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IDENTIFICATION AND ISOLATION OF RHIZOSPHERIC BACTERIA FROM PISIUM SATIVUM

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ABSTRACT

"Microbial Consortia"—groups of microorganisms—can do amazing feats. The field of agriculture might greatly benefit from the usage of microorganisms. Furthermore, combining them all makes them more effective. They would provide further advantages through their respective and reciprocal metabolic processes. Our goal is to separate a few possible microorganisms with various characteristics, such as the capacity to produce indole-acetic acid, mobilize potash, solubilize phosphate, and exhibit siderophore activity. These isolates could be used to create microbial consortia and formulations that work well as biofertilizers to progress the sector of agriculture. When attached to seeds, plant surfaces, or soil, biofertilizer—also known as bio-compost—contains living microorganisms that colonize the rhizosphere, or inside of the plant, and promote development by increasing the host plant's access to or supply of vital nutrients. Biofertilizers are supplements made using the standard processes of solubilizing phosphorus, obsessing over nitrogen, and combining chemicals that promote plant growth. As plants develop, soil microbial communities engage with them in the plant rhizosphere. They also interact more indirectly through plant litter, which offers resources and habitat to a wide variety of soil species.

Keywords: *biofertilizer, microbial inoculant, rhizosphere*

INTRODUCTION

A major issue with food security is the growing population combined with the loss of arableland brought on by urbanization and industrialization. As a result, there is intense pressure on agricultural fields to produce more crops. Huge quantities of expensive chemical fertilizers are used to meet the demand, but doing so results in environmental issues such as degraded soil quality, stunted microorganism growth, and eutrophication of aquatic habitats. Additionally, there are waste areas of non-arable ground that are either saline or acidic. Higher nutrient concentrations in the soil have been linked to nutrient accumulation near the root surface, increasing the plant's mass flow. The plant's root surface can receive major nutrients, such as calcium and magnesium, by mass movement in the same volume, and the rhizosphere can accumulate those ions (Halim et al., 2020). The world will have to endure the rising food demand as long as the human population keeps growing. Around one billion people were saved from starvation and undernourishment thanks to the Green Revolution, which took place seven decades ago. It also led to the invention of chemical fertilizers and other innovations.

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By 2050, it's predicted that there will be 10 billion people on the planet. It will be increasingly difficult to supply the tremendous demand for increased supplies of raw materials andfood as a result of the unprecedented strain this enormous population will place on the earth's limited natural resources. The increased application of chemical fertilizers, specifically nitrogen, phosphorus, and potassium, is responsible for the recent increase in yields per acre (Meena et al., 2020).

Beneficial microorganisms produce bioinoculants, sometimes referred to as microbial inoculants, which can directly or indirectly benefit the host plant by boosting nutrient availability and plant growth. Due mainly to the deposition of photosynthetic carbon in them, microbial species exhibit symbiosis or free-living relationships within the plant root system (Joshi et al. 2019). They are capable of converting confined nutritional components into accessible forms. Microorganisms have active or latent cells that effectively fix nitrogen, solubilize zinc, phosphate, and potassium in the rhizospheric root of the host plant (Patil and Solanki 2016). .It quickly multiplies the bacteria present when applied to soil, used as a seed treatment, or as a dip for seedling roots, which significantly aids in rhizosphere population growth. By evenly covering seeds with inoculants, bioinoculants aid in bioremediation and seed treatment (Dangi et al. 2019).

PHOSPHATE AVAILABILITY IN SOIL ROOT MICROBIOME

The spatially uneven distribution of resources (water and nutrients) in the soil is well known. For example, it is known that top-soil layers, also known as litter layers, are high in P because plant leftovers are continuously deposited in the majority of agricultural soils (Lynch and Brown, 2012). By influencing its availability, additional biotic and abiotic variables including as pH, temperature, microbial activity, and root spatial dispersion can further add to the regional variability of P (Lynch and Brown, 2012; Shen et al., 2011; Smith et al., 2003). Accordingly, shorter roots that grow toward P-rich soil strata and investigation of the root/rhizosphere-associated microbiome are necessary for effective P acquisition in order to mobilize the rarely accessible P present in the rhizosphere soil interface.

By increasing plant P uptake in rhizosphere soils, phosphate-solubilizing bacteria (PSB) are crucial to P cycling and plant growth. The majority of PSB generate indole-3-acetic acid (IAA), which promotes the growth of plant cells and RNA/protein synthesis, hence boosting plant growth (Sadaf.S et.al.,2009). Furthermore, P solubilization in soils depends on the low molecular weight organic acids and microbial metabolites that are produced in conjunction with PSB metabolic processes (Alori et al.,2017 & Jiang YF et al.,2020). Effective PSB treatment can prevent environmental harm that leads to water eutrophication and soil hardening by releasing the accumulated P left in soils by conventional P fertilizer (Billah M et al.,2019 & Etesami H et al.,2020). It has been demonstrated that PSB inoculation in fields can improve P fertilizer efficiency by combining it with organic and mineral P.

BIOLOGICAL FUNCTION OF IAA IN BACTERIA

Bacteria mostly communicate with plants that have the ability to produce IAA this confirms that IAA has environmental advantage upon those bacteria. Furthermore, the significance of bacterial IAA in bacterial activity that reaches beyond IAA's interaction with plants is probably indicated by the huge number of PGPB that possess IAA biosynthesis capacity.

MATERIAL & METHOD

1. **Sampling Sites-** Soil samples were collected in the month of December 2023, from the rhizosphere of pea from two different sites. The location of sites is
   Site 1 (Khirsu, Pauri Garhwal) – It is at a height of 1700 m with dense forests of pine and deodar. The villages of Khirsu were known for its agriculture activity.
   Site 2 (Tungra, Chakrata) – It is situated about 5 km away from Chakrata main tehsil of Dehradun where the people depend on their agriculture practices for their basic needs.

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2. **Soil Samples Collection** – Total 5 soil samples were collected from the rhizosphere of pea plants from the above-mentioned sites.
   Isolation and Enumeration of Rhizobacteria – The plant growth promoting bacteria from rhizosphere soil were isolated from different soil samples by serial dilution and spread plate methods on Rhizosphere Mimicking Agar (RMA) (Brescia et al., 2020).
3. **Morphological and Biochemical Characterization of Isolates** – For the identification of indigenous micro-organisms, bacterial isolates were grown on NAM. Bacterial isolates were further identified on the bases of Gram staining and various biochemical tests (Alder et al., 1967).
4. **Primary Screening of Rhizobacterial isolates**
   4.1 Estimation of Phosphate Solubilization – Phosphate solubilizing capability of the isolates was carried out by detecting the formation of transparent halos surroundings, bacterial colonies on the pikovskaya agar after 72 hours incubation at 28^0C (Pikovskaya, 1948).
   4.2 Indole -3-acetic acid production – IAA production by the bacterial isolates was determined by the Salkowski reagent method (Ehmann, 1977). Quantification was done by calculating Spectrophotometric method in which optical density of bacterial culture that was grown in LB broth with tryptophan.

RESULT

4.1. Sampling:

In the present study total 10 soil samples were obtained from the rhizosphere of pea plants in a sterile poly bags from 2 different sites (5 samples each site) for the analysis of soil microbial biomass (Fig 1).

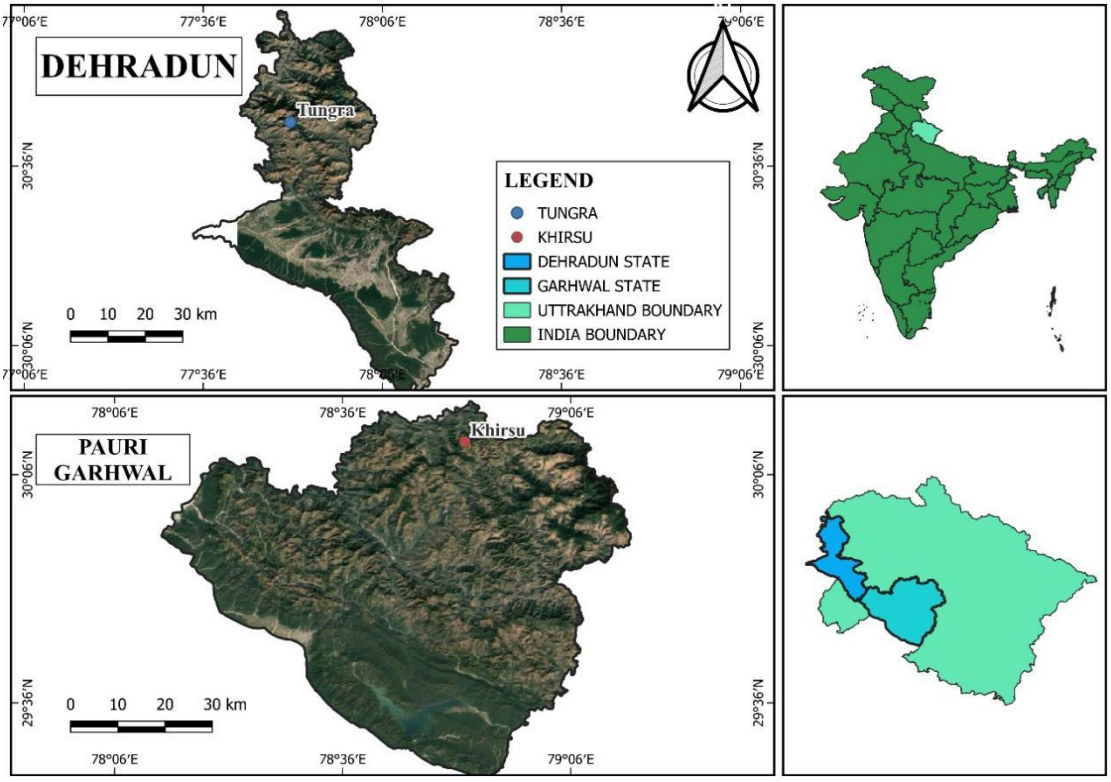

Fig 1: The Geographical representation of different sampling sites

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4.2. Isolation and Enumeration of Rhizobacteria:

The bacterial isolates were enumerated by using serial dilution method and the bacterial colonies were counted on Rhizosphere mimicking agar plates as colony forming unit and the maximum CFU value was found at site Khirsu ($33.8 \pm 0.6 \times 10^3$) (Table 1).

Table 1: Enumeration of Rhizosphere bacteria from different sites (Average of triplicates ± SE)

Samples	Colony Forming Unit		
	Dilution Factor		
	10^3	10^4	10^5
Site 1 - Khirsu			
Sample 1	$30.6 \pm 0.03 \times 10^3$	$22.2 \pm 0.2 \times 10^4$	$13.56 \pm 0.06 \times 10^5$
Sample 2	$29.3 \pm 0.1 \times 10^3$	$18.08 \pm 0.03 \times 10^4$	$11.03 \pm 0.33 \times 10^5$
Sample 3	$33.8 \pm 0.6 \times 10^3$	$21.1 \pm 0.01 \times 10^4$	$12.28 \pm 0.05 \times 10^5$
Sample 4	$28.8 \pm 0.02 \times 10^3$	$20.68 \pm 0.08 \times 10^4$	$9.33 \pm 0.33 \times 10^5$
Sample 5	$30.5 \pm 0.2 \times 10^3$	$24.88 \pm 0.06 \times 10^4$	$12.68 \pm 0.8 \times 10^5$
Site 2 -Tungra			
Sample 1	$25.03 \pm 0.05 \times 10^3$	$21.02 \pm 0.2 \times 10^4$	$14.02 \pm 0.18 \times 10^5$
Sample 2	$22.18 \pm 0.12 \times 10^3$	$19.33 \pm 0.13 \times 10^4$	$11.11 \pm 0.33 \times 10^5$
Sample 3	$23.34 \pm 0.04 \times 10^3$	$19.98 \pm 0.08 \times 10^4$	$12.82 \pm 0.42 \times 10^5$
Sample 4	$19.42 \pm 0.02 \times 10^3$	$12.71 \pm 0.3 \times 10^4$	$8.78 \pm 0.88 \times 10^5$
Sample 5	$21.98 \pm 0.7 \times 10^3$	$15.31 \pm 0.66 \times 10^4$	$10.03 \pm 0.3 \times 10^5$

4.3. IDENTIFICATION OF BACTERIAL ISOLATES:

Total 15 isolates of 10 bacterial genera were detected in the different samples of rhizosphere soil samples, which were been identified on the morphological and biochemical bases as *Bacillus*, *Pseudomonas*, *Rhizobium*, *Azotobacter*, *Micrococcus* sp., *Klebsiella*, *Clostridium*, *Azospirillum*, *Sphingobacterium*, and *Sporosarcina* (Table 2).

Table 2: Morphological and biochemical characteristics of bacterial isolates.

Table 2.a. Morphological characteristics

Isolates	Size	Shape	Elevation	Margin	Opacity	Texture	Color
PRBI-1	Small	Circular	Slightly	Irregular edges	Glossy	Smooth	Cream
PRBI-2	Large	Irregular	Slightly	Wavy edges	Opaque	Granular	Grey- White
PRBI-3	Large	Filamentous	Flat	Irregular edges	Translucent Opaque	Granular	White
PRBI-4	Large	Circular	Slightly	Regular edge	Glossy	Smooth	Red

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PRBI-5	Large	Oval	Flat	Irregular	Opaque	Smooth	White
PRBI-6	Large	Irregular	Flat	Undulate	Opaque	Dry	White
PRBI-7	Large	Circular	Flat	Round	Opaque	Smooth	Yellow
PRBI-8	Large	Irregular	Flat	Irregular	Opaque	Smooth	White
PRBI-9	Small	Circular	Convex	Round	Opaque	Smooth	White
PRBI-10	Small	Circular	Convex	Round	Opaque	Smooth	Yellow
PRBI-11	Small	Circular	Radiated from center	Round	Opaque	Smooth	Yellow
PRBI-12	Small	Circular	Convex	Round	Opaque	Smooth	Greenish
PRBI-13	Small	Circular	Convex	Round	Opaque	Smooth & Shiny	Yellowish green
PRBI-14	Large	Circular	Slightly	Regular	Glossy	Smooth	Pink
PRBI-15	Small	Round	Convex	Round	Transparent	Smooth	Grey- White

Table 2.b. Biochemical Characteristics of Bacterial Isolates

Bio chemical tests	Isolates														
	PRBI-1	PRBI-2	PRBI-3	PRBI-4	PRBI-5	PRBI-6	PRBI-7	PRBI-8	PRBI-9	PRBI-10	PRBI-11	PRBI-12	PRBI-13	PRBI-14	PRBI-15
Gram staining	+	+	-	+	-	-	+	+	-	+	+	-	+	-	-
Catalase	+	+	+	+	-	+	+	+	-	-	+	-	-	+	+
Citrate	+	+	+	+	-	+	+	-	-	-	-	-	+	+	-
Gas	-	-	+	+	-	+	+	+	-	-	-	-	+	+	+
Indole	-	-	-	-	-	-	-	-	+	+	-	+	+	-	-
MR	-	-	-	-	-	+	+	-	-	-	+	-	+	-	-
VP	+	+	-	-	+	-	+	+	+	+	-	-	-	+	+
Urease	-	-	-	-	-	+	-	-	-	-	-	-	-	-	+
Starch	+	+	-	-	-	-	+	-	-	-	-	-	-	-	+
Fructose	+	+	-	+	-	+	-	-	-	-	+	-	+	+	+
Dextrose	+	+	+	+	-	+	-	-	-	-	+	-	+	+	+
Lactose	+	-	+	+	-	+	-	-	-	-	-	-	+	-	+
Maltose	+	+	+	+	-	+	-	+	-	+	+	-	+	+	+
Mannitol	+	-	+	-	-	+	-	+	-	-	-	-	-	+	+
Raffinose	+	-	+	+	+	+	-	-	-	-	-	-	-	-	+
Sorbitol	+	-	+	-	-	+	-	-	-	-	+	-	+	+	+
Sucrose	+	+	+	+	-	-	-	+	-	-	+	-	+	+	+

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Arabinose	+	-	+	-	-	-	-	-	-	-	+	-	+	-	+
Mannose	+	-	+	+	-	+	-	+	-	-	-	-	-	-	+

4.4 PRIMARY SCREENING OF RHIZOBACTERIA ISOLATES:

Primary screening of different isolates was carried out on the bases of various PGPR activities such as Phosphate solubilization and Indole-3-acetic acid production. Out of 15 isolates that were screened PRBI – 6 shows maximum phosphate solubilization and indole-3-acetic acid production activity followed by PRBI-8, PRBI-2, PRBI-11, PRBI-13, and PRBI-12 respectively (Table 3a. and 3b.) (Fig 2 and 3).

Table 3.a. Phosphate solubilizing activity (Average of triplicate)

S.No.	Isolates	Zone of Solubilization (mm ± S.E)
1.	PRBI-1	1.3 ± 0.2
2.	PRBI-2	4 ± 0.01
3.	PRBI-3	1 ± 0.22
4.	PRBI-4	2.6 ± 0.04
5.	PRBI-5	1.9 ± 0.01
6.	PRBI-6	21.3 ± 0.03
7.	PRBI-7	3..2 ± 0.02
8.	PRBI-8	6.6 ± 0.33
9.	PRBI-9	2.8 ± 0.02
10.	PRBI-10	-
11.	PRBI-11	5.9 ± 0.33
12.	PRBI-12	2.4 ± 0.1
13.	PRBI-13	5.8 ± 0.12
14.	PRBI-14	3.3 ± 0.3
15.	PRBI-15	-

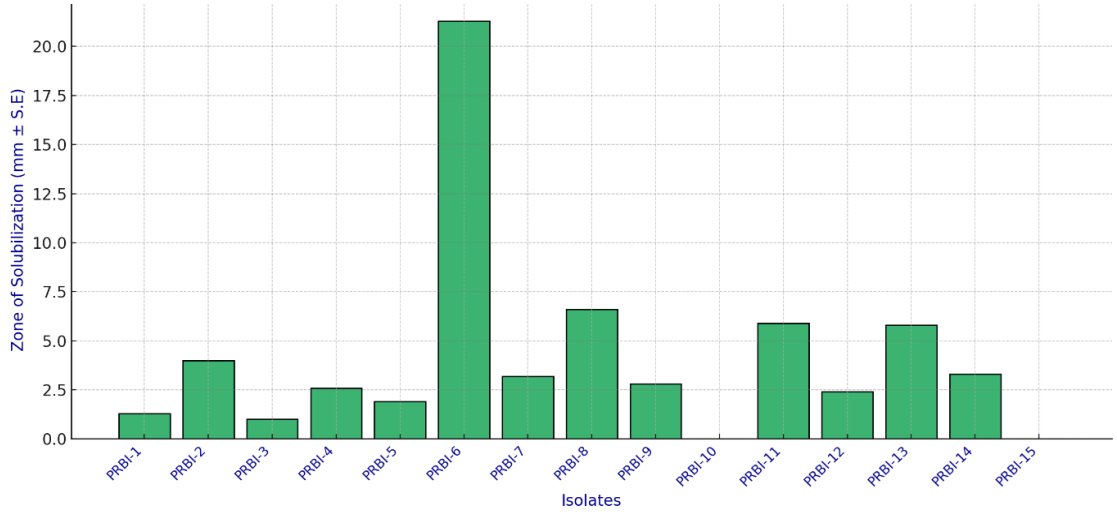

Fig 2: Graphical representation of phosphate solubilization ability of different isolates

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Table 3.b. Indole -3-acetic acid production

S.No.	Isolates	IAA production without tryptophan (A530))	IAA production with tryptophan(A530)
1.	PRBI-1	0.05	0.08
2.	PRBI-2	0.17	0.21
3.	PRBI-3	0.10	0.15
4.	PRBI-4	0.03	0.05
5.	PRBI-5	0.00	0.02
6.	PRBI-6	0.22	0.26
7.	PRBI-7	0.01	0.03
8.	PRBI-8	0.20	0.23
9.	PRBI-9	0.09	0.10
10.	PRBI-10	0.01	0.02
11.	PRBI-11	0.17	0.21
12.	PRBI-12	0.13	0.19
13.	PRBI-13	0.15	0.21
14.	PRBI-14	0.22	0.22
15.	PRBI-15	0.07	0.09

DISCUSSION

This study highlights the diversity and plant-growth-promoting potential of rhizobacteria isolated from the rhizosphere of Pisum sativum (pea) in two agroecological sites, Khirsu and Tungra. The higher bacterial density observed at Khirsu, compared to Tungra, likely reflects the influence of its dense forest ecosystem and fertile soil, emphasizing the role of site-specific factors in shaping microbial abundance and diversity.

Morphological and biochemical characterization revealed 15 isolates spanning genera such as *Bacillus*, *Pseudomonas*, *Rhizobium*, and *Azotobacter*, known for their roles in plant growth promotion. Among them, PRBI-6 emerged as a standout candidate, exhibiting superior phosphate-solubilizing activity (21.3 ± 0.03 mm) and high indole-3-acetic acid (IAA) production, particularly in the presence of tryptophan. The phosphate-solubilizing capability of PRBI-6 underscores its potential to mobilize fixed phosphorus in soil, a crucial trait for sustainable agriculture. Additionally, its ability to produce IAA, a vital phytohormone, enhances its role in promoting root elongation and overall plant development.

Other isolates, such as those from *Rhizobium* and *Azospirillum*, bring complementary benefits like nitrogen fixation, suggesting potential for synergistic effects when used in microbial consortia. Such formulations could address multiple nutrient limitations while reducing reliance on chemical fertilizers, offering a cost-effective and environmentally sustainable solution.

In conclusion, the rhizobacteria isolated in this study, particularly PRBI-6, demonstrate significant potential for bioinoculant development. Their ability to enhance nutrient availability and plant growth supports the transition toward sustainable agricultural practices. Future research should focus on field trials to validate these

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findings and optimize bioinoculant formulations for broader agricultural applications.

CONCLUSION

This study successfully demonstrated the potential of rhizobacteria isolated from the rhizosphere of Pisum sativum (pea) for promoting sustainable agriculture. The isolates exhibited diverse plant growth-promoting traits, with PRBI-6 showing exceptional phosphate-solubilizing activity and indole-3-acetic acid (IAA) production. These attributes highlight PRBI-6 as a promising candidate for bioinoculant development.

The diversity of the isolates, representing genera such as *Bacillus*, *Pseudomonas*, and *Rhizobium*, underscores the ecological significance of rhizosphere bacteria and their role in enhancing nutrient availability and plant growth. The findings provide a basis for developing microbial formulations tailored to specific crops and soil conditions, potentially reducing reliance on chemical fertilizers and supporting environmentally friendly agricultural practices. Future research should focus on conducting field trials to validate these laboratory findings and exploring the synergistic effects of microbial consortia. Such efforts are essential for translating these promising results into practical applications that address global food security challenges and promote sustainable farming systems.

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