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Characterization of Endophytic Bacteria For Salt-Tolerant And Plant Growth-Promoting Traits

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Abstract

Salinity stress is a major constraint to sustainable crop production worldwide. Endophytic microorganisms offer an eco-friendly alternative to chemical inputs by enhancing plant growth and stress tolerance. In this study, 56 endophytic bacterial isolates belonging to diverse genera including *Pseudomonas*, *Bacillus*, *Enterobacter*, *Microbacterium*, and *Rhizobium*—were obtained from three halophytic grasses (*Sporobolus marginatus*, *Urochondra setulosa*, and *Leptochloa fusca*). All isolates tolerated 0.5–5% NaCl, while 24 isolates sustained growth up to 10% NaCl, indicating strong halotolerance. A substantial proportion exhibited plant growth-promoting (PGP) traits, including nitrogen fixation, indole-3-acetic acid (IAA) production, phosphate solubilization, hydrogen cyanide production, and ACC deaminase activity. These findings highlight the potential of halophyte-associated endophytes as bioinoculants for improving crop performance under saline conditions.

Keywords: Salinity stress; Endophytic bacteria; Halophytes; Plant growth promotion; Sustainable agriculture

Introduction

Sustainable agriculture is essential to meet the food demands of a rapidly growing global population. However, excessive reliance on chemical fertilizers has led to soil degradation, environmental pollution, and loss of beneficial microbial diversity (1,2, 3). As a result, microbial-based alternatives such as plant growth-promoting endophytes (PGPE) are gaining increasing attention for their role in sustainable crop production (4,5, 6).

Endophytic bacteria colonize internal plant tissues without causing harm and contribute significantly to plant growth, nutrient acquisition, and stress tolerance (7,8,9). These microorganisms enhance plant resilience under abiotic stresses, particularly salinity, by producing phytohormones, solubilizing nutrients, and modulating stress-related ethylene levels via ACC deaminase activity (10,11,12).

Halophytic plants host specialized microbiomes adapted to high salinity and extreme environmental conditions. These microbial communities represent a valuable resource for identifying stress-tolerant bioinoculants (Rodríguez-Llorente et al., 2019). Recent studies demonstrate that halotolerant endophytes significantly improve plant growth and productivity under saline stress by enhancing physiological and biochemical processes (13,14,15,16).

Given the increasing extent of soil salinization globally, the exploration of halophyte-associated endophytes offers a promising strategy for sustainable agriculture. Therefore, the present study aims to isolate, characterize, and evaluate the salt tolerance and plant growth-promoting potential of bacterial endophytes from selected halophytic grasses.

Materials and Methods

Isolation and Identification of Endophytes:

Endophytic bacterial strains were recovered from the surface-sterilized roots, nodes, and leaves of three halophytic grass species, namely *Sporobolus marginatus* (KC1), *Urochondra setulosa* (KC2), and *Leptochloa fusca* (KC3). These grasses were originally collected from highly saline habitats of the Kutch Plains in Bhuj, Gujarat, India, and subsequently established in April 2021 at the experimental farm of the ICAR–Central Soil Salinity Research Institute (ICAR-CSSRI), Karnal, Haryana, India (29°43′N, 76°58′E; 245 m above mean sea level). The plants were maintained under saline conditions with an electrical conductivity of the soil extract (ECe) of 30 dS m^{-1}. Plant tissues, including roots, leaves, and nodes, were sampled between August and September 2021 (Figure 1) and transported to the Department of Biotechnology, Kurukshetra University, Kurukshetra, in sterile sealed bags. Upon arrival, the samples were processed for the isolation of endophytic bacteria and their subsequent evaluation for salt tolerance and plant growth-promoting characteristics.

The isolates were cultured on nutrient agar supplemented with NaCl concentrations ranging from 0.5% to 10% to assess salinity tolerance. Identification was performed through morphological, biochemical, and molecular (16S rRNA gene sequencing) analyses as particularized in our preceding study (17).

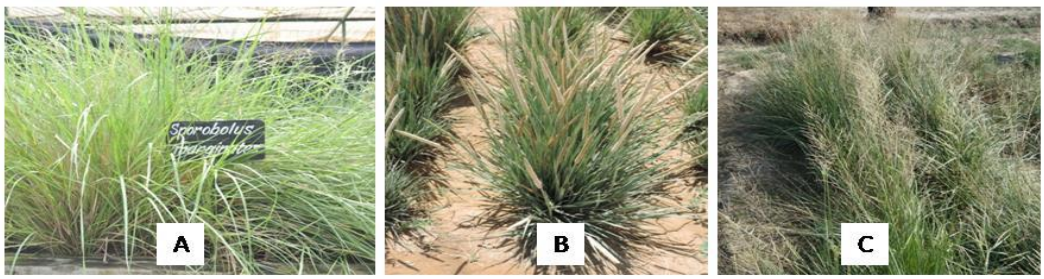


Figure 1: Halophytic grasses in the field (A- *Sporobolus marginatus*; B- *Urochondra setulosa* and C- *Leptochloa fusca*).

Screening for Plant Growth-Promoting Traits: The halotolerant isolated were further evaluated for their plant growth promoting potential by performing following tests-

- **Nitrogen Fixation:** Assessed using nitrogen-free mineral medium with bromothymol blue indicator (18).
- **Ammonia Production:** Determined by color change following addition of Nessler's reagent (19).
- **Phosphate Solubilization:** Evaluated using Pikovskaya's agar and quantified via spectrophotometry (20, 21).
- **IAA Production:** Measured using tryptophan-supplemented broth and Salkowski reagent (22).
- **HCN Production:** Detected using picric acid-treated filter paper (23).
- **ACC Deaminase Activity:** Assessed by growth on ACC-supplemented minimal medium (24).

Results and Discussion

Halotolerance and microbial diversity

A total of 56 endophytic bacterial isolates were recovered from the three selected halophytic grasses, including 24 isolates from *S. marginatus*, 15 from *U. setulosa*, and 17 from *L. fusca*. Among these, the maximum number of isolates were obtained from the root tissues of *S. marginatus*. Successful surface sterilization of explants was an essential step to ensure complete removal of epiphytic microorganisms. The efficiency of the sterilization procedure was confirmed by the absence of microbial growth on control plates inoculated with aliquots from the final rinse water and incubated at 37°C for 24–48 h. Initial characterization of the isolates was performed using morphological and biochemical traits. Further molecular and phylogenetic identification was conducted through 16S rDNA sequencing using PCR amplification with universal primers, following the methodology described by Chauhan et al (17). The isolated bacterial endophytes belonged to a wide range of genera, including *Pseudomonas, Bacillus, Enterobacter, Microbacterium, Rhizobium, Chryseobacterium, Brevibacillus, Klebsiella, Proteus,* and *Escherichia*. Among these, *Pseudomonas, Bacillus,* and *Enterobacter* were the dominant genera across all three grass species. Comparatively, *S. marginatus* harbored a richer diversity of endophytic bacteria than *U. setulosa* and *L. fusca*. Root tissues were generally the most favorable source for endophyte isolation, whereas node explants of *U. setulosa* and *L. fusca* yielded more isolates than leaf and root tissues. The isolation of these strains underscores the ecological richness of plant-associated microbiomes in saline environments. The dominance of genera such as *Pseudomonas, Bacillus,* and *Enterobacter* aligns with other findings that these taxa are highly adaptable and frequently associated with stress-resilient plants (25, 26, 27).

The isolates were subsequently evaluated for their halotolerance under different NaCl concentrations ranging from 0.5% to 10%. All isolates exhibited growth within the 0.5–5% salinity range. Notably, 24 isolates, mainly belonging to the genera *Pseudomonas*, *Bacillus*, and *Enterobacter*, continued to grow even at 10% NaCl concentration, indicating their strong salt-tolerance capacity. Comparable results have been reported globally, where halophyte-derived endophytes enhanced plant survival

and biomass under salinity stress (14, 28). Such tolerance is attributed to osmotic adjustment mechanisms, including compatible solute accumulation and ion homeostasis (16, 29).

Plant Growth-Promoting Traits: A large proportion of isolates exhibited PGP traits viz. Forty-three isolates demonstrated nitrogen fixation and ammonia production. These processes enhance nitrogen availability and are essential for plant growth under nutrient-limited conditions. Fourteen isolates exhibited phosphate solubilizing ability, with moderate efficiency. Quantitative analysis identified *Pseudomonas sp.* SS46 as the most efficient strain. The Table1 represents quantitative assessment of phosphate solubilization potential of selected bacterial isolates based on colony diameter, halo zone diameter, and calculated phosphate solubilization index (PSI). Isolates are categorized as low, moderate, or high solubilizers based on PSI values. The data highlight variability in solubilization efficiency among isolates derived from different host plants. A majority of isolates produced IAA, with several strains showing high production levels. Twenty-two isolates produced HCN, with varying intensities and a large proportion of isolates exhibited ACC deaminase activity. The presence of ACC deaminase activity in a majority of isolates is particularly significant, as this enzyme reduces ethylene levels and alleviates stress-induced growth inhibition (figure 2&3). The figure 4 exhibits qualitative and quantitative evaluation of key plant growth-promoting traits, including phosphate solubilization (mg ml^{-1}), indole-3-acetic acid (IAA) production (µg ml^{-1}), nitrogen fixation ability, ACC deaminase activity, and hydrogen cyanide (HCN) production. Selected isolates exhibiting multiple beneficial traits were prioritized for further characterization and potential application as bioinoculants.

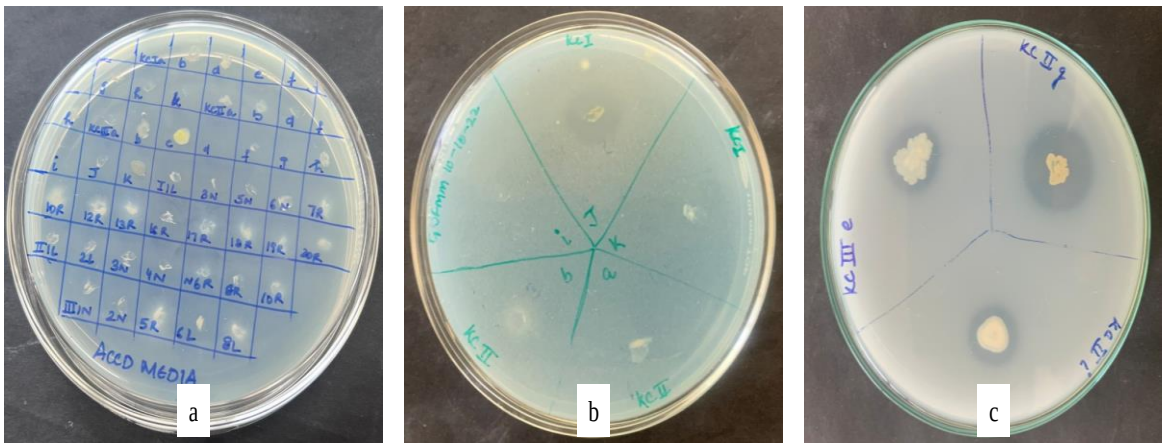


Figure 2: Screening of plant growth-promoting traits in endophytic bacterial isolates demonstrating key plant growth-promoting (PGP) activities of selected endophytic bacterial isolates: (a) growth on DF minimal medium supplemented with ACC indicating ACC deaminase activity; (b) growth on nitrogen-free mineral medium (NFM) confirming nitrogen fixation potential; (c) formation of clear halo zones on Pikovskaya's agar indicating phosphate solubilization. These assays collectively illustrate the multifunctional capabilities of halotolerant endophytes).

IAA production enhances root architecture and nutrient uptake, thereby improving plant growth under adverse conditions (30). Similarly, phosphate-solubilizing bacteria play a crucial role in mobilizing insoluble phosphorus, increasing its availability to plants (31). Recent studies confirm that ACC deaminase-producing bacteria enhance salinity tolerance by modulating plant hormonal balance. These multifunctional traits are critical for plant adaptation to stress conditions and have been widely reported in endophytic bacteria (11, 32, 33).

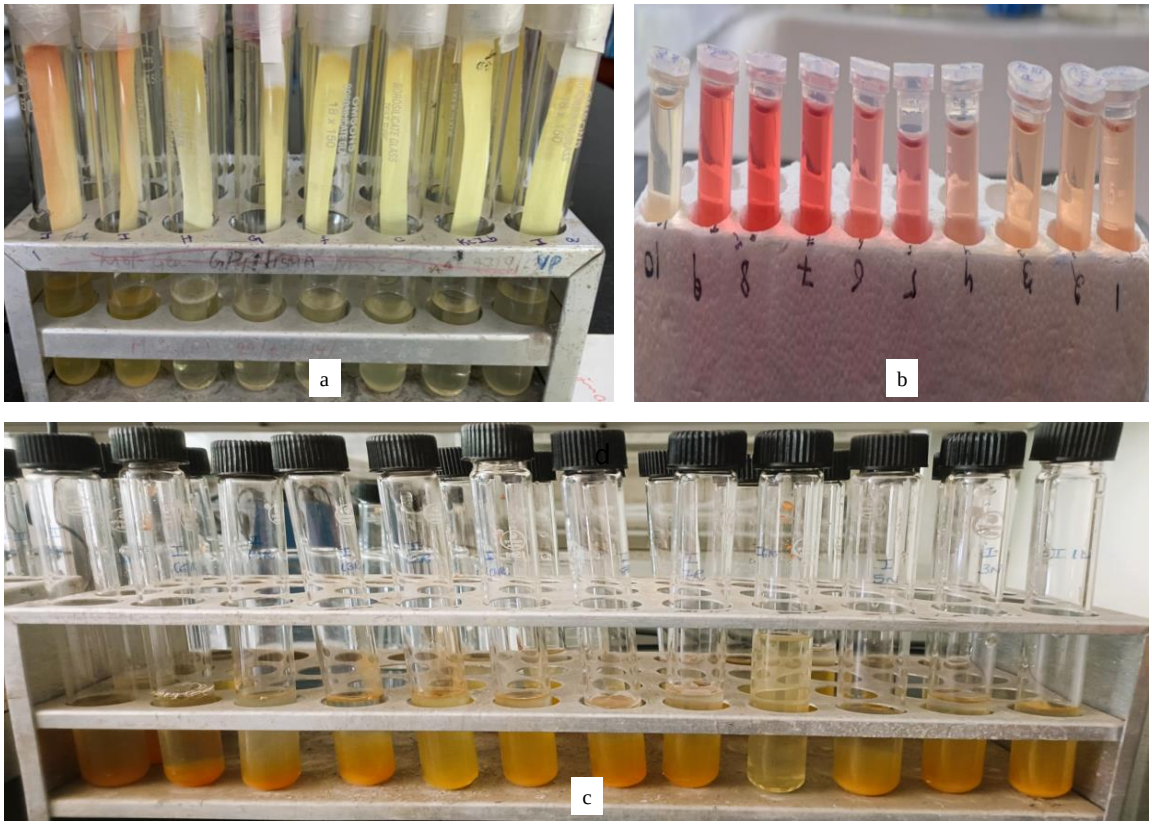


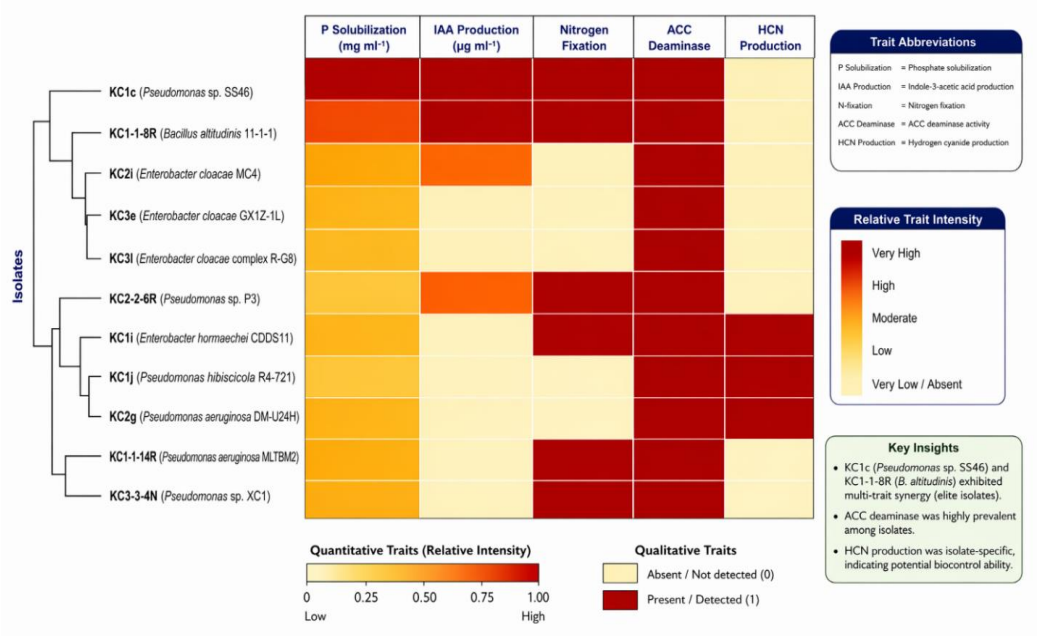
Figure 3: Biochemical characterization of PGP traits in bacterial endophytes(Qualitative assays showing production of secondary metabolites associated with plant growth promotion: (a) color change of picrate-impregnated filter paper from yellow to brown indicating hydrogen cyanide (HCN) production; (b) development of red coloration following Salkowski reagent addition indicating indole-3-acetic acid (IAA) production; (c) formation of yellow to brown coloration upon addition of Nessler's reagent indicating ammonia production. The intensity of color corresponds to the level of metabolite production.)

Table 1: Phosphate solubilization efficiency of endophytic bacterial isolates from halophytic grasses.

Isolate code	Bacterial identification	Colony diameter(mm)	Halo zone (mm)	PSI (index)
KC1c	*Pseudomonas* sp. strain SS46	7	18	3.57
KC1i	*Enterobacter hormaechei* subsp. *xiangfangensis* CDDS11	14	21	2.50
KC1j	*Pseudomonas hibiscicola* R4-721	15	23	2.53
KC1k	*Pseudomonas* sp. strain C8	8	9	2.13
KC1-1-6N	*Agrobacterium* sp. RA65	6	8	2.33
KC1-1-8R	*Bacillus altitudinis* 11-1-1	11	14	2.27
KC1-1-19R	*Enterobacter cloacae* R16	7	8	2.14
KC2g	*Pseudomonas aeruginosa* DM-U24H	8	20	3.50
KC2h	*Pseudomonas guguanensis* 4-n-1	16	17	2.06
KC2i	*Enterobacter cloacae* MC4	12	22	2.83
KC2-2-6R	*Pseudomonas* sp. strain P3	9	18	3.00
KC3e	*Enterobacter cloacae* GX1Z-1L	13	16	2.23
KC3l	*Enterobacter cloacae* complex R-G8	20	23	2.15
KC3-3-4N	*Pseudomonas* sp. XC1	9	11	2.22

Note: PSI (Phosphate Solubilization Index) = (Colony diameter + Halo zone diameter) / Colony diameter. Values represent measurements recorded after 7 days of incubation.

Figure 4: Heat map of Multi-trait Plant Growth Promoting (PGP) potential of endophytic bacterial isolates. The quantitate trait values were normalized (0-1) for heatmap visualization. Presence (1)/Absence (0) was used for qualitative traits.



Comparative Global Perspective: Globally, halotolerant endophytes have been reported to improve crop productivity under saline conditions in diverse agroecosystems, including rice, wheat, and legumes (14,28). Studies from Europe and Asia indicate that microbial consortia derived from halophytes outperform single-strain inoculants in promoting plant growth under stress (34, 15).

Compared to these studies, the present investigation highlights a similar trend, where isolates exhibiting multiple PGP traits (*Pseudomonas* sp. SS46 and *Bacillus altitudinis* 11-1-1) demonstrated higher functional potential. The ACC deaminase activity was highly prevalent among isolates and HCN production activity was isolate-specific, indicating potential biocontrol ability. This reinforces the concept that multifunctionality is a key determinant of microbial efficacy in stress mitigation (35,36,37).

Implications for Sustainable Agriculture: The findings of this study have significant implications for the development of biofertilizers tailored for saline soils. The selected isolates (*Pseudomonas* sp. SS46 and *Bacillus altitudinis* 11-1-1) showed superior performance across multiple parameters, making them strong candidates for future field applications. The integration of such microbial inoculants into agricultural systems can reduce dependency on chemical fertilizers, improve soil health, and enhance crop resilience to climate change-induced stresses. This aligns with global efforts toward sustainable intensification and environmentally friendly farming practices (38, 39,40).

Conclusion

This study demonstrates that halophytic grasses serve as reservoirs of halotolerant, plant growth-promoting endophytic bacteria. The identified strains, particularly *Pseudomonas* sp. SS46 and *Bacillus altitudinis* 11-1-1, exhibit strong potential for application as bioinoculants in saline agriculture. Their multifunctional traits, including nutrient mobilization and stress mitigation, position them as promising tools for sustainable crop production under challenging environmental conditions. The future research should focus on: Development of synthetic microbial consortia; Long-term field validation across agro-climatic zones and Integration into climate-resilient agricultural systems. With salinity projected to affect a large proportion of arable land in the coming decades, microbial solutions will play a crucial role in sustainable food production.

Authors contribution

Kajal Chauhan: Investigation, Data curation
Sulekha Chahal: Conceptualization, Supervision and Writing – original draft
Bindu Battan: Supervision, review & editing
Sunita Dalal: Review & editing

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Data Availability Statement

The data supporting the findings of this study are included within the article. Additional datasets generated and/or analyzed during the current study are available from the corresponding author upon reasonable request. The 16S rRNA gene sequence data generated for bacterial identification are reported in the reference study (Chauhan et al., 2023).

Conflict of Interest

The authors declare no conflict of interest.

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