Plant Growth Promoting Potential of Rhizosphere Bacteria Associated With Two Common Crops From The Kumaon Region of Uttarakhand

Pankaj Sanwal[1*] • Babita Rana[2] • Renu Chandola[2] • Nandita[2] • Bhawna Sanwal[1] • Gopal Krishna Joshi[2] • Tapan Kumar Nailwal[3*]

[1]*Bipin Tripathi Kumaon Institute of Technology, Dwarahat, Uttarakhand*
[2]*Department of Biotechnology, HNB Garhwal University, Srinagar, Garhwal, Uttarakhand*
[3]*Department of Biotechnology, Kumaun University, Nainital, Uttarakhand*

*Corresponding author Email Id: pansanwal@gmail.com

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Abstract: Plant growth promoting rhizosphere microorganisms offer unique advantages for the cultivation of crop plants. This study investigated the microbial diversity and functional capabilities of soil bacteria from rhizosphere samples associated with *gahat* and *mandua* crops. Colony-forming unit (CFU) counts on nutrient agar revealed significant variability among six soil samples, with bacterial populations ranging from 0.85×10^6 to 2.50×10^6 CFU/mL. Fifteen phosphate-solubilizing bacterial isolates were identified using modified Pikovskaya's agar, exhibiting solubilization efficiencies between 113% and 360%. Notably, isolate BPD22 from *mandua* rhizosphere soil demonstrated the highest efficiency at 360%. Gram staining indicated that 12 isolates were Gram-positive, while 3 were Gram-negative. Additionally, two isolates, BZM11 and BZM21, exhibited zinc solubilization efficiencies of 126% and 140%, respectively, on modified PVK media. These findings highlight the potential of indigenous soil bacteria in enhancing nutrient availability, suggesting their application as biofertilizers to promote sustainable agricultural practices.

Keywords: Rhizosphere • Microorganisms • *Gahat* • *Mandua* • Plant growth promotion • Crops

Introduction

Soil contains all possible microscopic forms of life depending on its texture, physical, chemical and biological properties. Root exudates are released in rhizosphere region, which serve as chemoattractants and nutrient sources favoring dense growth of microorganisms, particularly bacteria (Prashar *et al* 2014). The bacterial population of soil's rhizosphere region assists plant growth by converting nutrients into a form that is readily assimilated by them. In addition, they can also stimulate plant growth by producing phytohormones and suppressing the proliferation of pathogens (Hassan *et al* 2019). The plant growth promoting rhizobacteria (PGPR) thus provide a sustainable and attractive alternative to the chemical fertilizers that are harmful to the human beings as well as the entire eco-system (Saxena *et al* 2016). PGPRs have been developed, formulated and marketed as bio-inoculants for increasing the crop yield (Mishra & Arora 2016).

Phosphorus(P), an important plant growth limiting nutrient supports energy transfer, biosynthesis, photosynthesis, cell division, signal transduction and respiration. It helps in early root formation, growth, seed formation and improves food quality (Rawat *et al* 2018). Potassium (K) is one of the most important nutrient essential for plants regulatory mechanism, photosynthesis, protein synthesis, carbohydrate metabolism, tolerance to external stress etc. (Rawat *et al* 2023). Zinc (Zn) is also important micronutrient required for different metabolic processes of plants mainly respiration, photosynthesis, activation of enzymes etc. Zinc solubilizing bacteria (ZSB) have the potential of converting the unavailable form of zinc to the soluble form utilizable by plants (Masood *et al* 2022).

In Himalayan regions, farmers grow local crops well suited to the land and weather conditions, which keep the soil healthy and ensure enough food in fragile areas (Das *et al* 2018). Finger millet (*Eleusine coracana*), commonly known as *mandua* in the hilly regions of Uttarakhand, is a nutritionally rich millet which is widely cultivated in arid and semi-arid regions of Africa and Asia (Maharajan *et al* 2021). It is valued for its high content of dietary fiber, protein, antioxidants, calcium, iron, and various vitamins (Kumar *et al* 2024). The seeds of *mandua* contain 6-13% protein, 18% dietary fiber, 2.5–3.5% minerals, 0.34% calcium and 0.3-3% phenolics (Rawat *et al* 2020).

Horse gram (*Macrotyloma uniflorum Lam.*), locally known as *gahat* is a traditional pulse crop in the hilly region of the Himalayas. It is rich in proteins, iron, calcium, polyphenols, carbohydrates, essential amino acids, molybdenum, phosphorus and vitamins (Mehra & Upadhyaya 2013; Chahota *et al* 2020). *Gahat* is traditionally believed to help manage several ailments, including kidney and bladder stones, bronchitis, asthma, piles, leukoderma, and heart diseases.

Dwarahat block of Almora district is known for its reliance on traditional farming practices, particularly the cultivation of indigenous millets and pulses. Local farmers continue to grow *mandua*, barnyard millet (*Echinochloa frumentacea*, or *jhangora*), and foxtail millet (*Setaria italica*, or *kauni*). Additionally, pulses like *gahat*, black gram (*Vigna mungo*, *urad*), and green gram (*Vigna radiata*, moong) form an integral part of the agricultural system. Since all these are nutritionally rich crops and their rhizosphere soil may harbour a rich microbial diversity with abundance of plant growth promoting bacteria. Although these crops have been well characterized for their nutritional parameters (Dwivedi *et al* 2024) there is little information about the evaluation of plant growth promoting capabilities of their rhizosphere bacteria. The present study is therefore, carried out to investigate the plant growth promoting potential potential of *mandua* (*Eleusine coracana*) and *gahat* (*Macrotyloma uniflorum Lam.*) commonly cultivated in Dwarahat block of Almora district in the Kumaon region of Uttarakahnd, India.

Materials and Methods

Collection of soil samples

Soil samples were collected before sowing and after harvesting of two local crops *gahat* and *mandua* from agriculture fields located at Mirai and Dudholi villages of Dwarahat Block in district Almora. Uttarakhand, India (Table1, Fig 1).

Table 1 : Details of sampling sites and soil samples

S.N.	Sampling site	Nature of soil sample	pH	Temperature	Name of sample
1	**Mirai** (Lat:29.75, Lng:79.42, Altitude: 1426m)	Soil before sowing of crops	6.1	25°C	S1
		Rhizosphere soil after harvesting of *gahat*	5.9	22°C	M1
		Rhizosphere soil after harvesting of *mandua*	5.8	22°C	M2
2	**Dudholi** (Lat:29.80, Lng:79.47, Altitude: 1703m)	Soil before sowing of crops	7.2	24°C	S2
		Rhizosphere soil after harvesting of *gahat*	6.5	20°C	D1
		Rhizosphere soil after harvesting of *mandua*	6.6	20°C	D2

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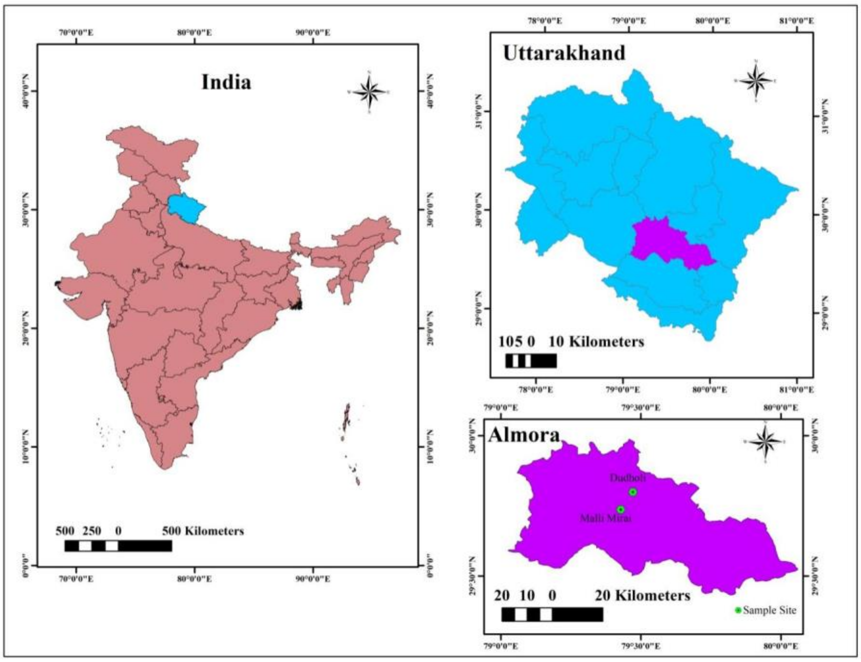

Fig. 1: Geographical location of sampling sites

Before sowing, the soil samples were collected from a depth of 0-20 cm with an auger and kept in sterile polyethylene bags. After harvesting intact roots along with the adhered soil were collected from similar depths and the rhizosphere soil was then carefully separated and transferred to sterile polyethylene bags. The polyethylene bags containing the soil samples were transported to the laboratory under cold conditions where the coarse material and pieces of roots were removed. The soil samples were homogenously mixed and passed through 2 mm sieve and stored at 4°C till further analysis.

Enumeration of bacterial population:

Standard plate count method was used to determine the total viable bacterial count. For this, the soil samples were first mixed in 10 mL of autoclaved distilled water and then serially diluted upto 10^6 fold dilutions. From each dilution 0.1 mL of suspension was aseptically transferred to nutrient agar plates and spread evenly. The plates were then incubated at 37 °C for 24 h under aerobic conditions. After incubation, bacterial colonies were counted using a colony counter and the colony forming units per milliliter (CFU/mL) were calculated using the formula (Ghorbani *et al* 2020):

$$\text{CFU/mL} = \frac{\text{Number of colonies on plate surface x Dilution factor}}{\text{Volume of bacterial suspension plated (mL)}}$$

Isolation and characterization of Phosphate solubilizing bacteria:

For isolation of phosphate solubilizing bacteria, modified Pikovskaya's (PVK) agar medium as described by Nautiyal (1999) was prepared and additionally supplemented with 0.5% Tri-calcium phosphate (TCP) as insoluble phosphate source, and 50 mg L^{-1} Cycloheximide as fungal growth inhibitor. The serially diluted soil samples as described earlier were plated on the modified PVK agar media and the plates were incubated at 30 ± 1°C for 7 days. The phosphate solubilization efficiency (Nguyen *et al*,1992) was calculated in the following manner.

Solubilization Efficiency = [Solubilization diameter (S) / Growth diameter (G)] x 100

Zn and K solubilization activity of soil bacteria

To assess the potassium solubilization ability of bacteria, Aleksandrov agar medium was prepared as described by Sarikhani *et al* (2018). Soil samples were serially diluted up to 10^{-6}, and 100 microliters of each dilution were inoculated onto the medium plates. The plates were then incubated at 30 ± 1°C for 7 days prior to observation. Similarly, to evaluate the zinc solubilization ability of bacteria, modified PVK medium supplemented with 0.1% insoluble zinc compound (Zinc carbonate) was prepared as described by Rawat *et al* (2023). Serially diluted soil samples were inoculated onto the medium, and the plates were incubated at 30 ± 1°C for 7 days before observation.

Results

Colony Forming Units

The collected soil samples were appropriately diluted and soil suspensions were spread onto nutrient agar plates to check the presence of culturable and viable bacterial populations in each of the soil samples tested (Fig. 2). The number of colony-forming units (CFUs) per milliliter varied significantly among all the six soil samples, ranging from 0.85 × 10^6 CFU/mL in the least populated sample to 2.50 × 10^6 CFU/mL in the most densely populated one, as summarized in Table 2.

Table 2 : Colony Forming Unit (CFU) count of soil bacteria

S.No.	Soil Sample	CFU/mL (10^6)
1	S1	1.05
2	S2	0.85
3	D1	2.50
4	D2	1.82
5	M1	1.10
6	M2	1.15

Fig. 2. Representative photograph of bacterial population obtained on Nutrient Agar medium

Isolation and characterization of Phosphate solubilizing microorganisms:

A modified Pikovskaya's (PVK) agar medium was used to evaluate the phosphate solubilizing ability of bacterial isolates obtained from soil samples collected after the harvesting of *gahat* and *mandua* crops. Based on the initial halo zone formation, which indicated phosphate solubilization efficiency, 15 bacterial isolates were selected for further analysis. The solubilization efficiency of bacterial isolates from *gahat* rhizosphere soil as presented in Table 3, ranged from 113% to 228%, whereas isolates from *mandua* rhizosphere soil showed a wider range of phosphate solubilization, from 123% to 360% (Fig. 3). Maximum phosphate solubilization efficiency was observed with the bacterial isolate BPD22 from the rhizosphere soil of *mandua* at Dudholi. Based on the Gram staining and microscopic observations, out of total of 15 bacterial isolates, 12 were gram positive while 03 (BPM23, BPD14 and BPD22) were gram negative (Table 3).

Table 3 : Phosphate Solubilization efficiency of bacterial isolates.

S.No.	Soil Sample	Name of Bacterial Isolate	Solubilization Efficiency (%)	Gram Staining
1	M1 (Collected from rhizosphere of *gahat*)	BPM11	200	+
2		BPM12	188	+
3		BPM13	228	+
4	M2 (Collected from rhizosphere of *mandua*)	BPM21	200	+
5		BPM22	125	+
6		BPM23	200	-
7		BPM24	156	+
8		BPM25	213	+
9	D1 (Collected from rhizosphere of *gahat*)	BPD11	113	+
10		BPD13	170	+
11		BPD14	227	-
12	D2 (Collected from rhizosphere of *mandua*)	BPD21	123	+
13		BPD22	360	-
14		BPD23	280	+
15		BPD24	200	+

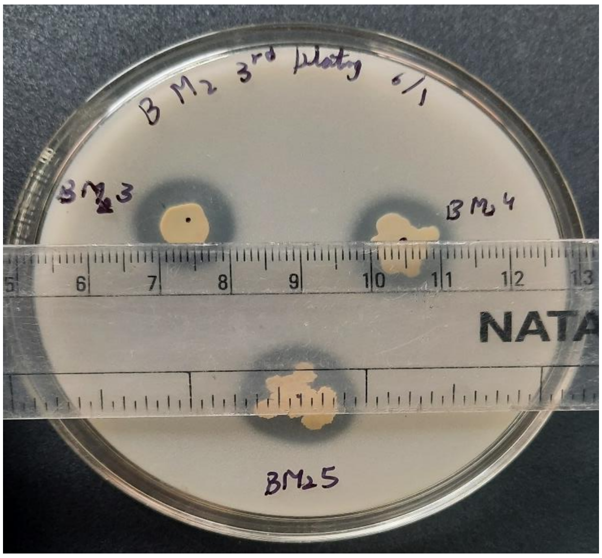

Fig. 3 : Representative plate showing phosphate solubilization by test bacteria BPM23, BPM24 and BPM25

Zn and K solubilization activity of soil bacteria

In the present finding, the bacterial isolates were able to grow on Aleksandrov agar medium, however, absence of halo zone around them indicate the absence of K solubilization activity in them. On the other hand, 2 bacterial isolates i.e. BZM1 and BZM2 (Table 4) grown on modified PVK medium exhibited clear zones around their colonies thereby indicating zinc solubilization activity (Fig. 4). The zinc solubilization efficiency for BZM11 and BZM21 was calculated as 126 % and 140 % for bacterial isolates BZM11 and BZM21, respectively.

Table 4 : Zinc Solubilization efficiency of bacterial isolates.

S.No.	Soil Sample	Name of Bacterial Isolate	Solubilization Efficiency (%)	Gram Staining
1	M1 (Collected from rhizosphere of *gahat*)	BZM11	126	+
2	M2 (Collected from rhizosphere of *mandua*)	BZM21	140	+

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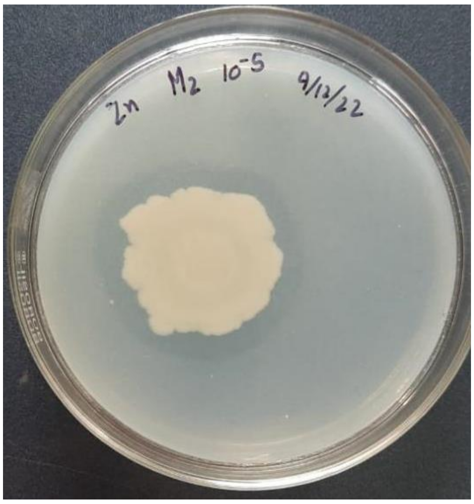

Fig. 4 : Representative Plate showing zinc solubilization by bacterial isolate BZM21

Discussion

The agriculture practices in the hill and mountain regions are often associated with issues like difficult terrain, inadequate infrastructure and low input applications (Patni *et al* 2018). The use of adequate microbial systems attributed with various plant growth promoting capabilities can spur the agriculture productivity in hilly regions. In the present investigation bacterial population associated with the rhizosphere soil of two common crops i.e. *mandua* and *gahat* were characterized for various plant growth promoting traits. While determining the total bacterial load as CFU, a variation was found in soil samples collected before sowing and after harvesting. After harvesting, the rhizosphere microbial community can be affected by various factors depending on the types of crops grown, fertilizers used and the crop residues (Dinca *et al* 2022). Packialakshmi *et al* (2021), have earlier reported 8.9 × 10^7 CFU/g from the rhizosphere soil of *mandua* which is much higher than our findings.

Our observations revealed a significant increase in colony-forming units (CFUs) in soil samples collected post-harvest compared to those taken before sowing the crops. For *gahat* (horse gram), this enhancement is likely due to the diverse root exudates and organic inputs characteristic of leguminous plants, which promote nutrient cycling and decomposition processes. Baheliya *et al* (2025) conducted a comprehensive study for evaluating the influence of leguminous crops on soil microbial populations, measured in colony-forming units per gram (CFU/g). Their findings highlighted the beneficial role of legumes in enhancing soil microbial load, thereby contributing to improved soil fertility and crop productivity. On the other hand, our cultivation of *mandua* (finger millet) also demonstrated elevated colony-forming unit (CFU) counts, which can be attributed to the decomposition of plant residues and root exudates serving as substrates for microbial proliferation. These findings align with the study by Niewiadomska *et al* (2020), who investigated the influence of cereal crops on soil microbial populations before sowing and after harvesting, emphasizing their vital role in nutrient cycling and overall soil fertility.

While detecting phosphate solubilizing abilities on modified PVK agar medium a variation in efficiencies ranging from 113 to 360% was recorded for 15 tested bacterial isolates. Previously, Karpagam and Nagalakshmi (2014) have documented phosphate solubilization efficiency in the range of 113 to 223% among bacterial isolates obtained from the rhizosphere soil of tomato plants. Similarly, Pandey and Putatunda (2018) observed phosphate solubilizing efficiency ranging from 103% to 283% in microbial isolates from rhizosphere environments. Chaudhary *et al* (2023) have demonstrated phosphate solubilization indices (SI) of 2.4 mm and 2.5 mm for two endophytic bacteria isolated from *mandua*. Notably, our current investigation recorded phosphate solubilization efficiencies ranging from 123% to 360%, which is slightly higher than these previous reports. These isolates could therefore hold the potential of being used as biofertilizers to enhance phosphorus availability in agricultural soils.

The test isolates used in the present study could not solubilize K but two of them exhibited Zn solubilization capabilities to the tune of 126 and 140 % using $ZnCO_3$ as substrate, respectively. Similar to our observation, zinc solubilization potential of 24 bacterial strains isolated from wheat and sugarcane rhizospheres was assessed by Kamran *et al* (2017) using $ZnCO_3$. Among these, six isolates exhibited clear solubilization zones, with *Rhizobium* sp. (LHRW1) demonstrating the largest zone diameter of 1.8 cm.

The findings of this study enhance our understanding of the plant growth promoting activities of rhizosphere bacteria associated with two commonly cultivated crops in the hilly regions of Uttarakhand. These bacteria have the potential to support sustainable agriculture by improving nutrient uptake and naturally enhancing soil fertility. This, in turn, reduces reliance on synthetic fertilizers, which are both expensive and environmentally harmful. The use of these beneficial microbes can lead to increased crop yields, improved produce quality, and more resilient farming systems. However, further research, including field studies, is necessary to validate their effectiveness as biofertilizers.

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