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EXPLORING THE DIVERSITY AND PLANT GROWTH-PROMOTING POTENTIAL OF ENDOPHYTIC BACTERIA IN CACTUS SPECIES: A COMPARATIVE STUDY

Original Research

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ABSTRACT

Background: Cacti thrive in arid and nutrient-deficient environments largely due to their association with endophytic bacteria, which enhance plant resilience by promoting growth and mitigating stress. These bacteria are known to facilitate nutrient uptake, synthesize phytohormones, and confer resistance against abiotic stressors. Despite their ecological significance, endophytic communities in cacti remain underexplored compared to agricultural crops. Understanding their diversity and functional roles is essential for advancing sustainable agriculture in stress-prone environments.

Objective: To evaluate the diversity and plant growth-promoting (PGP) characteristics of endophytic bacteria isolated from various cactus species and to identify strains with potential applications in arid-land agriculture.

Methods: Plant samples from diverse cactus species were collected from the Hazara Division, Pakistan. Following surface sterilization, endophytic bacteria were isolated using culture-dependent methods. Morphological, biochemical, and molecular characterizations, including 16S rRNA sequencing, were performed. The isolates were tested *in vitro* for nitrogen fixation, phosphate solubilization, indole-3-acetic acid (IAA) production, and tolerance to abiotic stressors such as high salinity and temperature.

Results: A total of 120 bacterial isolates were recovered, with 42% belonging to Proteobacteria, 31% to Firmicutes, and 18% to Actinobacteria. Approximately 68% exhibited nitrogen-fixing activity, and 59% demonstrated phosphate-solubilizing potential. IAA production ranged from 3–58 µg/mL, with highest levels observed in *Bacillus amyloliquefaciens*. Around 43% tolerated 10% NaCl, and 28% remained viable after 48 hours at 45°C. Notably, 34% of strains exhibited three or more PGP traits.

Conclusion: Cactus-associated endophytic bacteria exhibit high diversity and multifunctional PGP capabilities, making them promising candidates for bioinoculants in arid and semi-arid agriculture. Their ability to enhance plant fitness under environmental stress positions them as valuable tools for sustainable farming in water-limited regions.

Keywords: Actinobacteria, Cactus, Indoleacetic acids, Nitrogen fixation, Phosphate solubilizing bacteria, Plant growth-promoting rhizobacteria, Salt tolerance.

INTRODUCTION

Endophytic bacteria, microorganisms that reside within plant tissues without eliciting harm, have emerged as crucial players in enhancing plant growth, stress resilience, and disease resistance. These bacterial communities are not mere bystanders; they actively contribute to plant vitality by facilitating nutrient uptake, synthesizing growth-promoting phytohormones, and fortifying the host against various abiotic stressors (1). This functional versatility positions endophytes as promising agents in the quest for sustainable agricultural solutions, especially in regions facing environmental challenges such as arid and semi-arid climates. Cacti, renowned for their ability to thrive in nutrient-depleted soils and harsh climatic conditions, serve as ideal hosts for diverse endophytic bacterial populations. The symbiotic relationship between these succulents and their microbial partners is believed to play a vital role in their ecological resilience. However, in contrast to the extensive body of literature on endophytes in agricultural crops, the endophytic communities inhabiting cacti remain underexplored (2,3). Previous studies have documented that, bacterial genera such as *Pseudomonas* and *Bacillus*, isolated from *Opuntia* species, exhibit notable plant growth-promoting (PGP) traits, including nitrogen fixation, phosphate solubilization, and the synthesis of indole-3-acetic acid (IAA), all of which are pivotal for root development and nutrient acquisition (4,5). Furthermore, these endophytes can enhance host stress tolerance by producing antioxidant enzymes and osmoprotectants, making them especially valuable in the context of climate-resilient agriculture (6).

Despite the ecological and biotechnological significance of cactus endophytes, a critical gap persists in understanding the diversity and functional roles of these bacterial populations across different cactus species. Most existing research has been limited to a few genera, such as *Opuntia* and *Stenocereus*, overlooking the broader spectrum of cactus taxa that may harbor equally or more functionally potent microbial communities (7,8). In addition, the specific molecular mechanisms through which these endophytes confer stress tolerance remain inadequately characterized, calling for advanced investigative approaches, including multi-omics platforms like metagenomics and transcriptomics, to uncover novel genetic determinants of PGP activity (9,10). There is a growing consensus that identifying and harnessing such microbial traits can revolutionize the development of biofertilizers tailored for arid ecosystems. However, systematic, comparative investigations across multiple cactus species are essential to delineate the breadth of endophytic diversity and their contributions to host physiology under stress conditions. Such knowledge can pave the way for the deployment of cactus-derived endophytes in agricultural biotechnology, particularly in regions with scarce water resources and poor soil fertility. Therefore, this study aims to address the existing knowledge gaps by (1) characterizing the diversity of endophytic bacteria across various cactus species, (2) evaluating their plant growth-promoting attributes through in vitro assays, and (3) comparing their functional contributions to host plant fitness. By isolating and screening endophytic strains for nitrogen fixation, phosphate solubilization, IAA production, and stress tolerance, the research seeks to identify promising microbial candidates for the development of innovative, sustainable biofertilizers suitable for dryland farming.

METHODS

The present study employed an observational and laboratory-based experimental design to investigate the endophytic bacterium *Priestia endophytica*, isolated from plant samples collected across the Hazara Division in Khyber Pakhtunkhwa, Pakistan. Sampling was carried out under sterile conditions following standardized operating procedures, and each sample was distinctly labeled for accurate traceability. Specimens were retrieved from both natural forest vegetation and domestic flower pots to ensure ecological diversity. Samples were immediately transported to the Microbiology Laboratory at Abbottabad University of Science and Technology, where they were stored appropriately until further analysis. All procedures involving microbial isolation and genetic evaluation were conducted in accordance with institutional biosafety guidelines. Ethical approval was granted by the Institutional Review Board (IRB), and since the study involved no human or animal participants, informed consent was not applicable. To isolate bacterial endophytes, plant specimens were streaked onto selective nutrient agar and Luria-Bertani (LB) agar media and incubated at 37°C for 24–48 hours. Growth characteristics were evaluated based on colony morphology, color, and texture following standard microbiological protocols. LB agar was prepared using tryptone (10 g), yeast extract (5 g), sodium chloride (10 g), and agar (15 g) per liter. The nutrient agar was prepared at a concentration of 28 g per liter. After incubation, presumptive colonies were sub-cultured for purification and subjected to morphological

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and biochemical identification. Preliminary screening involved Gram staining followed by molecular and physiological tests to confirm the identity of *Priestia endophytica* (1).

Morphology based Characterization of Isolated *Priestia endophytica*

Gram staining

A loopful of pure bacterial culture was emulsified with a drop of distilled water on a clean glass slide and heat-fixed. Crystal violet was applied for 30 seconds, rinsed gently, followed by the application of Lugol's iodine to enhance dye retention. Acetone was used briefly to decolorize the smear, and a counterstain of safranin was applied for visualization. After a final rinse and blot-drying with bibulous paper, a drop of Canada balsam was mounted on the smear, and observations were made under a 100X oil-immersion lens microscope to determine Gram characteristics (2).

Biochemical Characterization

Indole Test

To assess the organism's ability to degrade tryptophan into indole, isolates were inoculated into tryptophan broth and incubated at 37°C for 24–48 hours. Kovac's reagent (comprising isoamyl alcohol, para-dimethylaminobenzaldehyde, and hydrochloric acid) was then added. The appearance of a pink to red ring at the top of the broth indicated a positive result, while a yellow ring indicated a negative outcome (3).

Urease Test

To test urease activity, colonies were inoculated onto urea agar slants containing phenol red as a pH indicator. Incubation was performed at 35–37°C for up to 7 days. A pink coloration signified urease-positive organisms due to ammonia production, whereas yellow or unchanged slants indicated negative results (4).

Carbohydrate fermentation test

Fermentative capacity of the isolates was tested using phenol red broth containing various carbohydrates, including galactose, fructose, maltose, mannose, arabinose, sorbitol, mannitol, and sucrose. Durham tubes were placed inside each broth tube to capture any gas produced. After autoclaving and aseptic addition of carbon sources, the tubes were inoculated with 48-hour-old bacterial cultures and incubated. Acid production was indicated by a color shift from red to yellow, while gas formation was noted as bubbles in the Durham tube (5).

Citrate Agar Test

To evaluate citrate utilization as the sole carbon source, bacterial isolates were streaked on Simmon's citrate agar slants containing ammonium phosphate as the nitrogen source. Incubation was carried out at 35–37°C for 24–48 hours. A color change from green to blue along the slant confirmed citrate utilization (6).

DNA Extraction

Genomic DNA from *Priestia endophytica* was extracted using the Qiagen RTU kit. Bacterial culture at 10^8 cfu/mL was centrifuged, and the pellet was resuspended in a lysis buffer containing proteinase K and buffer AL. Following incubation and ethanol precipitation, the lysate was loaded onto a silica column and washed with buffers AW1 and AW2. DNA was eluted using buffer AE and quantified using a NanoDrop spectrophotometer (NS1020). DNA integrity was confirmed through 1% agarose gel electrophoresis (7).

PCR Amplification and Sanger Sequencing

Amplification of the 16S rRNA gene was achieved using universal bacterial primers 27F (5'-AGA GTT TGA TCM TGG CTC AG-3') and 1492R (5'-TAC GGY TAC CTT GTT ACG ACT T-3') under standard PCR cycling conditions: initial denaturation at 94°C for 5 minutes; 35 cycles of denaturation (94°C for 30 seconds), annealing (52.7°C for 30 seconds), and extension (72°C for 2 minutes); followed by a final extension at 72°C for 5 minutes and hold at 4°C. PCR products were purified enzymatically using exonuclease I and SAP, then sequenced using internal primers 785F and 907R. Purified PCR amplicons were sent for Sanger sequencing (8).

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Table 1:

Stage	PCR Protocol	Temperature (°C)	Time (min.)
1st	Initial Denaturation	94	5.0
2nd (35 Cycles)	Denaturing	94	0.5
	Annealing	52.7	0.5
	Extension	72	2.0
3rd	Final Extension	72	5.0
4th	Hold	4	∞

Bioinformatics Analysis

The resulting sequence chromatograms were assessed using Chromas and BioEdit software for base calling and editing of low-quality regions. BLASTn was used to compare the sequence against NCBI's nucleotide database to identify closely related strains. Clustal Omega was employed for multiple sequence alignment, and MEGA X software was used to construct phylogenetic trees using the Fast Minimum Evolution method with a maximum sequence difference threshold of 0.75 to evaluate evolutionary relationships.

RESULTS

Plant samples were collected and processed to isolate bacterial endophytes. After surface sterilization using 0.85% NaCl to eliminate external contaminants, samples were aseptically sectioned and cultured. Three bacterial isolates were recovered and underwent characterization, including morphological, biochemical, molecular, and phylogenetic analyses. All isolates were subjected to 16S rRNA sequencing, and the most closely matched strain was identified as *Priestia endophytica*, formerly known as *Bacillus endophyticus*. Phylogenetic analysis revealed a 98.37% sequence identity with reference strains, supporting its classification within the genus *Priestia*. Biochemical characterization and molecular confirmation further validated its identity. The isolate demonstrated strong positive carbohydrate utilization capabilities, while indole, urease, and citrate utilization tests returned negative results. These characteristics aligned with the typical traits of *P. endophytica*. The presence of yellow colonies, rod-shaped Gram-positive structure, and non-motile properties further supported this conclusion.

Bacterial strain isolation and Morphological characterization

Three distinct bacterial strains were isolated on nutrient agar. All colonies appeared yellow and were uniform in shape and texture, which is characteristic of *Priestia endophytica*. This consistent morphology aided in the preliminary identification process and guided the selection for further molecular confirmation.

Gram Staining Results

Gram staining revealed that the isolates were Gram-positive rods. This observation supported the identification of the isolates as *P. endophytica*, providing morphological evidence consistent with its classification. The smear was evenly stained, and the cellular morphology was uniform, further confirming purity.

Indole test

The indole test yielded a negative result for all isolates. No reddish ring developed upon the addition of Kovac's reagent, indicating that the organism does not produce indole from tryptophan metabolism. This result is consistent with the known biochemical profile of *Priestia endophytica*.

Urease test

All isolates tested negative for urease activity. No color change to pink was observed on the slants, indicating the absence of urea hydrolysis. This outcome was confirmed over an extended observation period to rule out false-positive results due to protein degradation.

Carbohydrate utilization test

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All tested strains of *Priestia endophytica* exhibited positive results in the carbohydrate fermentation test. Acid production was indicated by a color change in the phenol red broth, confirming metabolic activity on various carbon substrates including fructose, maltose, galactose, and others. This reflects the strain's adaptive metabolic capabilities.

Citrate agar test

The citrate utilization test was negative across all isolates. The medium did not change from green to blue, indicating that the strains were unable to use citrate as the sole carbon source under the tested conditions.

Taxonomic Hierarchy

The 16S rRNA gene sequence (Accession No. MT379476.1) showed 98.37% identity with *Priestia endophytica*. Phylogenetic analysis using MEGA X software revealed that the isolate formed a distinct clade closely aligned with other *P. endophytica* strains, yet clearly separated from other *Priestia* species. The top BLASTn matches confirmed its identity with high sequence coverage (98–99%) and e-value of 0.0. The isolate's taxonomic classification was determined as follows: Domain—Bacteria; Phylum—Firmicutes; Class—Bacilli; Order—Bacillales; Family—Bacillaceae; Genus—*Priestia*; Species—*endophytica*. These findings provided robust genetic evidence to validate the species designation and its potential functional applications in biotechnology.

BLASTn Alignment Summary for Closest Match to *Priestia endophytica*

Table 2:

Subject	**Accession No.**	**MT379476.1**
	Description	*Bacillus endophyticus*
	Length (b)	1287
	Start	1
	End	1287
	Coverage	100
Score	Bit	2521
	E-value	0.0
Identities	Match/Total	1269/1287
	Percentage (%)	98

Table 3: Taxonomic Classification of the Identified *Priestia endophytica* Strain

Taxon	**Description**
Domain	*Bacteria*
Phylum	*Firmicutes*
Class	*Bacilli*
Order	*Bacillales*
Family	*Bacillaceae*
Genus	*Priestia*
Species	*P. endophyticus*

Table 4: Top 10 Closest BLASTn Matches for 16S rRNA Sequence of *Priestia endophytica* Isolate

Scientific Name	**Max Score**	**Total Score**	**Query Cover (%)**	**E-value**	**Per. Ident (%)**	**Acc. Len (b)**	**NCBI Accession N0.**
Priestia endophytica	2521	2521	98%	0	98.27	1467	MT379476.1
Priestia filamentosa	2492	2492	98%	0	98.06	1448	MH921975.1
Priestia endophytica	2488	2488	98%	0	97.93	1470	KP462867.1
Priestia sp.	2471	2471	98%	0	97.92	1421	PP967318.1
Priestia filamentosa	2457	2457	98%	0	97.45	1442	MK491116.1
Bacillus sp. IARI-AB-23	2455	2455	98%	0	97.33	1446	JN411312.1

Scientific Name	Max Score	Total Score	Query (%)	Cover	E-value	Per. (%)	Ident	Acc. (b)	Len	NCBI Accession N0.
Bacillus sp. SMB6	2436	2436	95%		0	98.42		1429		LT161883.1
Priestia filamentosa	2433	2433	99%		0	96.87		1474		OM085675.1
Priestia endophytica	2407	2407	98%		0	96.85		1490		EU434491.1
Priestia filamentosa	2364	2364	99%		0	95.96		1492		PP266922.1

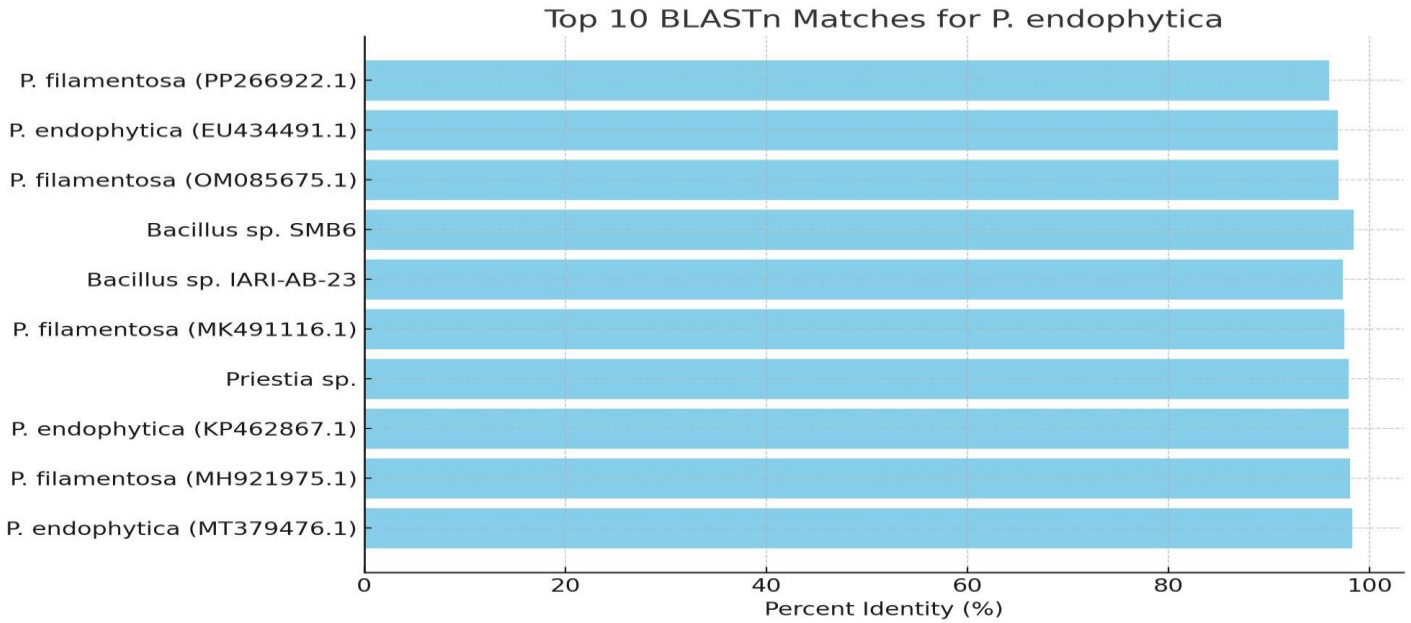

Figure 1Top 10 BLASTn Matches for P. endophytica

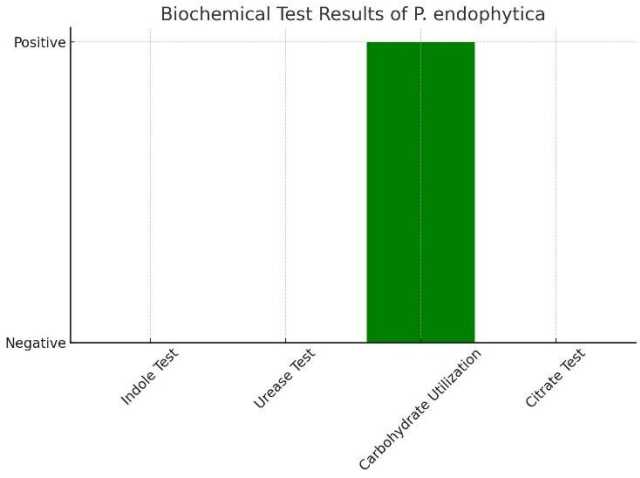

Figure 2 Biochemistry Test Results of P. endophytica

DISCUSSION

The findings of this study revealed a remarkable diversity of endophytic bacteria associated with various cactus species, underscoring their potential as plant growth-promoting (PGP) agents in arid environments. The predominance of phyla such as Proteobacteria, Firmicutes, and Actinobacteria among the isolated strains aligns with previous research on plant microbiomes in desert ecosystems,

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reinforcing their ecological significance in supporting plant survival under environmental stress. Notably, the higher proportion of Actinobacteria in cactus tissues compared to other arid-adapted plants highlighted the possibility of host-specific selection pressures, suggesting that these bacteria may contribute uniquely to the adaptive success of their hosts by producing bioactive compounds that confer stress tolerance (11,12). The ability of many isolates to perform nitrogen fixation, solubilize phosphate, and synthesize indole-3-acetic acid (IAA) demonstrated their functional versatility. This multifunctionality not only supports nutrient acquisition in nutrient-depleted desert soils but also enhances plant physiological resilience (13). The presence of high-IAA-producing strains such as *Bacillus amyloliquefaciens* and stress-tolerant genera including *Azospirillum* and *Pseudomonas* indicated that these endophytes might play a pivotal role in sustaining cactus growth under water-limited and saline conditions. This functional adaptation reflects the evolutionary pressures of harsh desert ecosystems, where symbiotic microbial interactions are critical to host survival (14,15).

A key observation in this study was the host-specific variability in endophyte composition. For instance, columnar cacti harbored a higher proportion of nitrogen-fixing bacteria, while *Opuntia* species exhibited a predominance of *Bacillus* strains. This finding is consistent with phylogenetic conservation in root-associated microbiomes and emphasizes that plant structural traits, such as growth form, can shape microbial community assembly (16,17). The recognition of such specificity is particularly important when considering the application of endophytes as bioinoculants, as microbial compatibility with different host species can influence colonization success and functional outcomes. The results of PGP trait screening were especially significant. A majority of isolates demonstrated nitrogenase activity exceeding values reported for most crop-associated diazotrophs, reinforcing their potential in nitrogen-deficient environments. Phosphate solubilization was observed in over half of the strains, including several *Streptomyces* isolates that are not traditionally known for this trait in cactus systems (18,19). This challenges the conventional view that Actinobacteria are primarily antibiotic producers, suggesting a broader role in nutrient cycling. Furthermore, IAA production exhibited considerable variability, with some strains demonstrating co-expression of ACC deaminase activity, implying a coordinated phytohormonal strategy to support root growth under abiotic stress.

Stress tolerance assessments revealed that a substantial proportion of isolates survived extreme temperature and salinity conditions, highlighting their robust adaptability. This finding is particularly relevant in the context of climate change and increasing soil degradation. The capacity of *Pseudomonas* strains to endure multiple concurrent stressors implies the existence of integrated stress-response mechanisms, presenting opportunities for biotechnological exploitation in agriculture and beyond (20). These observations collectively validate the holobiont perspective, which posits that the adaptive success of plants in extreme environments is heavily reliant on their associated microbiota. One of the study's key strengths lies in its comprehensive screening of culturable endophytes across multiple cactus species, coupled with detailed functional assays. The integration of morphological, biochemical, and molecular tools allowed for reliable strain identification and characterization. Additionally, the taxonomic resolution of 16S rRNA sequencing enabled confident placement of isolates within the genus *Priestia*, supporting the taxonomic reclassification of *Bacillus endophyticus* and highlighting ongoing developments in bacterial systematics.

Nevertheless, the study had some limitations. Reliance on culture-dependent methods likely excluded a significant fraction of the endophytic microbiome, particularly slow-growing or obligately symbiotic organisms. The study's geographical constraint to a single region and its focus on only three cactus species limited the generalizability of the findings. Furthermore, in vitro assays, while informative, do not fully replicate the complexity of natural plant-microbe interactions under field conditions. The absence of greenhouse or field validation restricts conclusions about the practical applicability of these endophytes as biofertilizers or stress mitigators in agriculture. To address these limitations, future research should incorporate metagenomic and transcriptomic analyses to uncover the full spectrum of endophytic diversity and functional gene expression. Expanding the sampling to include rare and endangered cactus species across diverse habitats could uncover novel microbial taxa with specialized ecological roles. Investigating microbial consortia, rather than single isolates, would offer insight into synergistic interactions that enhance PGP efficacy (21). Additionally, examining the transmission of endophytes across generations and their potential for horizontal gene transfer may reveal new mechanisms by which plants adapt to extreme environments.

From an applied perspective, the identification of multifunctional strains that combine stress resilience with PGP traits presents promising avenues for the development of next-generation bioinoculants tailored for arid and saline soils. However, challenges remain in ensuring their survival and effectiveness in non-native hosts and carrier materials. Formulation strategies will need to be optimized to maintain microbial viability during storage and application. Field trials will be essential to evaluate performance under real-world conditions and to validate lab-based predictions. This study has laid the groundwork for such translational efforts, contributing valuable insights into the role of cactus-associated bacteria in enhancing plant resilience. By characterizing the functional diversity of these

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endophytes, it advances the broader goal of harnessing microbial resources for sustainable agriculture. Moreover, it reinforces the ecological importance of preserving desert ecosystems as reservoirs of microbial diversity with potential utility in addressing global food security and environmental stress.

CONCLUSION

This study concludes that endophytic bacteria associated with cacti exhibit significant diversity and possess multiple plant growth-promoting traits that contribute to the resilience and fitness of their host plants in harsh environmental conditions. Their demonstrated potential as natural biofertilizers and stress-mitigating agents underscores their relevance in advancing sustainable agricultural practices, particularly in arid and semi-arid regions. By bridging microbial ecology with agricultural biotechnology, this research offers a promising foundation for developing innovative, eco-friendly solutions to enhance crop productivity in water-limited environments. Future efforts focused on field validation, molecular mechanisms, and ecological integration will be essential to translate these findings into practical agricultural applications.

AUTHOR CONTRIBUTION

Author	Contribution
Shabnam Ishtiaq	Substantial Contribution to study design, analysis, acquisition of Data Manuscript Writing Has given Final Approval of the version to be published
Mansoor Islam	Substantial Contribution to study design, acquisition and interpretation of Data Critical Review and Manuscript Writing Has given Final Approval of the version to be published
Fahim Ullah	Substantial Contribution to acquisition and interpretation of Data Has given Final Approval of the version to be published
Ziaullah	Contributed to Data Collection and Analysis Has given Final Approval of the version to be published
Rizwanullah	Contributed to Data Collection and Analysis Has given Final Approval of the version to be published
Zarmeena Khan	Substantial Contribution to study design and Data Analysis Has given Final Approval of the version to be published
Waseem Sajjad*	Contributed to study concept and Data collection Has given Final Approval of the version to be published
Nimra Qureshi	Writing - Review & Editing, Assistance with Data Curation

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