

Characterization of Rhizobacteria from Agricultural and Forest Ecosystems for Plant Growth-Promoting Potential

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Abstract

Plant growth-promoting rhizobacteria (PGPR) enhance plant development through multiple direct and indirect mechanisms, while their diversity and functional potential are strongly governed by soil type and land-use patterns. In the present study, rhizospheric soils were collected from an agricultural field cultivated with *Arachis hypogaea* L. and a forest site dominated by *Ficus benghalensis* L. at Bhintkhurd, Surat. A total of 40 bacterial isolates were obtained and screened for key PGPR traits, including phosphate solubilization, ammonia production, indole-3-acetic acid production, hydrogen cyanide production, and siderophore production. Among these, 11 isolates originated from agricultural soil and 29 from forest soil. Forest-derived isolates exhibited comparatively higher diversity and functional expression across most PGPR traits. Identification using MALDI-TOF mass spectrometry revealed the presence of *Bacillus subtilis*, *Bacillus cereus*, *Bacillus mojavensis*, and *Solibacillus silvestris*. Notably, isolates A10 and F11 demonstrated multifunctionality by expressing four PGPR traits simultaneously. The findings indicate that forest soils serve as a richer reservoir of diverse and functionally competent rhizobacteria. Multifunctional isolates identified in this study hold significant potential for application as biofertilizers in sustainable agricultural systems.

Keywords: PGPR, Rhizobacteria, Phosphate solubilization, Indole-3-acetic acid (IAA), Siderophore production, Biofertilizer, Forest soil, Agricultural soil, MALDI-TOF mass spectrometry, Sustainable agriculture.

Introduction

The rhizosphere is a dynamic zone of soil directly influenced by plant roots and represents one of the most biologically active interfaces in terrestrial ecosystems. Root exudates released into the rhizosphere support diverse microbial communities that influence plant growth, nutrient acquisition, and stress tolerance (Philippot et al., 2013; Berg & Smalla, 2009). The composition and activity of rhizospheric microorganisms are critical determinants of soil fertility and ecosystem functioning.

Among rhizosphere-associated microorganisms, plant growth-promoting rhizobacteria (PGPR) have gained considerable attention due to their ability to enhance plant growth and productivity while reducing reliance on chemical fertilizers and pesticides (Lugtenberg & Kamilova, 2009; Backer et al., 2018). These beneficial bacteria contribute to sustainable agriculture through a variety of mechanisms that improve

plant nutrition and health (Basu et al., 2021; Fasusi et al., 2021).

PGPR stimulate plant growth through both direct and indirect mechanisms. Direct mechanisms include biological nitrogen fixation, phosphate solubilization, siderophore production, and phytohormone synthesis, particularly indole-3-acetic acid (IAA), which enhances root growth and nutrient uptake (Olanrewaju et al., 2017; Saeed et al., 2021). Indirectly, PGPR suppress plant pathogens through the production of antimicrobial compounds, competition for ecological niches, and induction of systemic resistance in host plants (Backer et al., 2018; Aloo et al., 2022).

Soil microbial diversity and functionality are strongly influenced by land use and vegetation type. Agricultural soils are frequently subjected to tillage, fertilizer application, pesticide use, and monoculture practices, all of which can alter microbial community structure (Hartmann et al., 2015). In contrast, forest soils generally remain less disturbed and receive continuous inputs of organic matter from litter deposition and root turnover, creating habitats that support greater microbial abundance and diversity (Berg & Smalla, 2009; Compant et al., 2019). Consequently, forest ecosystems often serve as reservoirs of diverse microorganisms with beneficial plant-growth-promoting attributes.

The characterization of indigenous PGPR populations from different ecosystems is important for identifying efficient microbial strains suitable for biofertilizer development. Recent advances in microbial identification methods, particularly matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF MS), have facilitated rapid and reliable identification of environmental bacterial isolates (Singhal et al., 2015). The technology offers a cost-effective and reproducible alternative for microbial taxonomy and biodiversity studies.

Despite the ecological significance of South Gujarat, limited information is available regarding the diversity and plant growth-promoting potential of rhizobacteria associated with agricultural and forest ecosystems in this region. Therefore, the present study aimed to isolate and characterize rhizobacterial populations from the rhizosphere of *Arachis hypogaea* L. cultivated in agricultural soil and *Ficus benghalensis* L. growing in forest soil at Bhintkhurd, Surat. The isolates were evaluated for key PGPR traits including phosphate solubilization, ammonia production, indole-3-acetic acid production, hydrogen cyanide production, and siderophore production. Selected isolates were further identified using MALDI-TOF MS to assess their taxonomic diversity and potential application as biofertilizers for sustainable agriculture.

2 MATERIALS AND METHODOLOGY

2.1 Study Sites and Sample Collection

Rhizospheric soil was collected from two sites at Bhintkhurd, Surat: (i) an agricultural groundnut field (*Arachis hypogaea* L.) and (ii) a forest site dominated by the Indian banyan (*Ficus benghalensis* L.). Plants were carefully uprooted with sterile tools during the active growth stage, and tightly adhering rhizospheric soil was collected into sterile containers and transported to the laboratory.

2.2 Bacterial Isolation and Purification

Serial dilutions (10^{-1} to 10^{-6}) were prepared from 1 g soil in sterile distilled water. Aliquots (0.1 mL) were plated onto Nutrient Agar by spread-plate and pour-plate methods under laminar air flow at 35 $\pm$ 2 degrees C for 24-48 h. Morphologically distinct colonies were purified by repeated four-quadrant streaking until pure cultures were obtained.

2.3 PGPR Activity Assays

- **Phosphate Solubilization (PS):** Isolates were spot-inoculated on Pikovskaya's agar plates and incuba-

ted at 30°C for 4–5 days. Formation of a clear halo zone around colonies was considered positive for phosphate solubilization (Alori et al., 2021).

- **Indole-3-Acetic Acid (IAA) Production:** Bacterial cultures were grown in tryptophan-supplemented broth for 7 days. After incubation, Salkowski reagent was added, and development of a pink colour indicated IAA production (Patten & Glick, 2020).
- **Ammonia Production (AP):** Isolates were inoculated into peptone water and incubated for 48 h. Following incubation, Nessler's reagent was added, and the appearance of yellow to brown colour was recorded as positive for ammonia production (Raza et al., 2021).
- **Hydrogen Cyanide (HCN) Production:** Isolates were grown on glycine-amended agar plates with picric acid-soaked filter paper placed in the lid. A change in filter paper colour from yellow to brown after 4 days of incubation indicated HCN production (Backer et al., 2018).
- **Siderophore Production (SP):** Siderophore production was assessed using Chrome Azurol S (CAS) agar medium. Development of an orange halo around bacterial colonies was considered positive for siderophore production (Saha et al., 2022).

2.4 MALDI-TOF Identification

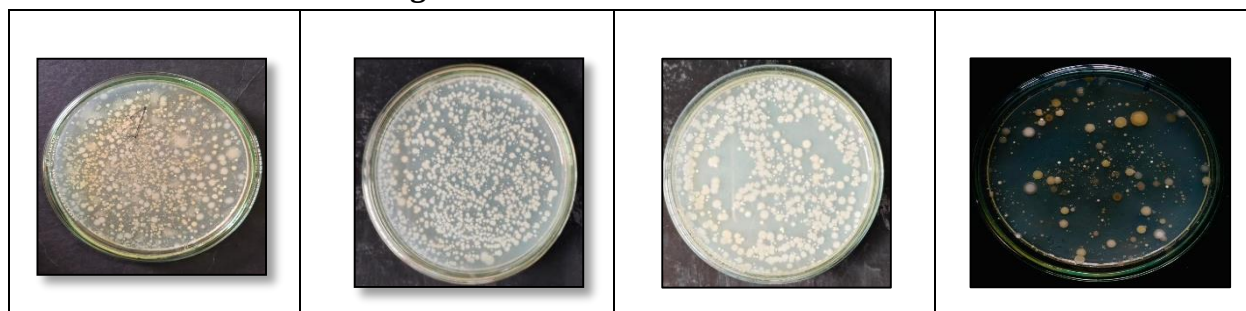
Selected bacterial isolates were subjected to identification using MALDI-TOF mass spectrometry. Colonies were spotted onto a MALDI target plate, overlaid with α -cyano-4-hydroxycinnamic acid (HCCA) matrix, and analysed using the MALDI-TOF system. Spectral profiles were compared with the Bruker Biotyper database. Identification scores ≥ 2.000 were considered reliable for species-level identification, scores between 1.700–1.999 indicated probable genus-level identification, and scores < 1.700 were considered unreliable (Singhal et al., 2021; Patel, 2022).

3 RESULTS

Isolation of Bacterial Colonies (After Purification)

A total of 40 morphologically distinct bacterial isolates were recovered through serial dilution and culture-based isolation techniques. Of these, 11 isolates originated from agricultural soil, whereas 29 isolates were obtained from forest soil. The substantially higher number of isolates recovered from forest soil suggests a greater bacterial abundance and diversity in the undisturbed forest ecosystem compared to the agricultural soil.

Fig 1: Colonies After Serial Dilution



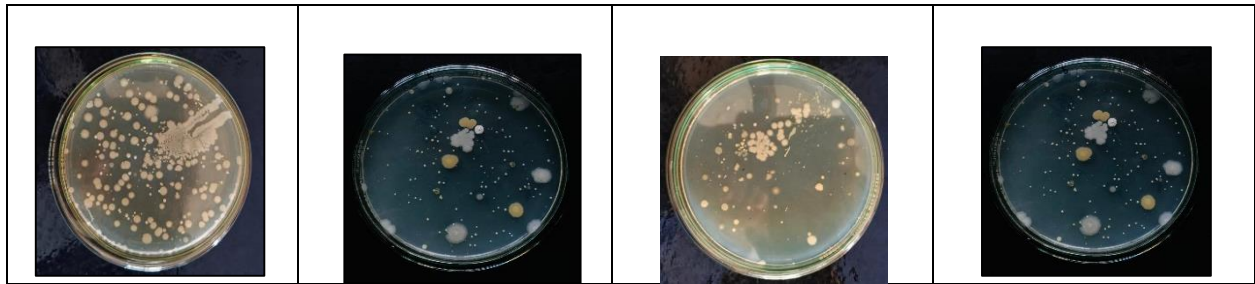


Fig. 2: Purified bacterial Colonies isolates

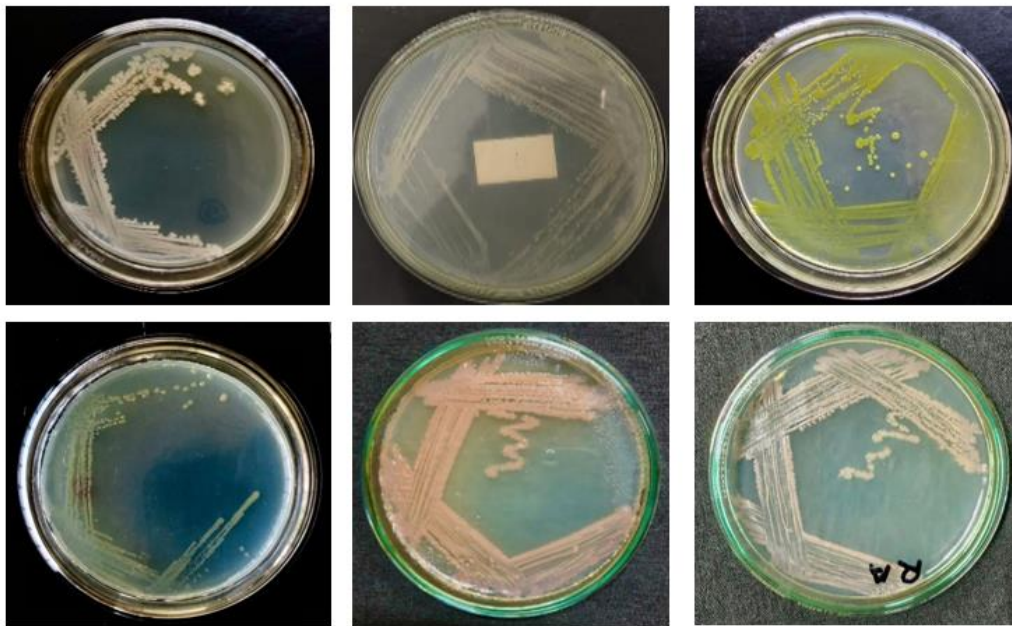
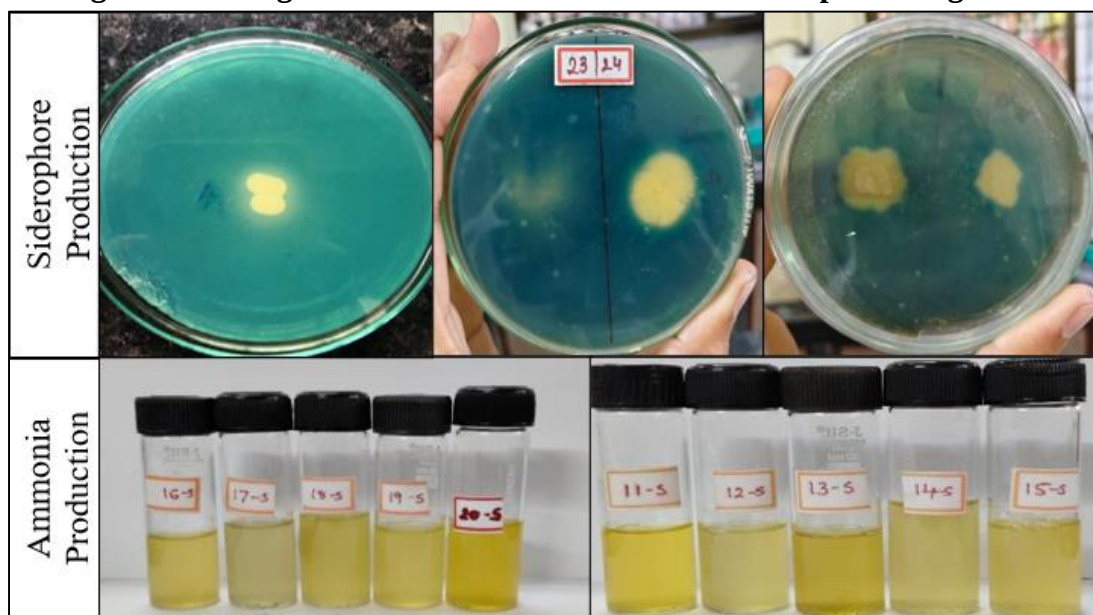


Fig.3: Screening of Bacterial isolates for Plant Growth promoting traits



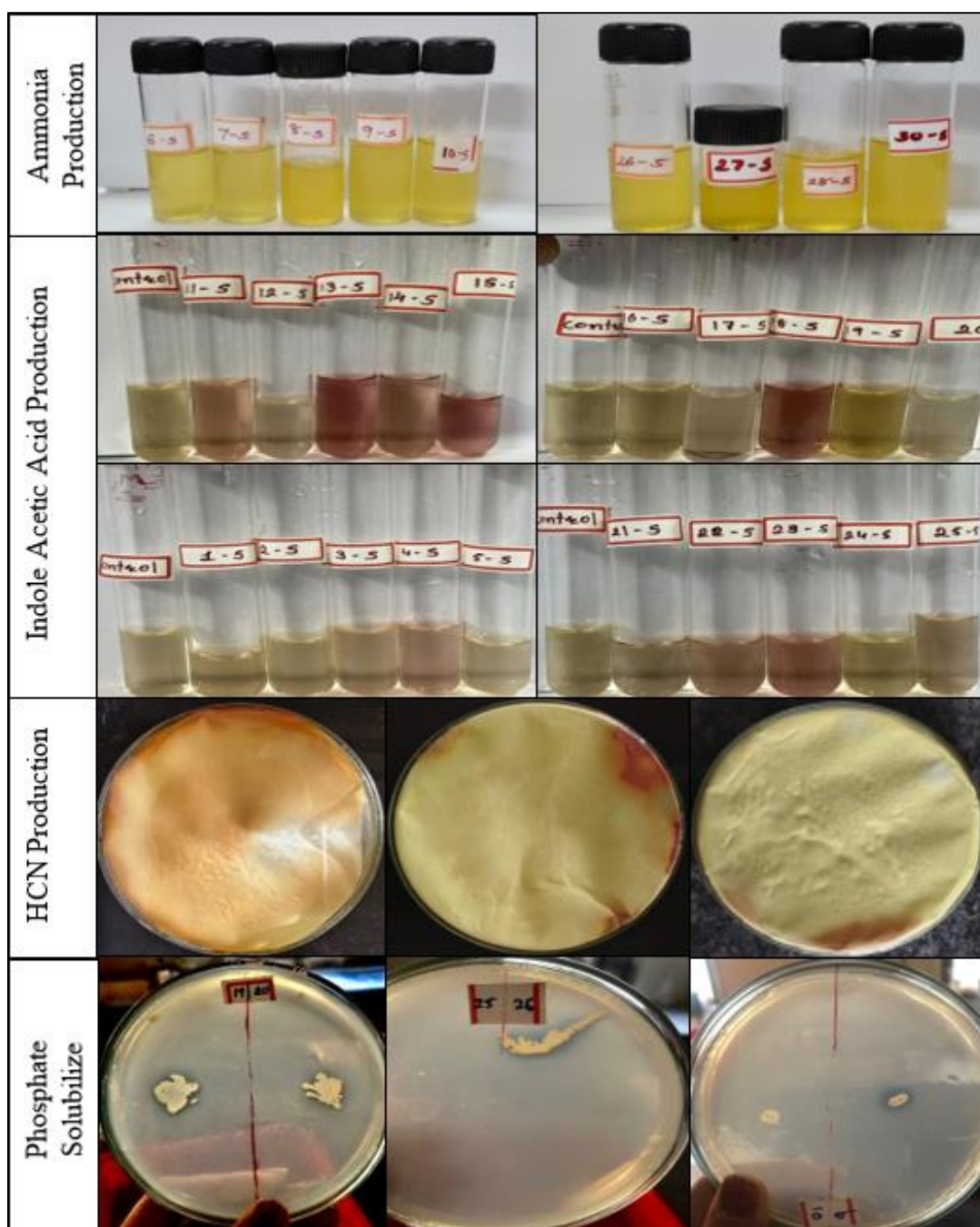


Table 1: PGPR Trait Profiling of bacterial isolates

F19	Yes	No	No	No	No
F20	Yes	Yes	No	Yes	No
F21	No	Yes	No	No	No
F22	No	No	Yes	No	No
F23	No	No	Yes	No	Yes
F24	No	No	No	No	Yes
F25	No	Yes	No	No	No
F26	No	No	No	No	No

PGPR Traits Table for All Colonies					
Bacteria colony	PS	AP	IAA	HCN	SP
A1	Yes	No	No	Yes	No
A2	Yes	No	No	No	No
A3	No	Yes	No	No	No
A4	No	No	No	Yes	No
A5	Yes	No	No	No	Yes
A6	Yes	No	No	Yes	No
A7	Yes	No	No	Yes	No
A8	Yes	No	No	Yes	No
A9	No	Yes	Yes	No	Yes
A10	Yes	Yes	Yes	No	Yes
A11	No	No	No	No	No
F1	No	No	No	No	No
F2	No	No	No	Yes	No
F3	No	Yes	Yes	No	No
F4	No	No	Yes	No	No
F5	Yes	Yes	No	No	No
F6	No	No	No	No	No
F7	Yes	No	No	Yes	No
F8	Yes	Yes	Yes	No	No
F9	Yes	No	No	No	Yes
F10	No	No	No	No	No
F11	Yes	Yes	Yes	No	Yes
F12	No	No	No	No	No
F13	No	Yes	Yes	No	Yes
F14	No	No	Yes	No	Yes
F15	Yes	No	Yes	No	Yes
F16	No	No	No	No	Yes
F17	Yes	No	Yes	No	No
F18	Yes	No	No	No	No
F27	No	Yes	No	No	No
F28	No	No	No	No	No
F29	No	No	No	No	No

Negative Test

Positive Test

Table 2. Summarises the PGPR trait frequencies in agricultural vs. forest soil isolates.

PGPR Trait	Agricultural (n=11)	Forest (n=29)	Total Positive
Phosphate Solubilization	7 (63.6%)	10 (34.5%)	17 (42.5%)
Ammonia Production	3 (27.3%)	9 (31.0%)	12 (30.0%)
IAA Production	2 (18.2%)	10 (34.5%)	12 (30.0%)
HCN Production	5 (45.5%)	3 (10.3%)	8 (20.0%)
Siderophore Production	3 (27.3%)	8 (27.6%)	11 (27.5%)

Isolates A10 and F11 were the most functionally versatile, testing positive for four traits each (PS, AP, IAA, SP). Isolates A9, F8, F13, F15, and F20 expressed three traits each. Agricultural isolates showed higher PS (63.6%) and HCN (45.5%) frequencies, while forest isolates displayed greater IAA (34.5%) and AP (31.0%) prevalence.

5.3 MALDI-TOF Identification Results

Of 22 isolates subjected to MALDI-TOF, nine were successfully identified. Table 2 presents the results.

Table 3. MALDI-TOF identification of selected PGPR-positive isolates.

Sample ID	Isolate	Organism	Score	Confidence
M2	A2	<i>Bacillus cereus</i>	1.95	Low (+)
M3	A3	<i>Bacillus cereus</i>	1.70	Low (+)
M7	A7	<i>Bacillus subtilis</i>	2.05	High (+++)
M8	A8	<i>Bacillus cereus</i>	1.85	Low (+)
M11	F3	<i>Bacillus mojavensis</i>	1.75	Low (+)
M12	F4	<i>Bacillus cereus</i>	1.81	Low (+)
M13	F5	<i>Solibacillus silvestris</i>	1.74	Low (+)
M21	F13	<i>Bacillus cereus</i>	1.87	Low (+)
M22	F14	<i>Bacillus cereus</i>	2.17	High (+++)

Bacillus cereus was the most frequently identified species (5 isolates). High-confidence identifications included *B. subtilis* (A7, score 2.05) and *B. cereus* (F14, score 2.17). A forest-specific species, *Solibacillus silvestris*, was identified from isolate F5. Twelve isolates remained unidentified, suggesting novel or understudied taxa.

DISCUSSION

The recovery of a substantially greater number of bacterial isolates from forest soil compared to agricultural soil suggests that forest ecosystems provide more favourable conditions for sustaining diverse microbial populations. Forest soils are characterized by continuous organic matter deposition, reduced anthropogenic disturbance, and greater habitat heterogeneity, all of which contribute to enhanced microbial richness and ecological stability (Jangid et al., 2011; Trivedi et al., 2020). In contrast, intensive agricultural practices may alter soil physicochemical properties and reduce microbial diversity through repeated disturbances and chemical inputs (Banerjee et al., 2019).

The higher frequency of phosphate-solubilizing isolates observed in agricultural soil may indicate adaptive responses of microbial communities to nutrient-limited conditions. Phosphate-solubilizing microorganisms play an important role in converting insoluble phosphorus into forms available for plant

uptake, thereby improving nutrient-use efficiency in agricultural systems (Sharma et al., 2013; Alori et al., 2017). Such microorganisms are often favoured in cultivated soils where phosphorus availability is a major constraint to crop productivity.

Forest-derived isolates exhibited comparatively greater IAA-producing ability, suggesting stronger plant–microbe interactions within natural ecosystems. Bacterial production of auxins has been reported to enhance root development, nutrient acquisition, and rhizosphere colonization, providing ecological advantages in competitive environments (Spaepen et al., 2007; Fukami et al., 2018). The prevalence of IAA-producing bacteria in forest soils may therefore reflect long-term adaptation to complex plant-associated niches.

The identification of multifunctional isolates possessing multiple PGPR traits is of particular significance. Previous studies have demonstrated that bacterial strains expressing more than one growth-promoting mechanism generally perform better under field conditions because they simultaneously improve nutrient availability, stimulate root growth, and enhance stress tolerance (Chaudhary et al., 2021; Akinrinlola et al., 2018). Therefore, isolates A10 and F11 may represent promising candidates for future biofertilizer development and greenhouse evaluation.

MALDI-TOF analysis revealed that *Bacillus* species constituted the dominant identifiable bacterial group. Members of the genus *Bacillus* are widely recognized for their ability to produce phytohormones, solubilize phosphate, synthesize siderophores, and suppress plant pathogens through various antagonistic mechanisms (Radhakrishnan et al., 2017; Saxena et al., 2020). Their capacity to form resistant endospores further enhances their survival under adverse environmental conditions and contributes to their widespread use in commercial microbial formulations.

The detection of *Solibacillus silvestris* among forest isolates highlights the taxonomic diversity present in forest rhizospheres. Although information regarding its plant growth-promoting potential remains limited, previous reports have documented its occurrence in forest and environmentally stressed habitats, suggesting ecological adaptability and potential functional significance (Krishnamurthi et al., 2009).

A considerable proportion of isolates remained unidentified through MALDI-TOF analysis. Similar limitations have been reported in environmental microbiology studies, where reference databases are often biased toward clinically important microorganisms and contain limited representation of environmental taxa (Rahi et al., 2016; Schlager et al., 2017). Consequently, complementary molecular approaches such as 16S rRNA gene sequencing and whole-genome analysis would improve taxonomic resolution and facilitate identification of potentially novel PGPR strains.

Overall, the results indicate that both agricultural and forest soils harbour beneficial rhizobacteria with significant plant growth-promoting potential. However, the greater diversity and broader functional characteristics observed in forest-derived isolates emphasize the value of natural ecosystems as reservoirs of microbial resources for sustainable agricultural applications.

CONCLUSION

Rhizobacterial isolates from both agricultural and forest soils at Bhintkhurd, Surat, harbor significant plant growth-promoting potential. Forest soil supported nearly three times more isolates and greater functional diversity across PGPR traits compared to agricultural soil. Multifunctional isolates A10 and F11, positive for PS, AP, IAA, and SP, are strong candidates as broad-spectrum biofertilizers. The dominance of *Bacillus* spp., including a high-confidence *Bacillus subtilis* identification, and the novel recovery of *Solibacillus silvestris* from forest soil, enrich our understanding of PGPR diversity in South Gujarat.

Future research should validate growth-promoting efficacy through greenhouse and field trials and develop stable formulations for sustainable agricultural application.

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