-/-	A/-	A/G	-/-	-/-	-/-	-/-	-/-	-/-	-/-
Arabinose	A/-	A/G	A/-	A/G	-/-	A/G	A/-	A/-	A/-	-/-	A/-
Galactose	A/-	A/G	A/-	-/-	A/-	A/G	-/-	A/-	A/-	A/-	A/-
Mannitol	A/-	A/G	A/-	A/G	-/-	A/G	-/-	A/-	A/-	-/-	-/-
Oxidase	+ve	+ve	+ve	+ve	+ve	-ve	+ve	-ve	+ve	-ve	-ve
Catalase	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve
Amylase	-ve	-ve	+ve	-ve	+ve	-ve	-ve	+ve	+ve	-ve	-ve
Citrate	-ve	+ve	-ve	-ve	-ve	+ve	+ve	+ve	+ve	+ve	+ve
Urease	-ve	+ve	-ve	-ve	-ve	+ve	-ve	+ve	+ve	+ve	+ve
Nitrate	+ve	+ve	+ve	+ve	+ve	+ve	-ve	-ve	-ve	-ve	-ve
Indole	-ve	-ve	+ve	+ve	-ve	-ve	-ve	+ve	+ve	-ve	-ve
VP	-ve	-ve	-ve	-ve	-ve	+ve	-ve	-ve	-ve	-ve	-ve
Methyl Red	-ve	-ve	+ve	+ve	-ve	-ve	-ve	-ve	+ve	-ve	-ve

Interpretation: A-Yellow color acid; G-Gas Bubble; A/- – indicates only Acid is present; A/G – indicates both Acid and Gas is Present; -/- – Both acid and gas are absent

3. RESULT and DISCUSSION

The procedure for PGPB isolation and selection, as well as the management of a significant number of data, present challenges in identifying the best-performing isolates. Numerous studies have examined how to separate and characterize PGPB from a range of crops' rhizospheres **[13]**. Two general strategies could be applied. First is a culture-dependent approach that involves isolating a large number of isolates, characterizing and identifying them, and then choosing the most promising candidates for field application **[14]**. The selection of some strains that perform well and have the needed traits and capabilities is the major outcome of this procedure. Yan et al. presented a different strategy **[15]**. It is based on an initial metagenomic analysis that emphasizes population diversity and the phylogenetic trees of each family and group. The ability to examine each strain's prevalence and account for actual soil and plant colonization is the key advantage of this strategy **[13]**.

In this investigation, a total of 18 isolates were found in the four samples that were taken from various villages in the Vadodara district. 11 isolates were identified as IAA producers, 4 isolates displayed strong phosphate solubilization, and 5 isolates were identified as siderophore producers out of the total number of isolates. All 11 isolates were attempted to be morphologically described **(Table 2)**. The Gram reaction of PGPR isolates was studied using microscopic examinations. Three isolates—VS3S3, VS4S1, and VS6K3—were Gram positive bacteria, while the remaining isolates were all Gram negative **(Table 1)**.

Tiwari et al. Six isolates in their investigation generated indole-3-acetic acid in concentrations ranging from 56 to 97 g/ml, however only one strain (PN13) shown phosphate solubilizing ability in Pikovskaya agar. Six isolates were also discovered to produce siderophores and to have antifungal properties against *Fusarium udum*. Almost all of the isolates showed catalase production, and just one sample, PN11, also produced HCN **[16]**. Seven of the nine isolates showed in vitro plant growth promotion activities, suggesting that they could be used as biofertilizers and microbial inoculants for pigeon pea crops because they promoted plant growth through a variety of mechanisms and provided a viable alternative to synthetic fertilizers and pesticides **[16]**

4. CONCLUSION

The current work demonstrates the importance of rhizobacteria in in vitro settings for a variety of PGPR characteristics. From the description above, it can be inferred that PGPR promote plant growth since it produces IAA, solubilizes phosphate, and produces siderophore. They are anticipated to eventually take the place of chemical fertilizers, insecticides, and growth regulators, all of which have negative consequences on sustainable agriculture. The conclusion is that by fixing nutrients and preventing their leaching out, the creation of formulations based on potentially active PGPR strains can increase the availability of nutrient concentration in the rhizosphere. The efficiency of maize cultivars in acquiring phosphorus from the soil was also improved by PGPR inoculation. The current study showed that PGPR might enhance the effects of plant growth-parameters on maize, however more research on the strain's molecular properties and application techniques for PGPR inoculation may help in utilizing these microorganisms.

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