

Screening of Rhizosphere-Associated Bacteria from *Rhododendron arboreum* for Plant Growth-Promoting Characteristics in the Northwestern Himalayas

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Abstract— The rhizosphere of native Himalayan plants represents a potentially valuable reservoir of microorganisms adapted to distinctive edaphic and climatic conditions. The present study was undertaken to isolate culturable bacteria from the rhizosphere of *Rhododendron arboreum* and evaluate their plant growth-promoting rhizobacterial (PGPR) characteristics. Thirteen rhizospheric soil samples were collected from different locations in Kangra district, Himachal Pradesh, India. A total of 56 morphologically distinct bacterial isolates were recovered and screened for nitrogen-fixing ability, phosphate solubilization, siderophore production, hydrogen cyanide (HCN) production and indole-3-acetic acid (IAA) production. Among the isolates, 18 (32.14%) exhibited phosphate-solubilizing activity, five (8.93%) showed nitrogen-fixing ability, while seven isolates each (12.50%) were positive for siderophore, HCN and IAA production. Six promising phosphate-solubilizing and six IAA-producing isolates were subsequently selected for quantitative evaluation. Considerable isolate-dependent variation was observed in both traits. Phosphate solubilization ranged from 307.86 to 879.57 $\mu\text{g mL}^{-1}$ during different incubation periods, with isolate Dha1(1) recording the highest overall mean (798.99 $\mu\text{g mL}^{-1}$), followed by D3(10) (701.85 $\mu\text{g mL}^{-1}$). Quantitative IAA production ranged from 18.72 to 68.37 $\mu\text{g mL}^{-1}$, with Dha1(1) exhibiting the highest mean production (64.13 $\mu\text{g mL}^{-1}$). The occurrence of isolates possessing multiple plant growth-promoting characteristics demonstrates the functional diversity of culturable bacteria associated with the *R. arboreum* rhizosphere. The findings identify the northwestern Himalayan *R. arboreum* rhizosphere as a promising source of indigenous PGPR for further characterization and evaluation as microbial inoculants for sustainable agriculture.

Keywords— *Rhododendron arboreum*; PGPR; rhizosphere; phosphate solubilization; IAA; siderophore; nitrogen fixation; Himalayas.

I. INTRODUCTION

The rhizosphere represents one of the most biologically active regions of soil and supports complex interactions among plant roots, microorganisms and soil constituents. Root exudates containing sugars, amino acids, organic acids and other metabolites create a nutrient-rich microenvironment that selectively recruits diverse microbial populations. Among these microorganisms, plant growth-promoting rhizobacteria (PGPR) have received considerable attention because of their ability to influence plant nutrition, development and health through multiple direct and indirect mechanisms. PGPR may enhance plant growth by biological nitrogen fixation, solubilization of insoluble phosphates, mobilization of mineral nutrients and production of phytohormones such as indole-3-acetic acid (IAA). They may also influence plant-microbe interactions through siderophore production, competition for nutrients and production of metabolites associated with suppression of certain phytopathogens. These functional characteristics have generated considerable interest in PGPR as components of microbial inoculants for sustainable agriculture. Natural and relatively undisturbed ecosystems are particularly interesting sources for exploring

beneficial microorganisms. Plants growing under mountain environments are exposed to fluctuations in temperature, soil acidity, nutrient availability, moisture and other environmental constraints. Their associated rhizosphere microorganisms may therefore possess physiological and functional characteristics that enable survival under such conditions.

Rhododendron arboreum Sm. is an important evergreen tree of the Himalayan region. Besides its ecological significance, different parts of the plant have traditionally been utilized for food and ethnobotanical purposes. The species occurs naturally across broad elevational gradients and is exposed to diverse soil and climatic conditions. Nevertheless, compared with major agricultural crops, relatively limited attention has been given to the cultivable plant growth-promoting bacteria associated with its rhizosphere. Previous investigation of the rhizospheric bacterial community of *R. arboreum* subsp. *delavayi* along the eastern Himalayan slope demonstrated a pronounced rhizosphere effect and showed that bacterial community structure was influenced by soil pH, total nitrogen and organic matter. Actinobacteria, Acidobacteria and Proteobacteria constituted important components of the rhizosphere bacterial community. These observations indicate that *Rhododendron*-associated rhizospheres support distinctive microbial communities that merit functional investigation.

Importantly, plant growth-promoting bacteria have also previously been recovered directly from *R. arboreum*. A *Burkholderia* isolate obtained from the rhizosphere of *R. arboreum* in the Kumaun Himalaya exhibited phosphate-solubilizing, IAA-producing and siderophore-producing characteristics. Bacterial endophytes associated with *R. arboreum* have similarly demonstrated nitrogen-fixing, phosphate-solubilizing and phytohormone-producing abilities, further suggesting that this Himalayan plant represents a reservoir of functionally diverse bacteria.

However, information regarding culturable PGPR associated with the rhizosphere of *R. arboreum* in the northwestern Himalayas, particularly Himachal Pradesh, remains comparatively limited. Exploring indigenous bacteria from this ecological niche may identify strains with useful plant growth-promoting properties and provide candidates for subsequent development of locally adapted microbial inoculants.

Therefore, the present investigation was undertaken to isolate culturable bacteria from the rhizosphere of *R. arboreum* collected from Kangra district of Himachal Pradesh and screen them for important PGPR characteristics, including phosphate solubilization, nitrogen fixation, siderophore production, HCN production and IAA production. Promising isolates were further evaluated quantitatively for phosphate solubilization and IAA production.

II. MATERIALS AND METHODS

2.1 Collection of rhizospheric soil

Thirteen rhizospheric soil samples associated with healthy *R. arboreum* plants were collected from selected locations in Kangra district, Himachal Pradesh, India. Soil adhering closely to the root system was carefully collected in sterile sampling bags and transported to the laboratory for microbiological analysis. Samples were processed as soon as possible after collection to minimize changes in the culturable bacterial population.

2.2 Isolation of rhizosphere bacteria

Rhizosphere bacteria were isolated using the serial dilution technique. Ten grams of rhizospheric soil was suspended in 90 mL of sterile distilled water and thoroughly homogenized to obtain an initial 10^{-1} dilution. Serial ten-fold dilutions were subsequently prepared under aseptic conditions. Appropriate dilutions were inoculated onto suitable bacteriological media and incubated at approximately $28 \pm 2^\circ\text{C}$. Colonies differing in morphology, pigmentation, margin, elevation and other cultural characteristics were selected and repeatedly streaked to obtain pure cultures. A total of 56 bacterial isolates were recovered and maintained for subsequent screening of plant growth-promoting characteristics.

2.3 Screening for phosphate-solubilizing activity

Phosphate-solubilizing ability was evaluated qualitatively using Pikovskaya's agar containing tricalcium phosphate as an insoluble phosphorus source. Fresh bacterial cultures were spot-inoculated onto the medium and incubated at $28 \pm 2^\circ\text{C}$. Formation of a clear halo surrounding bacterial growth was considered indicative of phosphate solubilization. Of the 56 bacterial isolates screened, 18 isolates exhibited phosphate-solubilizing activity.

2.4 Screening for nitrogen-fixing ability

The isolates were evaluated for their ability to grow on nitrogen-free medium. Bacterial cultures were inoculated onto glucose nitrogen-free mineral medium and incubated under suitable conditions. Growth and the characteristic reaction on the nitrogen-

free medium were used as preliminary indicators of nitrogen-fixing potential. Five isolates showed positive responses during qualitative screening.

2.5 Siderophore production

Siderophore production was evaluated using Chrome Azurol S (CAS) agar according to the principle described by Schwyn and Neilands (1987). Fresh cultures were inoculated onto CAS medium and incubated at approximately 28°C. Development of an orange/yellowish zone or characteristic colour change around bacterial colonies was recorded as a positive reaction for siderophore production. Seven isolates were positive for siderophore production.

2.6 Hydrogen cyanide production

HCN production was determined by inoculating bacterial isolates on nutrient agar supplemented with glycine (4.4 g L⁻¹). Sterile filter paper impregnated with alkaline picrate solution was placed inside the lid of each Petri plate without direct contact with the medium. The plates were sealed and incubated at approximately 28°C for 4–5 days. Development of an orange to reddish-brown colour on the filter paper was considered indicative of HCN production. Seven isolates showed a positive reaction.

2.7 Screening for IAA production

Bacterial isolates were screened for their ability to produce IAA using Salkowski reagent. Cultures were grown in an appropriate broth medium supplemented with L-tryptophan as an IAA precursor. Following incubation, cultures were centrifuged and the cell-free supernatant was reacted with Salkowski reagent. Development of pink to reddish coloration was considered indicative of IAA production. Seven of the 56 isolates were positive for IAA production.

III. RESULTS AND DISCUSSION

3.1 Isolation and qualitative screening of rhizosphere bacteria

A total of 56 culturable bacterial isolates were recovered from 13 rhizospheric soil samples collected from *R. arboreum*. Considerable variation was observed among isolates in their plant growth-promoting characteristics. Phosphate solubilization was the most frequently detected characteristic, occurring in 32.14% of the isolates. In contrast, nitrogen-fixing ability was detected in 8.93%, while siderophore, HCN and IAA production were each detected in 12.50% of the bacterial collection. The predominance of phosphate-solubilizing bacteria is of particular interest because phosphorus frequently occurs in soils in poorly available mineral forms. Phosphate-solubilizing microorganisms can increase phosphorus availability through several mechanisms, particularly the secretion of organic acids, proton release and chelation of mineral-associated cations. Consequently, phosphate-solubilizing bacteria are considered important components of microbial approaches to plant nutrient management (Table 1).

TABLE 1

QUALITATIVE SCREENING OF PLANT GROWTH-PROMOTING CHARACTERISTICS AMONG 56 BACTERIAL ISOLATES FROM THE *R. ARBOREUM* RHIZOSPHERE

PGPR characteristic	Positive isolates (n)	Positive isolates (%)
Phosphate solubilization	18	32.14
Nitrogen-fixing ability	5	8.93
Siderophore production	7	12.5
HCN production	7	12.5
IAA production	7	12.5

The recovery of bacteria with diverse functional traits also agrees with previous investigations of *Rhododendron*-associated microorganisms. Nailwal et al. reported a *Burkholderia* strain from *R. arboreum* rhizosphere in the Kumaun Himalaya exhibiting phosphate solubilization, IAA production and siderophore production. Furthermore, studies of *Rhododendron*

bacterial endophytes have demonstrated nitrogen-fixing and phosphate-solubilizing capabilities. The occurrence of multiple PGPR traits within the present culture collection therefore indicates that the *R. arboreum* rhizosphere represents a potentially valuable source of functionally diverse bacteria.

3.2 Quantitative evaluation of phosphate solubilization

Six promising phosphate-solubilizing isolates were selected for quantitative evaluation. Considerable variation was observed among isolates and incubation periods.

TABLE 2
QUANTITATIVE PHOSPHATE SOLUBILIZATION BY SELECTED RHIZOSPHERE ISOLATES ($\mu\text{g P mL}^{-1}$)

Isolate	Day 8	Day 14	Day 21	Day 29	Day 51	Mean
Dha1(1)	728.28	752.17	879.57	865.23	769.69	798.99
Dha1(4)	369.97	395.45	382.71	352.45	718.73	443.86
D3(10)	543.55	594.51	833.38	806.31	731.47	701.85
D6(4)	336.53	354.05	440.04	459.15	670.95	452.14
A4S2	307.86	349.27	354.05	307.86	559.48	375.7
GTW32/28	362.01	425.71	448	373.16	462.33	414.24

Phosphate solubilization ranged from 307.86 to 879.57 $\mu\text{g mL}^{-1}$ across isolates and incubation periods. Dha1(1) exhibited the highest overall phosphate-solubilizing ability, with a mean of 798.99 $\mu\text{g mL}^{-1}$, and attained a maximum of 879.57 $\mu\text{g mL}^{-1}$ on day 21. D3(10) was the second most effective isolate, recording a mean phosphate solubilization of 701.85 $\mu\text{g mL}^{-1}$, with a maximum of 833.38 $\mu\text{g mL}^{-1}$ on day 21. The other isolates exhibited comparatively lower mean phosphate solubilization: D6(4), 452.14 $\mu\text{g mL}^{-1}$; Dha1(4), 443.86 $\mu\text{g mL}^{-1}$; GTW32/28, 414.24 $\mu\text{g mL}^{-1}$; and A4S2, 375.70 $\mu\text{g mL}^{-1}$. The variation demonstrates that phosphate-solubilizing ability was strongly isolate dependent. The temporal pattern was also different among isolates. Dha1(1) and D3(10) attained their maximum values relatively earlier, whereas several other isolates exhibited their highest measured values at day 51.

Such variation can reflect differences among bacterial strains in organic-acid production, proton extrusion, chelation and metabolism of the available carbon source. However, these mechanisms were not directly measured in the present experiment and therefore remain possible explanations rather than demonstrated mechanisms.

3.3 Quantitative production of indole-3-acetic acid

Six promising isolates were also evaluated quantitatively for IAA production.

TABLE 3
IAA PRODUCTION BY SELECTED *R. ARBOREUM* RHIZOSPHERE ISOLATES ($\mu\text{g mL}^{-1}$)

Isolate	Day 3	Day 5	Day 8	Day 14	Mean
Dha1(1)	68.37	66.51	59.07	62.56	64.13
Dha1(4)	20.93	26.27	22.91	35.81	26.48
D3(10)	49.76	44.07	18.72	56.74	42.32
D6(4)	46.86	46.39	48.6	55.69	49.39
A4S2	34.41	31.86	24.65	33.02	30.99
GTW32/28	22.9	30.81	28.14	41.74	30.9

IAA production varied from 18.72 to 68.37 $\mu\text{g mL}^{-1}$, demonstrating substantial variation among the isolates. Dha1(1) was the most efficient IAA producer, with the highest mean production of 64.13 $\mu\text{g mL}^{-1}$ and a maximum value of 68.37 $\mu\text{g mL}^{-1}$ on day 3. D6(4) was the second most effective isolate, with an average of approximately 49.39 $\mu\text{g mL}^{-1}$, followed by D3(10) with 42.32 $\mu\text{g mL}^{-1}$. IAA is an important auxin involved in plant root development. Bacterial IAA can influence primary root elongation, lateral-root initiation and root-hair formation, potentially increasing the root surface available for water and nutrient acquisition. Nevertheless, the magnitude of IAA production in culture should not by itself be interpreted as evidence of plant growth promotion because plant response depends on bacterial colonization, IAA concentration, plant genotype and environmental conditions. The marked differences observed among isolates are consistent with the known strain-dependent nature of bacterial IAA biosynthesis.

3.4 Overall PGPR potential of the *R. arboreum* rhizosphere

The combined qualitative and quantitative results demonstrate substantial functional heterogeneity among culturable bacteria recovered from the *R. arboreum* rhizosphere. Among the 56 isolates, phosphate solubilization was the most common PGPR characteristic. The simultaneous occurrence of nitrogen-fixing, siderophore-producing, HCN-producing and IAA-producing bacteria suggests that the rhizosphere supports organisms potentially capable of contributing to plant nutrition, root development and rhizosphere interactions through different mechanisms. Particularly noteworthy was Dha1(1), which exhibited the highest mean values for both quantitative phosphate solubilization and IAA production. D3(10) also demonstrated high phosphate-solubilizing ability, while D6(4) showed comparatively strong IAA production. The present findings complement ecological evidence demonstrating that the *R. arboreum* rhizosphere harbours a distinct bacterial community. Debnath et al. found significant differences between bulk-soil and *R. arboreum* rhizosphere bacterial communities and demonstrated that soil pH, nitrogen and organic matter influenced community structure along Himalayan altitudinal gradients. Taken together, these observations support further exploration of native Himalayan plants as reservoirs of beneficial microorganisms. Indigenous isolates may be especially interesting for microbial inoculant development because they originate from local ecological conditions. However, in vitro PGPR traits alone are insufficient to establish biofertilizer efficacy. Promising isolates require taxonomic identification, biosafety assessment, rhizosphere-colonization studies and controlled plant experiments followed by replicated field validation.

IV. CONCLUSION

The present investigation demonstrated that the rhizosphere of *Rhododendron arboreum* in the northwestern Himalayas harbours a diverse population of culturable bacteria possessing important plant growth-promoting characteristics. Of 56 isolates obtained from 13 rhizospheric soil samples, 18 (32.14%) were phosphate solubilizers, five (8.93%) exhibited nitrogen-fixing ability, and seven isolates each (12.50%) were positive for siderophore, HCN and IAA production. Quantitative evaluation further revealed pronounced strain-dependent differences. Dha1(1) exhibited the highest mean phosphate solubilization (798.99 $\mu\text{g mL}^{-1}$) and IAA production (64.13 $\mu\text{g mL}^{-1}$), identifying it as a particularly promising candidate for further investigation. The study therefore establishes *R. arboreum* rhizosphere as a valuable ecological niche for exploring indigenous PGPR in the Himalayan region. Promising isolates identified through the present screening should subsequently be subjected to molecular identification, physiological characterization, biosafety assessment and plant-based validation before their potential use as microbial inoculants can be established.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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