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Research Article

Isolation and Characterization of *Rhizobacteria* from Soil and Its Efficiency as Plant Growth-Promoting *Microbes*

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ABSTRACT

The rhizosphere is the soil environment where the plant root is available and is a zone of maximum microbial activity, resulting in a confined nutrient pool in which essential macro-and micronutrients are extracted. The microbial population in the rhizosphere is relatively different from that of its surroundings due to root exudates that function as a source of nutrients for microbial growth. Those resulting in symbiotic and non-symbiotic nitrogen fixation are considerably important among all these interactions. In recent years, the use of bacteria (*rhizobacteria)* to promote plant growth has increased in several regions of the world and has acquired relevant importance in developing countries that are the producers of raw materials for food. In the present study *Rhizobacteria* was isolated from the soil and the production of IAA and phosphate solubilisation was studied. The effect of PGPR was shown on mat seeds (matki) and black-eyed pea seeds (chawali) on the growth of plants.

Keywords: *Rhizobacteria,* PGPR, IAA, Mat seeds, Black-eyed pea seeds.

INTRODUCTION

Soil is an energetic living framework and it is not a basic asset in agriculture and food security but is also supports of all life handled. Pathogenic microorganisms influencing plant wellbeing are a major and unremitting risk to sustainable farming and biological system steadiness around the world. The chemical fertilizers utilized in agribusiness to increment yields kill pathogens, pests, and weeds and harm the biological system enormously. Because of current open concerns around the side impacts of agrochemicals, there is an expanding intrigue in moving forward the understanding of agreeable exercises among plants and rhizosphere microbial populations. So, there is a critical requirement for natural operators around the world. The utilization of plant development to advance Rhizobacteria (PGPR) is a better way to unravel this issue. They play a vital part in incrementing soil ripeness, advancing plant development, and concealing phytopathogens for the advancement of ecofriendly sustainable agribusiness.[1]

In present-day development, unpredictable utilization of fertilizers, especially nitrogenous and phosphorus, has driven to considerable contamination of soil, air and water. Intemperate utilization of these chemicals harms soil microorganism, influences soil richness status, and contaminates the environment. They are imperative to increase soil readiness, plant advancement progression, and concealment of phytopathogens for the headway of eco-friendly sustainable agribusiness.[1]

The confinement of water accessibility in a few ranges of the world, along with the increase of human population and the extension of agrarian, vitality, and mechanical segments, have, as of now, significantly heightened the demand for water in the final decades. Dry season is a major constraining factor for trim generation as it causes unsettling influences on plant development and editing disappointment. Water shortage may cause morphological, biochemical, and physiological wounds on plants, influencing different critical cellular forms. In expansion, water shortfall can diminish the estimate of crops and organs, delay blossoming and fertilization, and decay grain yield and quality. These negative impacts are frequently related with diminishes in the microbial action of the soil.[2]

PGPR has been profoundly compelled to reach a certain range for their application. It is due to conflicting properties of PGPR, whereas its impacts depend on different variables like its survival in soil framework, capacity to associate with already present microflora of that place, the factor associated with the environment. They could also act and prove to be beneficial by producing various inhibitor compounds, bacteriocins, lytic enzymes, siderophores, and phosphate solubilization, and could also play a role in synthesizing phytohormones.[3] PGPR regulates growth through various indirect and direct mechanisms. It may include the addition of compounds related to microbe metabolism.[4] Temperature rises have brought major rises in global temperatures and the appearance of various abiotic factors that have a negative impact on agricultural output.[5]

The positive impacts of PGPR, a naturally occurring soil bacterium, on plant imperativeness and output, have been considered altogether. In addition to securing plants from pathogens and harsh conditions, they can boost supplement accessibility, spur plant development, fortify root improvement, and more. Several diverse mechanisms are included in how PGPR helps plants. Auxins, cytokinins, and gibberellins, which empower root and shoot development, are shaped by a few PGPR. In sustainable agricultural methods, the application of PGPR as bio-fertilizers and biopesticides has pulled in a lot of attention. In the establishment of the world economy and horticulture are crucial in providing nourishment, fiber, bioenergy, and other necessities to the growing human population. However, chemical pesticides and fertilizers are utilized as often as possible in routine agricultural practice, which can be awful for the wellbeing of people and the environment and the supportability of agricultural output. Therefore, maintainable and naturally inviting strategies are becoming progressively important to increment plant development and progress agricultural efficiency. In a long time, PGPR has surfaced as a practical choice for cultivating success in the long term.[6]

The mode of action of PGPR is due to their direct and indirect mechanisms. The direct mechanism involves the synthesis of certain plant growth-promoting substances or nutrients from the environment and making them available to plants. The indirect mechanism involves prevention of the deleterious effect of one or more phytopathogens by PGPR by one or several mechanisms.[7]

Substituting synthetic mineral fertilizers with PGPR in hydroponic lettuce production can conserve fertilizer without compromising plant development, yield, or product quality. This approach can potentially enhance the sustainability of soilless culture systems. The investigation involved a gradual reduction of synthetic mineral fertilizers by 20, 40, 60, and 80%, with PGPR employed as a substitute for the diminished mineral fertilizers.[8]

The rhizome-microbiome is crucial to agriculture because a wide variety of root exudates and plant cell debris attract unique and distinct patterns of microbial colonization. New studies have shed light on the mechanisms via which signaling by PGPR can induce various plant responses, both at the local and systemic levels. Insufficient information is available regarding the impact of the PGPR mechanism and molecules on metabolic pathways in root characteristics. More research and understanding of the processes involved in PGPR-mediated phytostimulation would help to create more effective rhizobacterial strains that could function in a variety of agroecological settings.[9]

The PGPR attributes multiple plant-growth-promoting (PGP) traits to plants, which increases their resilience to overcome the effects of stresses under prevailing environmental conditions. The mechanisms elicited by PGPR include the production of biofilm for enhancing soil binding to roots and regulation of relative water content, triggering of osmotic response via production and accumulation of osmolytes, enhancement in nutrient uptake, and maintenance of ion homeostasis. It induces a protective system to mitigate the existing stresses in a time-sensitive and cost-effective manner. With the rising emphasis on sustainability, environmental safety, and food security, the employment of bio-inoculants will help to overcome the constraints resulting from a persistent change in the climatic conditions and offer a promising alternative to achieve sustainability in agriculture in an environmentally friendly manner.[10]

MATERIAL AND METHODS

Two types of soil samples were collected from Sample 1: The soil sample (BLACK) was collected from the sugarcane field in Wagheri (Karad). PGBS1. Sample 2: A soil sample (RED) was collected from a garden in Akurdi. PGRS2.

Isolation of Bacteria from the Rhizosphere Soil

Soil samples were collected from sugarcane field that are (BLACK SOIL) and garden (RED SOIL). 10 grams of soil were taken into 200 ml of conical flask and 40 ml of sterile distilled water was added to it. The flask was shaken for 10 minutes on a rotary shaker. Serial dilution was performed upto 10^{-5} for both the samples PGBS1 and PGRS2. About 0.1 mL of sample PGBS1 and PGRS2 was inoculated by spread plate method on sterile Congo Red Yeast Extract Mannitol Agar plates (CREYMA). Both the samples were incubated for 3 days at 28°C. After incubation, isolated colonies were obtained and are maintained on CREYMA slant. Bacterial colonies were streaked on other CREYMA plates and plates were incubated at 28°C for 3 days. Bacterial colonies were observedd over the streak plate, well isolated colonies were picked up and re-streaked on fresh CREYMA plates and incubated at 28°C for 3 days. Characterization and identification of microorganisms carried out by gram staining and motility.[11] Further biochemical tests were performed to identify PGBS1 and PGRS2.

IAA Production and Phosphate Solubilization:

IAA production of bacteria

A single colony of bacterial culture was grown on LB liquid medium. A loop full of culture was inoculated into 100 mL of a conical flask containing LB liquid medium with the help of a sterile inoculated needle. The flask was then incubated for 7 days at room temperature. The culture in the flask showed a dense milky white color. The growth was observed.[12]

Phosphate solubilization of bacteria

Plates were prepared with Pikovskya's medium. The culture was streaked on plates and was incubated for seven days. After seven days of incubation, white color colonies were observed on plates.[13]

RESULT AND DISCUSSION

Isolation and Characterization of the Isolate

By performing colony characteristic and biochemical tests, the genus and species were identified using Bergey's manual of systematic bacteriology.

Identification of Isolate

Two bacterial isolates were successfully isolated from the rhizosphere soils of the sugarcane field and garden soil. They were designated as PGBS1 and PGRS 2. As shown in Table 1 and 2 and Figs. 1 and 2

The isolate was tentatively identified as Pseudomonas spp. and Rhizobium by performing the cultural, morphological and microscopic observation. As shown in Table 3.

Table 1: Colony characteristics of the isolate

Soil sample	*Size*	*Margin*	*Shape*	*Consistency*	*Opacity*	*Gram character*	*Motility*
PGBS1	2 mm	Entire	Circular	Sticky	Opaque	Gram-negative	Motile
PGRS2	2 mm	Entire	Circular	Sticky	Opaque	Gram-negative	Motile

Table 2: Biochemical characterization of the isolate

Soil sample	*Indole*	*MR*	*VP*	*Citrate*	*Catalase*	*Nitrate*	*Oxidase*	*Growth at 5% NaCl*
PGBS1	-	-	-	-	+	-	+	-
PGRS2	-	+	-	+	+	+	+	-
Sample		Glucose		Mannitol		Dextrose		
PGBS1		+		+		-		
PGRS2		+		+		-		

(+) stands for positive result.
(-) stands for negative result

Fig. 1: Red soil

Fig. 2: Black soil

Subculture of Plant Growth Producing *rhizobacteria*

Production of IAA by the isolate

IAA production was done in LB broth. Confirmation of IAA was done by changing the color of the dense milky white color observed after 7 days of incubation. As shown in Table 4.

Solubilization of phosphate by isolate

Solubilization of Phosphate was done on Pikovskya's medium. The presence of white colonies on the plate after 7 days of incubation confirmed phosphate solubilization.

The IAA production and phosphorous solubilization of PGPR isolates. As shown in the table, isolates PGBS1 and PGRS2 induce the production of IAA, and isolates PGBS1 and PGRS2 have the ability to solubilize the phosphorous.

Table 3: Identification of isolate

Sample	*Plant growth-producing rhizobacteria*
PGBS1	Pseudomonas spp.
PGRS2	Rhizobium

Table 4: Production of IAA and solubilization of phosphate

Isolate	*IAA production*	*Phosphate solubilization*
PGBS1	+	+
PGRS 2	+	+

(+) stands for positive result.
(-) stands for negative result

CONCLUSION

Rhizobacteria was isolated from the sugarcane field of Wagheri (Karad) and the botanical garden of Dr.D.Y.Patil Arts, Commerce and Science College Akurdi, Pune-44. Two bacterial isolates were successfully isolated from rhizosphere soils of the sugarcane field and garden soil. They were designated as PGBS1 and PGRS2. These bacteria were characterized and identified as *Pseudomonas spp* and *Rhizobium*. Confirmation of IAA was done by changing the color of the dense milky white color observed after seven days of incubation. The ability of indole acetic acid and phosphate solubilization was studied. *Rhizobium* has the ability to solubilize phosphate.

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CONFLICTS OF INTEREST

The authors declare no conflict of interest.

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