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Mitigating salt stress in maize: A multifaceted role for a halotolerant *Paenibacillus* sp. isolate from arid saline Sebkh

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ABSTRACT Soil salinity severely limits crop productivity in arid regions. This study investigates a halotolerant *Paenibacillus* sp. isolate for mitigating salt stress in maize (*Zea mays* L.). The bacterium was characterized for key plant growth-promoting rhizobacteria (PGPR) traits, indole-3-acetic acid (IAA) production, phosphate solubilization, siderophore secretion, and ammonia release, under NaCl concentrations up to 900 mM. Its efficacy was tested in maize seed germination and seedling growth assays under four salinity levels (0-150 mM NaCl). The isolate maintained high IAA production and phosphate solubilization up to 600 mM NaCl and exhibited enhanced siderophore secretion with increasing salinity. Inoculation did not affect final germination percentages but alleviated germination delays under 100 mM NaCl. Crucially, bacterial inoculation significantly improved seedling growth under all salt stresses, with shoot length showing up to a fourfold increase at 150 mM NaCl compared to uninoculated controls. These results demonstrate that this *Paenibacillus* sp. confers substantial salt tolerance to maize during early development, highlighting its strong potential as a bioinoculant for sustainable agriculture in saline environments.

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Introduction

Soil salinization represents a mounting global challenge that threatens agricultural productivity and food security, particularly in arid and semi-arid regions where irrigation practices, high evapotranspiration, and poor drainage facilitate salt accumulation in the root zone (Taïbi et al. 2021). According to the FAO, more than 20% of irrigated land is already affected by salinity, and this proportion is expected to rise with ongoing climate change and land mismanagement (FAO 2021; Souana et al. 2020). Salinity imposes both osmotic and ionic stresses on plants, disrupting water uptake, impairing nutrient acquisition, and ultimately stunting growth and yield (Munns and Tester 2008; Taïbi et al. 2016). Among staple crops, maize (*Zea mays* L.) is particularly sensitive to salinity during germination and early seedling development, stages critical for stand establishment and crop performance (Farooq et al. 2015). In light of these constraints, there is growing interest in sustainable strategies that can enhance crop resilience without relying on chemical amendments or genetic modification.

One promising avenue involves the use of plant growth-promoting rhizobacteria (PGPR), a diverse group of soil microbes capable of stimulating plant growth directly through phytohormone production, nutrient

solubilization, and nitrogen fixation, or indirectly by mitigating abiotic stress via antioxidant modulation and stress hormone regulation (Vurukonda et al. 2016). Under saline conditions, halotolerant PGPR have attracted particular attention due to their ability to survive and function in high-salt environments while supporting plant health. Several studies have demonstrated that such microbes can alleviate the detrimental effects of salt stress on crops by producing indole-3-acetic acid (IAA), siderophores, and other bioactive compounds (Egamberdieva et al. 2017; Nadeem et al. 2010). For example, inoculation with salt-tolerant *Bacillus* and *Pseudomonas* strains has been shown to improve seed germination, nutrient uptake, and biomass accumulation in maize and other cereals under saline conditions (Bano and Fatima 2009; Egamberdieva 2007).

Despite these promising findings, most research has focused on a limited number of bacterial genera, and there is a relative paucity of data on the performance of *Paenibacillus* species in saline agroecosystems. *Paenibacillus* is a diverse genus of facultatively anaerobic, endospore-forming bacteria known for their ability to fix nitrogen, solubilize phosphate, and produce plant-beneficial metabolites (Grady et al. 2016). While some strains have demonstrated plant growth-promoting activity under non-stress conditions, their capacity to function as halotolerant bioinoculants remains underexplored.

Furthermore, few studies have characterized the resilience of PGPR traits across a salinity gradient or assessed the effects of *Paenibacillus* inoculation on both germination kinetics and seedling development under salt stress.

This study addresses these gaps by investigating the halotolerance and plant growth-promoting potential of a *Paenibacillus* sp. isolate recovered from a saline environment. Specifically, we assess the bacterium's ability to maintain key PGPR traits, including IAA production, siderophore secretion, phosphate solubilization, and ammonia generation, under increasing NaCl concentrations. We also evaluate the effects of seed inoculation on maize germination dynamics and early seedling growth under controlled salinity treatments. By characterizing both microbial functionality and plant responses, this work aims to elucidate the potential of *Paenibacillus* as a bioinoculant for enhancing crop performance in salt-affected soils.

Material and methods

Sampling site and bacterial isolation

The bacterial isolate designated as *Paenibacillus* sp. strain C5 was recovered from the root systems of *Salsola longifolia* Forssk., a halophytic species native to the Sebkh of Ain Sekhoune, situated within the Chott Chergui basin in northwestern Algeria (34°16'N, 00°33'E). This hyper-saline depression is typified by minimal organic matter, extreme temperature fluctuations, and soil conductivity often exceeding 50 dS m⁻¹ (Benkhettou et al. 2022). To enhance the probability of isolating halotolerant bacteria, plant specimens were collected from the central, most salt-impacted zone of the Sebkh. Root samples were aseptically excavated, stored in sterile polyethylene bags, transported on ice, and processed within 24 hours of collection.

Bacterial isolation and morphological characterization

Following transport, roots were rinsed with sterile distilled water to remove adherent soil particles. Segments approximately 5-10 mm in length were excised under sterile conditions and placed directly onto nutrient agar plates. Incubation was carried out at 28 °C for 48 h to allow bacterial growth. Distinct colonies were sub-cultured repeatedly to obtain axenic cultures. Colony characteristics including pigmentation, texture, and margin type were recorded. Morphological features were examined microscopically using Gram staining and standard slide preparation techniques. Pure cultures were preserved at -80 °C in 30% glycerol for long-term storage.

Molecular identification of bacterial isolate

For taxonomic identification, the bacterial isolate was

cultured in nutrient broth for 48 hours at 28 °C, followed by centrifugation to harvest cell pellets. Genomic DNA was extracted using the Gene Elute™ Plant Genomic DNA Purification Kit (Sigma-Aldrich, St. Louis, MO, USA), with minor protocol modifications to optimize bacterial cell lysis. DNA quality and concentration were verified spectrophotometrically, and samples were stored at -20 °C. Amplification of the 16S rRNA gene was performed using standard primers under the following PCR conditions: initial denaturation at 95 °C for 10 min; 30 cycles of 95 °C for 1 min, 50 °C for 30 s, and 72 °C for 1 min; and a final extension at 72 °C for 7 min. PCR products were confirmed on 1% agarose gels, purified, and subjected to Sanger sequencing. The resulting partial 16S rRNA gene sequence of isolate C5 (approximately 470 bp) was obtained and compared with sequences available in the NCBI database using BLASTn. The analysis revealed the highest similarity (98.26%) with *Paenibacillus lautus* strain NRRL NRS-666 (GenBank accession NR_115599.1). Based on this level of similarity, the isolate was assigned to the genus *Paenibacillus* and designated as *Paenibacillus* sp. The 16S rRNA gene sequence of strain C5 has been submitted to GenBank and the accession number will be provided upon acceptance (BioSample SAMN56374401, BioProject PRJNA1433589).

Functional assessment of plant growth-promoting traits

Salt tolerance

Salt tolerance was evaluated by inoculating nutrient broth supplemented with NaCl at concentrations of 0, 300, 600, and 900 mM. A bacterial suspension from 2-day-old cultures, adjusted to OD₆₀₀ = 0.08-0.10, was used to inoculate the media. Cultures were incubated at 28 °C with shaking at 180 rpm for 2 days. Bacterial growth was quantified spectrophotometrically at 600 nm.

IAA production

To assess auxin-like compound biosynthesis, cultures were grown in nutrient broth amended with 0.1% L-tryptophan under varying NaCl concentrations (0, 300, 600, 900 mM). The inoculated media were incubated for 2 days at 28 °C and 150 rpm. Supernatants were collected post-centrifugation (8,000 rpm, 10 min, 4 °C) and reacted with Salkowski reagent. Absorbance at 530 nm was recorded after 30 minutes of incubation in the dark. IAA concentration was estimated using a standard curve (Nautiyal 1999).

Phosphate solubilization

To evaluate phosphorus solubilization, the isolate was pre-cultured in LB broth, washed, and resuspended in phosphate-free NBRIP medium (OD₆₀₀ = 0.5). Aliquots were

incubated in NBRIP supplemented with varying salt concentrations (0-900 mM NaCl) at 28 °C, 150 rpm for 2 days. After centrifugation, soluble phosphorus was quantified using the ammonium molybdate blue method, with absorbance at 355 nm. KH_2PO_4 standards were used for calibration.

Ammonia production

Bacterial suspensions (200 μL) were inoculated into 10 mL of sterile peptone water and incubated for 5 days at 28 °C. Supernatants were reacted with Nessler's reagent, and absorbance was measured at 425 nm to estimate ammonia levels using an ammonium sulfate standard curve.

Siderophore secretion

Bacterial cultures were incubated in LB medium containing NaCl (0-900 mM) for 5 days at 28 °C. Post-incubation, the supernatant was combined with equal volumes of CAS reagent, and absorbance was recorded at 630 nm after 20 minutes. Percent siderophore units (psu) were calculated as:

$$\text{psu} = [(A_{\text{ref}} - A_{\text{sample}}) / A_{\text{ref}}] \times 100$$

where A_{ref} is the absorbance of the reference CAS solution.

Germination under salt stress

Maize (*Zea mays* L.) hybrid DKC4598 (DEKALB®, Bayer CropScience), widely cultivated in Algeria, was used in the experiment. Maize seeds were surface-disinfected with 1.5% sodium hypochlorite, rinsed thoroughly with sterile distilled water, and soaked for 1.5 h in a freshly prepared bacterial suspension adjusted to $\text{OD}_{600} = 0.10$ (corresponding to approximately 1×10^8 CFU mL^{-1}) under gentle agitation. Mock-inoculated seeds were soaked in sterile saline-free buffer for the same duration. Seeds were then placed on sterile filter paper moistened with NaCl solutions (0, 50, 100, or 150 mM) in triple-compartment Petri dishes. Plates were incubated at 25 °C in darkness, and germination was monitored every 6-12 h for up to 96 h. Data were used to calculate:

Germination percentage (GP) (Ranal et al. 2009):

$$\text{GP (\%)} = (\text{Number of seeds germinated} / \text{Total seeds}) \times 100$$

Mean germination time (MGT) (Iqbal et al. 2023):

$$\text{MGT (h)} = \Sigma(ni \times ti) / \Sigma ni$$

where ni is the number of seeds germinated at time ti (in

hours) and Σni is the total number of seeds germinated over the entire observation period.

Germination rate index (GRI) (Maguire 1962):

$$\text{GRI} = \Sigma(Ni / ti)$$

where Ni is cumulative germinated seeds by time ti (h).

Coefficient of velocity of germination (CVG) (Ranal et al. 2009):

$$\text{CVG} = (\Sigma ni / \Sigma(ni \times ti)) \times 100$$

where ni is the number of seeds germinated at time ti (in hours) and Σni is the total number of seeds germinated. $\Sigma(ni \times ti)$ is the sum of each germinated seed count multiplied by its germination time.

Seedling growth evaluation

Surface-sterilized seeds were germinated on vertically oriented filter paper in germination bags moistened with NaCl solutions (0, 50, 100, or 150 mM), matching the germination assay treatments. Seed inoculation and mock treatments were as described above. After 7 days under a 16 h light/8 h dark cycle at 25 °C, root and shoot lengths were measured using a digital caliper.

Assessment of salinity mitigation

The bacterium's capacity to mitigate salinity stress was quantified using the Salinity Mitigation Index (SMI) computed for shoot and root lengths at each salinity level (50, 100, and 150 mM NaCl). SMI was expressed as the percentage change relative to the uninoculated control at the same NaCl concentration:

$$\text{SMI (\%)} = [(L_{\text{inoculated}} - L_{\text{control}}) / L_{\text{control}}] \times 100$$

where $L_{\text{inoculated}}$ and L_{control} are the mean seedling lengths (mm) of inoculated and uninoculated seedlings, respectively. Positive SMI values indicate mitigation (growth promotion under salinity).

Statistical analysis

Normality and homogeneity of variance were verified using Shapiro-Wilk and Levene's tests. One-way ANOVA was used to examine the influence of salt on bacterial PGP traits. For germination and growth data, two-way ANOVA assessed the interaction between inoculation and NaCl concentration. Significant effects ($p < 0.05$) were further explored using Tukey's HSD test. Pearson correlation was employed to explore associations between bacterial traits and plant responses.

Table 1. Salt tolerance and plant growth-promoting traits of the *Paenibacillus* sp. isolate at varying NaCl concentrations.

NaCl (mM)	Culture density (OD ₆₀₀)	IAA (µg mL ⁻¹)	Siderophore production (%)	P-solubilization (mg mL ⁻¹)	Ammonia (mM NH ₄)
0	0.056 ± 0.007 ^c	8.58 ± 0.42 ^a	72.4 ± 0.8 ^c	0.0393 ± 0.0068 ^{ab}	0.411 ± 0.052 ^a
300	0.369 ± 0.021 ^a	8.99 ± 0.26 ^a	79.4 ± 6.5 ^{bc}	0.0400 ± 0.0044 ^{ab}	0.415 ± 0.021 ^a
600	0.137 ± 0.034 ^b	7.56 ± 1.06 ^{ab}	89.7 ± 4.50 ^{ab}	0.0520 ± 0.0052 ^a	0.293 ± 0.050 ^b
900	0.016 ± 0.006 ^c	6.93 ± 0.44 ^b	95.5 ± 0.46 ^a	0.0343 ± 0.0025 ^b	0.192 ± 0.010 ^c

Values are means ± standard deviation (n = 5). Values are means ± standard deviation (n = 5). Different letters within a column indicate significant differences according to Tukey's HSD test (p < 0.05). OD₆₀₀: optical density at 600 nm; IAA: indole acetic acid-like compounds; P-solubilization: phosphate solubilization.

Results

Bacterial salt tolerance and PGPR traits

The *Paenibacillus* sp. isolate grew across a broad salinity range, with optimal growth observed at moderate salt concentrations (Table 1). Bacterial growth, measured via turbidity (OD₆₀₀), peaked at 300 mM NaCl (0.369 ± 0.021) and declined significantly at higher salinity. At 600 mM NaCl, OD₆₀₀ dropped by around 63% (to 0.137 ± 0.034), and at 900 mM NaCl it reached a near-minimal level (0.0157 ± 0.0006), statistically similar to the control (0.056 ± 0.007). Production of indole-3-acetic acid (IAA) analogues remained high (8-9 µg mL⁻¹) up to 300 mM NaCl, demonstrating no significant change between control (8.58 ± 0.42 µg mL⁻¹) and 300 mM NaCl (8.99 ± 0.26 µg mL⁻¹). However, at 900 mM NaCl, IAA contents fell to 6.93 ± 0.44 µg mL⁻¹, a significant reduction compared to lower salt conditions (Table 1).

Siderophore production tended to increase with salinity. The isolate's siderophore yield was around 72% without NaCl, around 79% at 300 mM NaCl, and rose to 89.7 ± 4.50% at 600 mM NaCl. At 900 mM NaCl, it reached the highest level (95.5 ± 0.46%), which was significantly greater than both 0 and 300 mM NaCl. In contrast, phosphate solubilization showed only minor variations with salt. Soluble phosphorus remained around 0.034-0.040 mg mL⁻¹ from 0 to 300 mM NaCl, increased slightly at 600 mM NaCl (0.052 ± 0.005 mg mL⁻¹), then dropped to 0.034

± 0.003 mg mL⁻¹ at 900 mM NaCl. This slight decrease at 900 mM NaCl was the only statistically significant change in P-solubilization (600 vs. 900 mM NaCl).

Ammonia production declined progressively under salt stress. Ammonium contents were around 0.41 mM NH₄ at 0-300 mM NaCl, then fell to 0.293 ± 0.050 mM at 600 mM NaCl (which is approximately 30% lower than the control value) and further to 0.192 ± 0.010 mM at 900 mM NaCl. The ammonia production at 600 and 900 mM NaCl was significantly lower than at 0 or 300 mM NaCl, indicating that high salinity markedly reduces ammonia production by the isolate (Table 2).

Seed germination under saline conditions

Maize seed germination was adversely affected by increasing NaCl concentration, while bacterial inoculation had little influence on final germination outcomes. In the non-saline control, germination reached about 70% by 96 h (final germination 70.0 ± 10.0%). A mild salt stress of 50 mM NaCl caused a slight reduction in germination (final germination around 63% in controls), and more severe stress led to pronounced germination losses. At 100 mM NaCl, only 43.3 ± 5.8% of non-inoculated seeds germinated, and a similar proportion (43.3 ± 23.1%) germinated at 150 mM NaCl (Table 3).

Inoculated seeds showed a comparable pattern; around 73.3% final germination at 0 mM NaCl, decreasing to 60.0 ± 10.0% at 50 mM NaCl, 50.0 ± 10.0% at 100 mM NaCl, and 36.7 ± 5.8% at 150 mM NaCl. No significant differences in final germination were evident between inoculated and uninoculated treatments at any given salt level (Table 4).

Germination kinetics, however, revealed subtle shifts with salt and inoculation. In the absence of salt, germination was relatively rapid; mean germination time (MGT) was around 51-54 h, and the coefficient of velocity of germination (CVG) was around 1.9-2.0 % h⁻¹. Low salinity of 50 mM NaCl had minimal impact on germination speed, as MGT remained around 51 h and CVG around 2.0 % h⁻¹ in both treatments. At 100 mM NaCl, uninoculated seeds germinated more slowly, MGT lengthened to 64.8 ± 2.1 h

Table 2. One-way ANOVA results for the effect of salinity on bacterial growth and PGP traits (OD₆₀₀ and the indicated metabolite assays).

Trait	F	p-value	Significance
OD ₆₀₀	182.60	1.05 × 10 ⁻⁷	***
IAA (µg mL ⁻¹)	6.85	0.0133	*
Siderophore (%)	20.18	0.000435	***
P-solubilization (mg mL ⁻¹)	6.83	0.01345	*
Ammonia (mM NH ₄)	23.84	0.000242	***

Significance levels: ns = not significant; * p < 0.05; ** p < 0.01; *** p < 0.001.

Table 3. Maize germination parameters under different salinity and inoculation treatments.

NaCl (mM)	Treatment	Final germination (%)	MGT (h)	CVG (% h ⁻¹)	GRI
0	Control	70.0 ± 10.0 ^a	51.19 ± 5.29 ^a	1.967 ± 0.203 ^a	3.03 ± 0.41 ^a
	Inoculated	73.3 ± 5.8 ^a	54.07 ± 5.32 ^a	1.861 ± 0.177 ^a	3.00 ± 0.30 ^a
50	Control	63.3 ± 15.3 ^a	51.33 ± 10.26 ^a	2.006 ± 0.438 ^a	2.69 ± 0.72 ^a
	Inoculated	60.0 ± 10.0 ^a	50.91 ± 8.04 ^a	1.999 ± 0.338 ^a	2.64 ± 0.63 ^a
100	Control	43.3 ± 5.8 ^a	64.80 ± 2.08 ^a	1.544 ± 0.050 ^a	1.61 ± 0.19 ^b
	Inoculated	50.0 ± 10.0 ^a	53.07 ± 4.41 ^a	1.894 ± 0.165 ^a	2.14 ± 0.34 ^b
150	Control	43.3 ± 23.1 ^a	53.71 ± 12.73 ^a	1.940 ± 0.501 ^a	1.78 ± 0.71 ^b
	Inoculated	36.7 ± 5.8 ^a	70.67 ± 2.31 ^a	1.416 ± 0.047 ^a	1.25 ± 0.14 ^b

Values are means ± standard deviation (n = 5). Values are means ± standard deviation (n = 5). Different letters within a column indicate significant differences according to Tukey's HSD test (p < 0.05). Final germination (FGP) is the percentage of seeds germinated by 96 h. Mean germination time (MGT) is in hours. CVG = coefficient of velocity of germination. GRI = germination rate index.

(versus around 51 h at 0-50 mM), and CVG dropped to 1.54 ± 0.05 % h⁻¹, indicating a significant delay (Table 3).

Interestingly, inoculated seeds at 100 mM germinated faster than controls (MGT 53.1 ± 4.4 h, CVG 1.89 ± 0.16 % h⁻¹), essentially maintaining the germination rate observed at lower salinity. Under the higher salinity of 150 mM NaCl, germination was protracted in the inoculated group; MGT increased to 70.7 ± 2.3 h and CVG fell to its lowest value (1.42 ± 0.05 % h⁻¹). Uninoculated seeds at 150 mM NaCl showed MGT (53.7 ± 12.7 h) and CVG (1.94 ± 0.50 % h⁻¹) similar to their 50 mM NaCl results, as only the faster-germinating portion of seeds managed to emerge under this severe salt stress. Consistent with these timing metrics, the germination rate index (GRI) declined with rising salinity for both treatments. GRI dropped from around 3.0 (at 0 mM) to around 2.7 at 50 mM NaCl, then to around 1.6 (control) - 2.1 (inoculated) at 100 mM NaCl, and fell below 1.8 at 150 mM NaCl in both cases, reflecting the overall reduction in germination speed and completeness at high salt (Table 3).

Taken together, higher NaCl concentrations significantly suppressed and slowed germination, while bacterial inoculation did not significantly alter the final germination percentage. Notably, inoculation did mitigate the salt-induced delay at 100 mM NaCl (accelerating germination relative to controls).

Seedling growth and salinity mitigation

Salinity also severely stunted early seedling development, and in this context the inoculated seedlings showed markedly greater growth than controls under moderate and high salt stress (Table 5). Without bacterial inoculation, shoot elongation declined steadily from an average of 29.8 mm in non-saline conditions to 25.0 mm at 50 mM NaCl, 18.7 mm at 100 mM NaCl, and only 7.9 ± 1.5 mm at 150 mM NaCl. A similar downward trend was observed in root length, which fell from 34.1 mm (0 mM NaCl) to 33.2 mm (50 mM NaCl), 18.3 mm (100 mM NaCl), and 16.6 ± 5.4 mm at 150 mM NaCl in uninoculated seedlings.

Inoculation with *Paenibacillus* sp. significantly promoted seedling growth, especially at higher salinities. At 0 mM NaCl, inoculated seedlings had slightly longer shoots (36.2 ± 4.6 mm) and roots (40.1 ± 10.1 mm) than controls, though the difference was modest. The growth advantage conferred by the bacterium became more pronounced at 50 mM NaCl; inoculated shoot length averaged 28.9 ± 6.3 mm versus 25.0 ± 2.6 mm in controls, and inoculated roots (65.9 ± 8.6 mm) were roughly double the length of uninoculated roots (33.2 ± 3.8 mm). Under 100 mM NaCl, shoots remained short in both treatments (around 19 mm), but inoculation maintained a small positive effect (19.6 ± 0.7 mm versus 18.7 ± 3.5 mm). In roots at 100 mM NaCl, the inoculated seedlings (26.5 ± 4.4 mm) grew around 45% longer than controls (18.3 ± 1.3 mm). The most dramatic

Table 4. Two-way ANOVA for maize germination indices: final germination percentage (FGP), mean germination time (MGT), coefficient of velocity (CVG), and germination rate index (GRI).

Factor	FGP	MGT (h)	CVG	GRI
NaCl concentration	F = 8.457 **	F = 3.140 ns	F = 1.752 ns	F = 12.506 ***
Inoculation (<i>Paenibacillus</i> sp.)	F = 0.000 ns	F = 0.424 ns	F = 0.376 ns	F = 0.011 ns
NaCl × Inoculation	F = 0.381 ns	F = 4.015 *	F = 2.344 ns	F = 1.216 ns

Significance: ns = not significant; * p < 0.05; ** p < 0.01; *** p < 0.001.

Table 5. ANOVA of maize seedling growth (shoot and root lengths) under salinity with NaCl and inoculation treatment.

Factor	Shoot length	Root length
NaCl concentration	F = 5.369 **	F = 8.496 **
Inoculation (<i>Paenibacillus</i> sp.)	F = 10.374 **	F = 20.656 ***
NaCl × Inoculation	F = 3.721 *	F = 2.698 ns

Significance: ns = not significant; * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

differences were observed at 150 mM NaCl. While uninoculated seedlings at this salinity barely developed (shoots 7.9 mm, roots 16.6 mm on average), inoculated seedlings achieved significantly greater growth (Table 6).

The mean shoot length in the inoculated group was 32.6 ± 12.2 mm, more than four-fold higher than the control, and root length reached 42.2 ± 21.7 mm, about 2.5 times the length of uninoculated roots. This indicates that the bacterium substantially alleviated salt stress effects on seedling growth.

To quantify this benefit, a salinity-mitigation index (SMI) was calculated based on the relative growth improvement due to inoculation. The SMI for shoot length rose sharply with increasing salinity; at 50 mM NaCl the inoculated shoots were on average around 17% longer than controls, at 100 mM NaCl around 7% longer, and at 150 mM NaCl they were about 329% longer (more than four times, reflecting the inoculation rescue of shoot growth at extreme salt) (Table 7).

Accordingly, shoot SMI at 150 mM NaCl was significantly higher than at the lower salt concentration. In contrast, the root length SMI values were high but more erratic, and did not differ statistically across salt concentrations. Inoculation roughly doubled root length at 50 mM NaCl (SMI $\approx 99\%$), increased it by around 47% at 100 mM NaCl, and by around 167% at 150 mM NaCl, though variation was considerable in the highest group (Table 8).

Overall, these findings demonstrate that increasing salinity strongly inhibits maize germination and seedling development, but the *Paenibacillus* sp. isolate can substantially improve seedling growth, particularly root development, under saline conditions, partially mitigating

Table 7. Salinity-mitigation index (SMI) for maize seedling shoots and roots at each salt treatment.

NaCl (mM)	Shoot SMI (%)	Root SMI (%)
50	17.1 ± 35.0^b	99.1 ± 20.8
100	7.2 ± 18.6^b	46.7 ± 34.9
150	329.2 ± 193.5^a	166.9 ± 160.4

Values are means $\pm$ standard deviation ($n = 5$). Different letters within a row of means (for a given trait) indicate significant differences between NaCl treatments (Tukey HSD, $p < 0.05$).

Table 6. Maize seedling shoots and roots lengths under different salt and inoculation treatments.

NaCl (mM)	Treatment	Shoot length (mm)	Root length (mm)
0	Control	29.77 ± 11.83^a	34.13 ± 7.25^b
	Inoculated	36.20 ± 4.63^a	40.08 ± 10.15^b
50	Control	25.04 ± 2.61^{ab}	33.18 ± 3.75^b
	Inoculated	28.86 ± 6.28^{ab}	65.93 ± 8.64^a
100	Control	18.67 ± 3.52^b	18.28 ± 1.25^b
	Inoculated	19.57 ± 0.74^b	26.53 ± 4.44^b
150	Control	7.89 ± 1.53^b	16.59 ± 5.45^b
	Inoculated	32.59 ± 12.20^a	42.18 ± 21.66^b

Values are means $\pm$ standard deviation ($n = 5$). Different letters within a column indicate significant differences according to Tukey's HSD test ($p < 0.05$).

the detrimental effects of salt stress.

Correlation between PGPR traits and maize seedling responses

Pearson correlation analysis was conducted to examine potential relationships between bacterial plant-growth-promoting traits and maize seedling growth parameters under salinity stress (Fig. 1). The analysis revealed several significant associations between bacterial physiological characteristics and plant responses. Root elongation showed a moderate positive correlation with bacterial density (OD600, $r = 0.55$) and indole-3-acetic acid production (IAA, $r = 0.34$). Ammonia production was also weakly

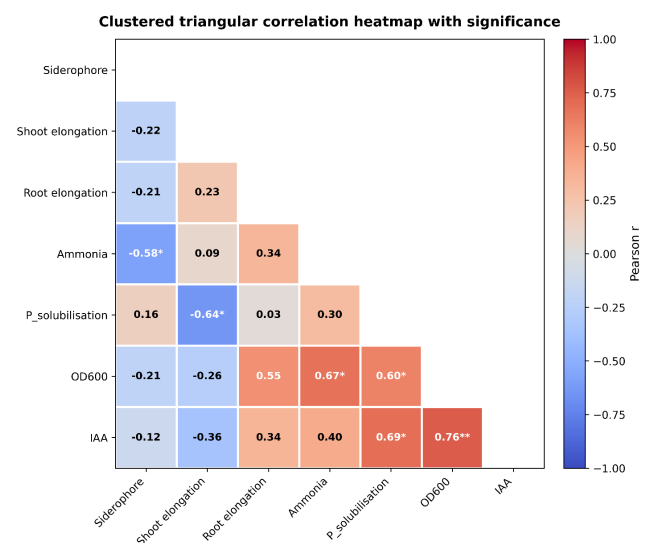


Figure 1. Clustered triangular heatmap showing Pearson correlation coefficients among bacterial plant growth-promoting traits and maize seedling growth parameters under salinity stress. Variables were hierarchically clustered based on correlation distance ($1 - r$). Only the lower triangle of the matrix is displayed. Asterisks indicate significance levels (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$).

positively correlated with root elongation ($r = 0.34$). In contrast, shoot elongation was negatively correlated with phosphate solubilization capacity ($r = -0.64$). These results suggest that bacterial traits related to auxin production and metabolic activity may contribute to improved root development under saline conditions.

Discussion

This study demonstrates that a halotolerant *Paenibacillus* sp. isolated from an arid *sebkha* can retain key plant-growth-promoting functions under high NaCl and substantially mitigate early salt injury in maize. Importantly, the work integrates (i) a salinity gradient assessment of multiple PGP traits with (ii) plant bioassays that separate germination dynamics from post-germination growth, allowing a clearer interpretation of when and how bacterial benefits emerge.

At the microbial level, the strain displayed broad halotolerance (up to 900 mM NaCl) while maintaining high IAA production and phosphate solubilization at moderate-to-high salinity. Trait stability across salinity is a critical selection criterion for bioinoculants intended for salt-affected soils, because osmotic stress often suppresses metabolite production even when growth persists (Radhakrishnan and Krishnasamy 2024).

The bacterium grew optimally at moderate salinity (300 mM NaCl) and tolerated even higher salt, an essential prerequisite for any PGPR intended for saline soils. Similar robust osmotolerance has been documented in halophilic rhizobacteria; for instance, Paul and Nair (2008) reported that a coastal-soil *Pseudomonas* could colonize roots at high salinity by accumulating osmolytes and stress proteins. Our isolate's broad salt tolerance thus suggests it could survive in salt-stressed rhizospheres, an important strength for practical use. Moreover, the *Paenibacillus* isolate maintained or even enhanced several PGP traits under salt stress.

Production of IAA-like molecules remained high up to 300 mM NaCl, declining only at the extreme 900 mM concentration. This resilience is noteworthy because phytohormone synthesis in many bacteria is inhibited by osmotic stress. Consistent with our findings, Egamberdieva (2007) observed that a *Bacillus* strain promoted maize growth in saline soil while producing auxins that stimulated root development. The slight drop in IAA at 900 mM in our study likely reflects a general metabolic limitation at very high osmolarity, but the ability to synthesize growth hormone analogues at moderate salinity could be crucial for sustaining plant root elongation under stress. The isolate's phosphate-solubilizing activity was similarly stable; soluble phosphate concentrations

remain statistically unchanged from 0 to 600 mM NaCl, with only a minor reduction at 900 mM. Many salt-tolerant rhizobacteria likewise continue to mobilize phosphorus under moderate salt stress, ensuring that nutrient acquisition by plants is not compromised (Egamberdieva 2007). This steadiness in P-solubilization suggests our *Paenibacillus* isolate could help alleviate the P deficiency often associated with saline soils, where chemical P availability is limited by high pH and ionic interactions.

Perhaps the most striking trait response was in siderophore production, which significantly increased with rising salinity. At the highest NaCl concentration, the bacterium produced around 95% siderophore units, nearly 30% rise compared to non-saline conditions. This trend is not commonly reported and may indicate an adaptive response whereby the bacterium boosts iron-scavenging mechanisms under salt stress (Hoffmann et al. 2002). Saline soils frequently induce iron deficiency in plants because high salt and pH reduce Fe bioavailability (Shrivastava and Kumar 2015). Siderophore secretion by rhizobacteria can alleviate this by chelating Fe^{3+} and making it more accessible to plants. In fact, recent work has highlighted that siderophore-producing microbes mitigate iron limitation and enhance plant physiology under salinity (Sultana et al. 2021). Our isolate's elevated siderophore output under salt may therefore confer a dual benefit; improving its own survival and simultaneously supplying iron to the maize host. This trait could help counteract salt-induced chlorosis and support better growth, as also noted by Sultana et al. (2021) for other halotolerant PGPR. In contrast, the bacterium's ammonia production declined progressively with salinity, dropping by over 50% at 900 mM NaCl. This outcome is consistent with classical studies showing that excessive salt inhibits biological nitrogen fixation and related enzymes (Zahran 1999). Zahran (1999) observed that high NaCl concentrations disrupt nitrogenase activity, leading to lower ammonia release, a pattern mirrored in our isolate. Thus, while our *Paenibacillus* sp. isolate likely provides some nitrogen to plants at low-medium salt, its N-fixation capacity may be curtailed in extreme salinity. This limitation underscores that not all PGP functions are equally salt-tolerant; nitrogen metabolism appears more salt-

Table 8. ANOVA of salinity-mitigation index (SMI) for shoot and root growth. SMI (%) represents the relative increase in seedling length due to inoculation at each salt concentration.

Response (SMI)	F	p-value	Significance
Shoot length SMI	7.733	0.0218	*
Root length SMI	1.193	0.3662	ns

Significance: ns = not significant; * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

sensitive than siderophore or IAA biosynthesis in this strain.

In the seed bioassay, increasing salinity severely reduced final germination, and, in our experiments, bacterial inoculation did not significantly improve the final germination percentage under any salt condition. By 150 mM NaCl, only 40-50% of seeds germinated regardless of inoculation, indicating that the osmotic and ionic stresses were the dominant determinants of seed viability. This aligns with general observations that maize, a moderately salt-sensitive crop, suffers substantial germination loss beyond 100 mM NaCl (Shrivastava and Kumar 2015). Some previous studies have reported that certain PGPR can enhance seed germination under salt stress, for example, Bano and Fatima (2009) noted higher germination and seedling emergence in maize when co-inoculated with beneficial *Rhizobium* and *Pseudomonas*, attributed to better osmotic adjustment and hormonal stimulation. In our case, however, any such positive effect on final germination was minimal. One explanation may be that our inoculation method (coating seeds with bacteria prior to germination) did not fully overcome the severe water deficit and ion toxicity imposed by NaCl. It is known that if salt levels exceed a critical threshold, even PGP microbes cannot rescue seeds that are physiologically too stressed to germinate (Bano and Fatima 2009). Another possibility is that our strain's benefits manifest more in post-germination growth rather than in the binary outcome of germination itself. Indeed, although final germination percentages were similar, inoculation did influence germination kinetics at intermediate salinity. At 100 mM NaCl, inoculated seeds germinated faster (MGT around 53 h) than uninoculated seeds (MGT around 65 h), effectively maintaining a germination speed comparable to non-saline conditions. This suggests the bacterium helped seeds overcome germination delays caused by salt. The mechanism for this acceleration might involve bacterial production of stress-alleviating compounds. Many PGPR secrete 1-aminocyclopropane-1-carboxylate (ACC) deaminase, which lowers plant ethylene levels and thereby prevents the ethylene-induced inhibition of germination under stress (Nadeem et al. 2010). Although we did not assay ACC deaminase in our isolate, numerous *Paenibacillus* and *Bacillus* strains are known to possess this trait (Nadeem et al. 2010; Sukweenadhi et al. 2018). It is plausible that our *Paenibacillus* sp. isolate produced ACC deaminase or other phytohormones in the seed microenvironment, reducing the deleterious ethylene buildup and allowing quicker radicle protrusion at 100 mM NaCl. By contrast, under the highest salt concentration (150 mM), inoculation did not improve germination rate; intriguingly, the inoculated seeds had a longer mean germination time than controls. This could indicate that

at extreme salinity, the bacterial presence was insufficient to hasten germination and might have even slightly prolonged it, perhaps because the inoculant enabled a few additional, otherwise non-germinating seeds to eventually germinate (thereby increasing final percentage marginally, but raising the average time). Alternatively, the severe stress might have overwhelmed any bacterial mitigation, leading to stochastic germination where only the inherently most salt-tolerant seeds germinated rapidly (mostly in the control group) while others (benefiting from bacteria) did so later. In any case, these germination results highlight that the *Paenibacillus* provided a measurable benefit at moderate salt stress (mitigating germination delay), but its influence waned as salinity reached levels acutely inhibitory to maize seeds. This finding is in line with earlier reports that PGPR can speed up germination and improve uniformity under mild-to-moderate stress, even if ultimate germination percentages remain unchanged (Nadeem et al. 2010; Bano and Fatima 2009).

In contrast to the modest germination effects, the seedling growth results unequivocally demonstrate that *Paenibacillus* isolate inoculation conferred substantial salinity tolerance to young maize plants. Salinity alone severely stunted maize seedling development, as seen in uninoculated controls, a well-documented phenomenon since salt stress inhibits cell expansion, disrupts nutrient uptake, and causes ion toxicity in seedlings (Shrivastava and Kumar 2015). By 150 mM NaCl, control seedlings were extremely dwarfed (on average only 8 mm shoots and 17 mm roots), essentially failing to establish. Remarkably, inoculated seedlings under the same stress grew fourfold taller (shoot around 33 mm) and had roots around 2.5 times longer (around 42 mm) than non-inoculated counterparts. This significant alleviation of salt injury by the bacterium underscores the potency of the plant-microbe interaction. Similar plant growth rescue by PGPR under salinity has been reported in other studies. For example, Bano and Fatima (2009) observed improved shoot elongation and biomass in salt-stressed maize due to bacterial inoculation, which they attributed to better Na⁺/K⁺ homeostasis and enhanced antioxidant defense in the inoculated plants. Likewise, Egamberdieva (2007) found that inoculation with a *Bacillus polymyxa* strain significantly increased maize shoot and root growth in a mildly saline soil, improving nutrient uptake in the process. Our results extend these findings to a higher salinity regime and a different bacterial genus, demonstrating that even a *Paenibacillus* isolate can effectively mitigate salt stress in maize. The pronounced root growth promotion we observed (e.g. nearly double root length at 50-100 mM NaCl with inoculation) is especially notable. Root architecture is crucial under salt stress, as longer roots can explore a larger soil volume for water and nutrients and potentially

avoid surface salt accumulation. PGPR-produced IAA is a likely factor in stimulating root elongation and branching (Sukweenadhi et al. 2018). Sukweenadhi et al. (2018) reported that a *Paenibacillus yonginensis* strain producing high IAA modulated root system architecture and activated stress-responsive genes in ginseng, thereby improving salt tolerance. It is conceivable that our *Paenibacillus* sp., which maintained IAA synthesis under salt, similarly promoted root growth in maize, enabling better water uptake and vigor despite osmotic stress. Additionally, siderophore-mediated iron nutrition could have contributed to more robust seedling development, as adequate iron is essential for chlorophyll formation and enzyme function; a recent review noted that siderophore-producing rhizobacteria enhance overall plant physiological processes in saline soils by relieving iron limitation (Sultana et al. 2021).

Mechanistically, several non-mutually exclusive pathways could underlie the observed growth mitigation. Sustained bacterial IAA production under salinity can stimulate root system development, increasing the effective soil volume explored and improving water and nutrient acquisition. Elevated siderophore production may alleviate iron limitation, indirectly supporting chlorophyll biosynthesis and antioxidant enzyme function under salt stress. In addition, many *Paenibacillus* spp. are known to produce stress-alleviating enzymes and metabolites (e.g., ACC deaminase, exopolysaccharides, compatible solutes, and antioxidant-modulating signals) (Wang et al. 2024). Although these traits were not quantified here, they represent testable hypotheses that align with the observed phenotype and would strengthen causal inference in future work.

Overall, the magnitude of salinity mitigation by this *Paenibacillus* isolate is noteworthy. The “salinity-mitigation index” for shoot length reached around 329% at 150 mM NaCl, quantitatively illustrating that inoculation more than tripled shoot growth relative to uninoculated controls under extreme stress. The large proportional benefit observed at high salinity highlights the potential of this isolate as a candidate bioinoculant for saline conditions. This underscores the potential significance of our isolate as a bioinoculant for enhancing crop resilience in highly saline environments. In practical terms, if these results translate to soil-based conditions, the bacterium could enable maize (and possibly other crops) to not only survive but actively grow in soils with salt levels that would otherwise be prohibitive. This finding contributes to the growing body of literature showing that harnessing naturally salt-tolerant rhizobacteria is a viable strategy to ameliorate salinity stress in agriculture (Shrivastava and Kumar 2015).

Conclusion

The findings of the present study provide compelling evidence that *Paenibacillus* sp. isolate from saline environments can function as an effective plant growth-promoting rhizobacterium (PGPR) under salt stress, with significant implications for improving crop resilience in salinity-affected areas. The isolate not only exhibited robust growth across a wide NaCl gradient but also retained key PGPR functions, including IAA production, phosphate solubilization, and siderophore secretion, under elevated salinity. Notably, its capacity to synthesize siderophores increased progressively with salinity, highlighting an adaptive response with potential agronomic relevance in iron-limited saline soils. Although ammonia production declined at higher salt levels, the preservation of multiple other PGPR traits suggests that this strain remains metabolically competent under osmotic stress, a valuable attribute for bioinoculants intended for saline agriculture.

Importantly, the inoculation of maize seeds with *Paenibacillus* sp. isolate resulted in significant mitigation of salt-induced growth suppression at the seedling stage. While the bacterium did not markedly alter final germination percentages, it accelerated germination kinetics under moderate salinity and markedly enhanced shoot and root elongation under severe salt stress. This growth promotion was particularly evident at 150 mM NaCl, where inoculated seedlings exhibited more than triple the shoot length of controls, underscoring the bacterium's ability to counteract the inhibitory effects of high salinity. Such a level of mitigation, quantified through the salinity-mitigation index (SMI), is rarely reported for a single microbial strain and attests to the isolate's potential utility as a biofertilizer in salt-affected soils.

The novelty of this work lies in the identification and functional characterization of a naturally halotolerant *Paenibacillus* strain with proven efficacy in promoting maize seedling growth under elevated salinity. By demonstrating both physiological resilience and functional competence under stress, the isolate bridges a critical gap between *in vitro* PGPR screening and practical application under abiotic constraints. These findings contribute to the expanding literature advocating for the integration of indigenous, salt-tolerant microbes into sustainable agriculture strategies, particularly for regions facing escalating soil salinization due to climate change and irrigation practices.

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