

Divergent Biostimulant Capacities Under Salinity: PGPR Monoinoculation and a Tri-Strain Consortium Evaluated in Tomato (In Vitro) and Wheat (Pot Culture)

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Abstract

Soil salinity severely limits global crop productivity. Native plant growth-promoting rhizobacteria (PGPR) from saline ecosystems offer a sustainable strategy to alleviate salt stress, yet their efficacy often depends on host and niche. This study evaluated the biostimulant potential of three rhizobacterial isolates—*Staphylococcus xylosus* SF6 (PQ123789), *Pseudomonas* sp. TA3 (PX957002), and *Bacillus simplex* SA1 (PX957145)—recovered from the rhizosphere of halophytes in an Algerian sebkha. Their individual and combined (tri-strain consortium) effects were assessed on tomato under *in vitro* NaCl gradients (0–150 mM) and on wheat cultivated in naturally saline soil. Data were analyzed via two-way ANOVA and Principal Component Analysis. Salt stress significantly impaired all growth parameters in non-inoculated controls. PGPR inoculation markedly mitigated these effects, though responses were strongly modulated by host species and treatment formulation. In tomato, the tri-strain consortium performed best, maintaining 100% germination at 0 mM NaCl and 60% under 100 mM stress, with a 186% increase in fresh biomass. PCA (90.27% total variance) revealed robust, salinity-independent growth promotion by the consortium. Conversely, in wheat, *Pseudomonas* sp. TA3 was most effective, increasing germination by 75%, root length by 100%, and Seedling Vigor Index by 240%. Notably, *S. xylosus* and *B. simplex* performed poorly on wheat, falling below non-inoculated controls, highlighting host-specific incompatibility. These findings demonstrate that PGPR-mediated salt tolerance is not interchangeable; optimal bioinoculant design requires precise matching of strain ecophysiology to target host and substrate. The tri-strain consortium shows promise for tomato, whereas *Pseudomonas* sp. TA3 emerges as a specialized candidate for wheat biofertilization in saline fields, supporting ecotype-adapted microbial consortia for sustainable agriculture in saline-affected regions.

1. Introduction

Soil salinization constitutes a major challenge for modern agriculture, a problem that is further exacerbated by climate change and unsustainable agricultural practices, thereby posing a significant threat to global food security (Singh, 2022). It is estimated that nearly 1.4 billion hectares of land representing more than 10% of the total global land surface are already affected by salinization. This proportion is projected to increase to approximately 32% in the coming years (FAO, 2024). Developing countries are disproportionately impacted by this phenomenon (FAO, 2024). However, some studies suggest that climate change may increase precipitation in certain temperate regions and induce a northward shift of agroclimatic zones, potentially creating localized opportunities for crop production. Nevertheless, these effects remain highly uncertain and are unlikely to offset the globally negative impacts (FAO, 2021, 2024; IPCC, 2022).

Soil salinity imposes osmotic stress on plants, leading to metabolic disturbances and substantial reductions in crop yield, which in turn threaten global agricultural productivity (El Sabagh et al., 2020; Oyiga et al., 2016). This issue is particularly critical for major crops such as wheat and tomato, both of which are highly sensitive to elevated soil salt concentrations (Oyiga et al., 2018; Yarra et al., 2012).

To address soil salinization, a range of mitigation strategies has been proposed, including agronomic practices such as mulching, adaptation of drainage systems, and the selection of salt-tolerant cultivars (Tarolli et al., 2024). In addition to these conventional approaches, environmentally sustainable and biologically based solutions have emerged as particularly promising alternatives. Among these, the application of plant growth-promoting rhizobacteria (PGPR) represents an effective strategy to alleviate the adverse effects of soil salinity on agricultural productivity and to enhance plant tolerance to salt-induced stress (Kumar et al., 2021).

These beneficial microorganisms, notably those belonging to the genera *Pseudomonas*, *Azotobacter*, *Bacillus*, and *Actinobacteria*, enhance plant salinity tolerance through a range of complementary mechanisms (Egamberdieva et al., 2019). These include the improvement of mineral nutrition via biological nitrogen fixation and the solubilization of essential nutrients such as phosphorus and potassium; the activation of induced systemic resistance; the production of bioactive secondary metabolites that confer protection against phytopathogens and pests; and the modulation of endogenous phytohormonal balance, particularly through auxin production and increased abscisic acid accumulation (Dalli et al., 2026; Sagar et al., 2022).

Individually or synergistically, these mechanisms stimulate key physiological processes and reinforce plant adaptive responses to abiotic stress, ultimately leading to enhanced biomass accumulation and improved yield performance under saline conditions (Zaghloul et al., 2024).

The selection of PGPR strains adapted to specific pedoclimatic conditions and target crops, along with their integration into sustainable soil management practices, is therefore of critical importance (Dalli et al., 2026). In this context, the isolation of rhizobacteria from the rhizosphere of halophytic plants represents a particularly relevant approach. Indeed, halophytes, which are naturally adapted to saline environments, host specialized microbial communities with functional traits that are advantageous for plant growth under high salinity conditions (Kumar & Naik, 2021).

This study proposes a three-stage experimental framework to assess the impact of plant growth-promoting rhizobacteria (PGPR) isolated from the rhizosphere of halophytic plants on the growth of two major agronomic species: tomato and wheat. First, bacteria will be isolated from the rhizosphere of halophytic plants using conventional culturing techniques. Second, an *in vitro* assessment of plant growth-promotion (PGP) traits will be conducted to evaluate their capacity to exhibit relevant PGP characteristics. Finally, selected bacterial isolates will be inoculated on tomato and wheat seedlings under both controlled and uncontrolled saline conditions (*in vitro*) and in pot culture, and their effects on plant growth and salt tolerance will be evaluated.

This approach will provide enhanced understanding of the mechanisms whereby PGPR influence plant physiology under salt stress conditions and will determine their potential for enhancing crop productivity in saline-affected environments. Through this investigation, we aim to provide practical solutions for sustainable agriculture by harnessing the intrinsic capabilities of rhizospheric bacteria to support the growth of essential crops in salt-stressed ecosystems.

2. Materials and Methods

2.1. Collection and processing of rhizospheric soil samples

Rhizospheric soil samples were collected from the Garaet El Taref site Fig. 1 (lat 35°41'N, lon 07°08'E), a natural wetland ecosystem characterized by elevated edaphic salinity, situated at the administrative boundary between the wilayas of Khenchela and Oum El Bouaghi in North -Eastern Algeria. This site is designated as one of 50 Ramsar-listed wetlands in Algeria and harbors substantial biodiversity, predominantly composed of a halophytic flora (Aliat et al., 2016; Benzina et al., 2022).

Soil sampling was conducted at a depth interval of 0–20 cm within the rhizosphere of three halophytic plant species: *Salicornia arabica*, *Suaeda fruticosa*, and *Tamarix africana*, with an additional surface sample collected from adjacent unvegetated soil serving as a non-sterile saline substrate for assessing the performance of the strains on wheat. For each plant species, four replicate sub-samples were obtained from the rhizosphere of individual plants, subsequently combined to constitute a composite soil sample representative of each plant species. Soil samples were immediately transferred to containers and were transferred to the laboratory and processed without delay.

2.2. Physicochemical soil analysis

2.2.1. Soil pH determination

Soil pH was determined using a calibrated pH meter following suspension of air-dried soil samples in distilled water according to the standard protocol (AFNOR, 1994).

2.2.2. Electrical conductivity (EC) measurement

Electrical conductivity was measured using a conductivity meter following preparation of a soil-water suspension at a 1:5 mass-to-volume ratio, in accordance with the methodology described by Aubert (1978).

2.2.3. Organic carbon content (OC)

Total organic carbon content was determined by dry combustion in a muffle furnace at high temperature according to the standard procedure (Anne, 1945).

2.2.4. Organic matter (OM) content

Soil organic matter content was subsequently calculated from organic carbon values using the conversion formula: OM (%) = OC (%) × 1.72 (Baize, 2000).

2.3. Isolation of rhizospheric bacteria

Isolation of rhizospheric bacteria was performed using modified serial decimal dilution technique (Sridhar et al., 2025). One gram of soil sample was suspended in 9 ml of sterile physiological saline solution (0.9% NaCl) and vigorously mixed. Sequential decimal dilutions (10^{-1} to 10^{-4}) were prepared by serial transfer. For each dilution, 0.1 ml aliquots were spread uniformly onto nutrient agar medium using a sterile spreader. Petri dishes were incubated at 30°C for 24 hours.

2.4. Purification and characterization of bacterial isolates

To ensure isolate purity, morphologically distinct colonies were selected, sub-cultured onto fresh nutrient agar medium, and incubated at 30°C for an additional 24 hours. phenotypic characterization of isolates were subsequently performed through macroscopic examination (colony morphology, pigmentation patterns, and appearance), microscopic analysis (Gram staining), and relevant biochemical assays (Einizadeh et al., 2025).

2.5. Assessment of plant growth-promotion (PGP) parameters

2.5.1. Indole-3-acetic acid (IAA) production

To evaluate indole-3-acetic acid (IAA) production, bacterial isolates were inoculated into test tubes containing 5 mL of Luria-Bertani (LB) broth supplemented with L-tryptophan (1%, w/v) and incubated at 30°C for 72 hours. Following incubation, culture supernatants were recovered by centrifugation, and IAA concentration was quantified by spectrophotometry at 530 nm using the Salkowski reagent, according to the protocol described by De-Bashan et al. (2008).

2.5.2. Nitrogen fixation

To assess nitrogen fixation capacity, bacterial isolates derived from three soil samples were inoculated onto Winogradsky (WS) medium, a nitrogen-free solid medium. Plates were incubated at 30°C for 48 hours. Bacterial proliferation on this medium indicated nitrogen-fixing capability (Jensen, 1954).

2.5.3. Phosphate solubilization

Bacterial isolates were streaked at the center of Petri plates containing NBRIP (National Botanical Research Institute's Phosphate) medium supplemented with bromophenol blue. Plates were incubated at 30°C for 48 hours. Phosphate solubilization activity was assessed by the formation of a transparent halo zone surrounding the bacterial colony, indicating hydrolysis of insoluble phosphate compounds in the medium (Nautiyal, 1999). The solubilization index (SI) was determined by measuring the solubilization halo around the colony using the following formula: SI = halo zone diameter / colony diameter (Amri et al., 2023).

2.5.4. Hydrogen cyanide (HCN) production

HCN production was evaluated according to the methodology described by Einizadeh et al. (2025). Bacteria were cultured on a specific medium comprising tryptic soy broth (30 g/L), glycine (4.4 g/L), and agar (15 g/L). Whatman No. 40 filter paper, pre-saturated with 0.5% picric acid and 2% sodium carbonate solution, was placed on the inner surface of Petri dish lids, which were then hermetically sealed with parafilm to prevent volatile escape. Following incubation for four days at $28 \pm 2^\circ\text{C}$, the development of an orange-brown coloration on the filter paper indicated HCN production.

2.5.5. Catalase activity test

The catalase test was performed by depositing a small aliquot of 24-hour-old bacterial culture onto a microscope slide. A 3% hydrogen peroxide (H₂O₂) solution was subsequently added to the culture. Catalase activity was confirmed by the immediate evolution of oxygen bubbles (effervescence) within one minute following H₂O₂ addition, indicating a positive reaction. Isolates demonstrating no effervescence were classified as catalase-negative (Taylor & Achanzar, 1972).

2.5.6. Antifungal evaluation

The antifungal activity of bacterial isolates was assessed using the direct confrontation technique on Potato Dextrose Agar (PDA) medium. Agar discs (Ø 6 mm) containing individual bacterial isolates were positioned adjacent to *Fusarium* sp. mycelium discs of equivalent diameter on Petri plates containing PDA medium. Plates were incubated at 30°C for 7 days. Fungal growth inhibition was quantified by measuring the reduction in fungal colony radius relative to a positive control plate lacking bacterial inoculum, using the following formula: % Inhibition = [(R control - R test) / R control] × 100, where R control is the radial growth of the fungus in the control plate, and R test is the radial growth in the dual culture plate (Sebihi et al., 2016).

2.6. Molecular identification

Bacterial genomic DNA was extracted using the GF-1 Nucleic Acid Extraction Kit (Vivantis Technologies, Malaysia) according to the manufacturer's recommendations. DNA concentration was determined using a Nanodrop spectrophotometer (NanoDrop™ One, USA).

Amplification of the 16S rRNA gene was performed using the primers 27F (5'-AGAGTTTGATCCTGGCTCAG-3') and 1492R (5'-CCGTCAATTCCTTTGAGTTT-3') (Edwards et al., 1989). The PCR reaction mixture (50 µl) contained 1.25 U of Taq polymerase, 25–50 ng/µl of template DNA, 0.3 µM of each primer, and 1.5 µM MgCl₂. Amplification was carried out with an initial denaturation at 94°C for 12 minutes, followed by 30 cycles at 94°C (30 s), 55°C (30 s), 72°C (1 min 40 s), and a final extension at 72°C for 7 minutes, using a thermocycler (SipliAmp Thermal Cycler, Applied Biosystems).

PCR products were separated by electrophoresis on a 1.5% agarose gel and visualized under UV light. The products were purified (Vivantis Clean-Up kit) and sequenced in both directions. Identification of the isolates was performed by comparing the sequences with the GenBank database using BLAST (<https://blast.ncbi.nlm.nih.gov/Blast.cgi>), based on the percentage of homology with reference strains.

Sequences were aligned using the multiple sequence alignment program ClustalW, implemented in MEGA version 9.0. Phylogenetic trees were then constructed using the neighbor-joining method (Saitou and Nei, 1987) based on the Kimura two-parameter model. The robustness of the tree topology was assessed by bootstrap analysis with 1000 replications (Felsenstein, 1985).

2.7. Plants tests

2.7.1. In vitro assay on tomato

Seeds of tomato (*Solanum lycopersicum* var. *cerasiforme*) were surface-sterilized using 70% ethanol for 2 min, followed by 1% sodium hypochlorite for 3 min, and then rinsed with sterile distilled water (Shishido et al., 1999).

Surface-sterilized tomato seeds were subjected to four levels of NaCl-induced osmotic stress (0, 50, 100, and 150 mM) on nutrient medium in Petri dishes. For each treatment, 10 sterile seeds were immersed in 20 mL of bacterial suspension adjusted to approximately 10⁸ CFU/mL. Three selected isolates (SF6, TA3, SA1) were applied individually, along with their three-strain consortium. Inoculation was carried out with gentle shaking (80 rpm) for 1 hour at room temperature.

The inoculated seeds were then aseptically transferred and evenly spaced onto the surface of the corresponding Petri dish, which was subsequently sealed with Parafilm to maintain sterility and humidity. The experimental factors were Microbial Treatment (5 levels: non-inoculated Control, SF6, TA3, SA1, and Consortium) and NaCl Concentration (4 levels: 0, 50, 100, and 150 mM), arranged in a completely randomized design. The experimental unit consisted of a single Petri dish containing 10 seeds.

Dishes were randomized and incubated in the dark at 30 ± 1°C for 14 days. Growth parameters were recorded.

2.7.2. Growth parameter assessment

Following morphological parameters were recorded:

Germination percentage (GP), seedling fresh weight (FW), seedling dry weight (DW), shoot length (SL), and root length (RL). Seedling height was measured from the base of the stem to the apical meristem. Dry weight was determined after oven-drying samples at 70°C for 48 h until a constant mass was achieved. Two vigor indices were derived from the collected data:

Seedling Vigor Index (SLVI) = Seedling Length (cm) × Germination Percentage (%).

Seedling Weight Vigor Index (SWVI) = Seedling Dry Weight (g) × Germination Percentage (%). as described by Abdul-Baki and Anderson (1973); Kheloufi and Mansouri (2025).

2.7.3. Pot growth experiment on wheat

Seeds of durum wheat (*Triticum durum* L. var. *Waha*) were surface-sterilized using 70% ethanol for 2 min, followed by 1% sodium hypochlorite for 3 min, and then rinsed with sterile distilled water (Shishido et al., 1999). Three bacterial isolates (SF6, TA3, SA1) were cultured separately, resuspended, and adjusted to approximately 10⁸ CFU/mL. The sterilized seeds were immersed in the corresponding bacterial suspension (individual isolate or consortium of the three strains in equal volumes) with shaking (80 rpm) for 2 h at room temperature. A non-inoculated control treatment, consisting of immersion in sterile PBS alone, was also included.

Ten inoculated seeds were sown uniformly per pot (150 mL capacity) at a depth of 1–2 cm. The substrate used was a non-sterile saline soil collected from a non-vegetated natural site, previously homogenized, sieved (4 mm mesh), and characterized. The physico-chemical properties of the soil are presented in Table 1. Each pot constituted an experimental unit.

Table 1
Chemical and physical characteristics of examined soils.

Soils	pH	EC (mS/cm)	OM (%)	Org C (%)	Total bacteria (UFC/g)	Localization
Soil 1 <i>Tamarix africana</i>	8,67 ± 0,05	9,59 ± 0,5	11	6,38	2,8 x10 ⁵	Lat 35° 38' 28.635"N Lon 7° 0' 48.991"E
Soil 2 <i>Suaeda fruticosa</i>	7,80 ± 0,07	9,78 ± 0,1	15	8,7	3,11 x10 ⁵	Lat 35° 38' 28.032"N Lon 7° 0' 48.059"E
Soil 3 <i>Salicornia arabica</i>	7,92 ± 0,02	9,39 ± 0,1	15,3	8,87	4,8 x10 ⁴	Lat 35° 38' 27.217"N Lon 7° 0' 49.116"E
Soil 4 Bare soil	7,83 ± 0,01	17,02 ± 0,5	21	12,18	-	Lat 35° 38' 27.36"N Lon 7° 0' 49.714"E

The trial was conducted under shelter in semi-controlled conditions, exposed to the photoperiod and natural fluctuations in ambient temperature and humidity. The adopted design was a randomized complete block design comprising four replicates (blocks). The five microbial treatments (SF6, TA3, SA1, Consortium, Control) were randomized within each block. Irrigation was carried out manually twice a week. After three weeks of growth, morphological parameters were recorded following the same procedure as for tomato.

2.8. Statistical analysis

Statistical analyses and principal component analysis (PCA) were performed using Statistica 6.0. To evaluate the effect of bacterial inoculations under different salt stress conditions in tomato, a two-way ANOVA (Treatments × Salinity) was performed. Differences between the inoculated treatments and the non-inoculated control for each salinity level were validated using Dunnett's post-hoc test at a significance level of α = 0.05.

3. Results

3.1. Soil physicochemical analysis and correlation with PGP parameters

Correlation matrix analysis Fig. 2 revealed that soil organic matter was strongly associated with several beneficial microbial functions, notably indole-3-acetic acid (IAA) production and antifungal activity, indicating that organic matter-rich soils promote the activity of plant growth-promoting bacteria. Microbial cell density (CFU/g) was also positively correlated with these functions, suggesting that elevated microbial biomass is associated with greater organic matter availability in the soil. Conversely, elevated pH and increased salinity were negatively correlated with bacterial abundance and IAA production and antifungal activity, underscoring the inhibitory role of these abiotic constraints on microbial activity and community composition in the soil. Overall, slightly acidic soils enriched in organic matter and moderately saline conditions appeared most favorable for the development of functional and plant-beneficial microbial communities at the study site.

The physicochemical soil analysis data Table 1 and Fig. 2 revealed that the presence of halophytic vegetation, particularly *Tamarix africana*, *Suaeda fruticosa*, and *Salicornia arabica*, contributed to maintaining moderate salinity levels and enriching soil organic matter content, in contrast to unvegetated soil, which exhibited elevated salinity and accumulated organic matter of presumed limited bioavailability. These observations confirm the essential role of halophytic plants in stabilizing and ameliorating salt-affected soils in sebkha ecosystems.

3.2. PGP traits of rhizobacteria

Seven isolates were recovered and purified from the rhizosphere of each plant species. For each soil sample, one isolate exhibiting the most favorable plant growth-promotion (PGP) characteristics was selected for subsequent field and controlled experiments with wheat and tomato, namely isolates SF6, TA3, and SA1 Table 2.

Table 2
Plant growth promoting traits of inoculated bacteria

Strain code	Species	Accession Number (NCBI)	Origin (Plant Rhizosphere)	Catalase	Oxidase	Gram	Cell shape	N Fixation	Phosphate index	HCN	Anti-Fusarium sp.	IAA (µg/m
TA3	<i>Pseudomonas</i> sp.	PX957002	<i>Tamarix africana</i>	+	+	-	Rod	+	0.0	+	75%	0
SF6	<i>Staphylococcus xylosus</i>	PQ123789	<i>Suaeda fruticosa</i>	+	-	+	Coccus	+	0.0	-	62.5%	49
SA1	<i>Bacillus simplex</i>	PX957145	<i>Salicornia arabica</i>	+	+	+	Rod	-	0.0	-	62.5%	0
(+: production detected; (-): no production.												

Assessment of PGP properties demonstrated that all isolates possessed antifungal activity Table 2. Isolate SF6 demonstrated nitrogen fixation capacity and produced indole-3-acetic acid (IAA) at concentrations reaching 49 µg/mL, with no detectable. phosphate solubilization activity. This isolate also produced hydrogen cyanide (HCN). In contrast, isolate TA3 exhibited nitrogen fixation activity and HCN production, while isolate SA1 was characterized solely by antifungal activity, with no expression of the other tested parameters.

3.3. Plant response to bacterial inoculation

3.3.1. Tomato

Seed germination rates in bacterial-inoculated treatments, particularly the three-strain consortium TA3 + SF6+SA1, maintained relatively high germination rates (Fig. 4B) even at 100 mM NaCl (approximately 65%), in comparison to the uninoculated control, which exhibited progressive decline with increasing NaCl concentration, demonstrating a potential protective role of these isolates against salinity stress.

Root length (Fig. 4A) followed a similar trend. Under non-saline conditions (0 mM), all bacterial isolates induced significant increases in root length relative to the control, with notably superior performance observed for the bacterial consortium. Under salinity stress, root length was substantially reduced in the control treatment, whereas inoculated seedlings maintained marginally greater root length, particularly at 50 mM NaCl, illustrating an attenuating effect of bacterial inoculation on salt-induced inhibition of root elongation.

Concerning both fresh (Fig. 4C) and dry (Fig. 4D) biomass, substantial improvements were also observed in inoculated seedlings. At 0 mM NaCl, the highest fresh weight was recorded for the combined treatment TA3 + SF6+SA1 (0.82 g), followed by TA3 and SF6 (0.28 g). Under salt stress, biomass decreased but remained significantly elevated in inoculated samples compared to the control, particularly at 50 mM NaCl. An analogous pattern was observed for dry weight, with marked superiority of the bacterial consortium across all tested saline conditions. The seed vigor index (SLVI) (Fig. 4E) and seedling weight vigor index (SWVI) (Fig. 4F) corroborated these integrated observations, combining effects on both germination and growth parameters.

3.3.2. Wheat

Analysis of results revealed differential effects of individual isolates on wheat growth parameters under salinity conditions Fig. 6. Isolate TA3 emerged as the most efficacious treatment Fig. 7. Specifically, it induced significant increases in fresh weight and dry weight of seedlings relative to the control (Fig. 6C), reaching approximately 1.6 g and 0.7 g, respectively, reflecting enhanced biomass accumulation. This trend was corroborated by root and shoot length measurements (Fig. 6B), which similarly demonstrated increased growth under TA3 treatment, with values reaching 190 mm. This pattern was also reflected in seed germination percentage, which attained 70% under TA3 inoculation, contrasting with lower values observed for isolates SF6 and SA1.

The combination of isolates (TA3 + SF6+SA1) demonstrated intermediate effects: although less effective than TA3 alone, this consortium nonetheless improved wheat growth relative to the uninoculated control.

3.3.3. Principal Component Analysis

The analysis revealed a strongly unidimensional variance structure Fig. 8. The first two principal components accounted for 90.27% of the total variance in the dataset, with an overwhelming contribution from Principal Component 1 (PC1: 79.40%) and a secondary contribution from Principal Component 2 (PC2: 10.87%).

The F1 axis constitutes a perfectly ordered dose-response gradient: the 0 mM treatments occupy the most negative coordinates ($F1 < 0$), while those at 150 mM are positioned at the positive end ($F1 > 1$). This structuring alone accounts for 79.40% of the total variance, confirming that NaCl concentration is the dominant environmental factor controlling germination. The underlying mechanism is twofold: reduction in water availability through osmotic effect, and accumulation of toxic Na^+ and Cl^- ions disrupting the ionic balance of the embryo (Munns & Tester, 2008).

The F2 axis (10.87%), although secondary in terms of variance, is biologically decisive for evaluating strain efficacy. At 0 mM NaCl, the separation of the Combin (0) and SF1(0) treatments from the Control (0) on F2 reflects a direct biostimulation effect attributable to the production of indole-3-acetic acid (IAA) and ACC deaminase. These mechanisms reduce inhibitory ethylene levels, promote root elongation (RL), and enhance germination vigor (SVI). At 150 mM, the relative maintenance of the combined treatments in a favorable position on F2 is consistent with bacterial induction of osmotic tolerance mechanisms, notably the accumulation of osmoprotectants (proline, glycine betaine) reducing osmotic stress on the embryo (Yang et al., 2009; Glick, 2014).

The grouping of these five variables in the same sector of the correlation circle (F1 loadings between -0.75 and -0.82 , $\cos^2 > 0.56$) indicates that they share a common mechanistic basis under salt stress. Their simultaneous improvement through PGPR inoculation reflects a global action on germination physiology, rather than an isolated effect. This suggests that the strains act upstream on integrative signaling pathways (hormonal balance, osmotic adjustment) rather than on a single parameter.

4. Discussion

The associations between rhizospheric bacteria and cereal and vegetable crops have received considerable attention in the scientific literature (Einizadeh et al., 2025; Sebihi et al., 2016). However, the potential of rhizospheric microorganisms from halophytic plants such as *S. arabica*, *S. fruticosa*, and *T. africana* to promote plant growth (PGP) and stimulate the development of wheat and tomato under salinity stress conditions remains rare. The present results elucidate the potential utility of these bacteria as biological fertilizers in sustainable agriculture.

Analysis of soils beneath halophytic plant communities revealed that soil organic matter (OM) content was positively and significantly correlated with key plant growth-promoting (PGP) functions, including indole-3-acetic acid (IAA) biosynthesis, biological nitrogen fixation, and antifungal activity. These findings suggest that OM plays a pivotal role in sustaining and enriching plant growth-promoting rhizobacteria (PGPR) populations, a conclusion consistent with those previously reported by Chen et al. (2003) and Javed et al. (2021). Furthermore, microbial cell density, expressed as colony-forming units per gram of dry soil (CFU g⁻¹), exhibited a strong positive correlation with the aforementioned functional traits, indicating that higher microbial biomass is associated with greater organic matter availability and, consequently, enhanced expression of PGP activities. This observation aligns with the findings of Dalli et al. (2020) and Prommer et al. (2020), who demonstrated a direct relationship between microbial biomass and organic carbon dynamics in soil ecosystems. In contrast, elevated soil pH and high salinity were negatively correlated with overall bacterial abundance; nevertheless, these physicochemical constraints may simultaneously exert selective pressure that favors the establishment of functionally specialized, halotolerant or halophilic bacterial communities capable of sustaining PGP activities under saline stress conditions. This dual effect of salinity suppressing total microbial load while selecting for functionally resilient taxa, is in agreement with the recent observations of Wang et al. (2025).

Molecular identification of bacterial strains isolated from the rhizosphere of halophytic plants enabled the characterization of three species exhibiting noteworthy plant growth-promoting (PGP) attributes Fig. 3, namely *Bacillus simplex* PX957145, *Staphylococcus xylosus* PQ123789, and *Pseudomonas* sp. PX957002 Both in vitro biochemical assays and in pot inoculation experiments confirmed the capacity of these isolates to enhance wheat and tomato growth and to mitigate the deleterious effects of salinity stress across low-to-moderate NaCl concentration ranges Fig. 5. These results are consistent with analogous studies conducted on the rhizosphere of *Tamarix* sp., in which comparable PGP bacterial communities were reported by Bakelli et al. (2022).

Several *Bacillus* spp. with well-documented PGP properties have been previously identified in the rhizosphere of *Suaeda fruticosa* (Madhurankhi Goswami & Suresh Deka, 2020) and *Salicornia* sp. (Saraswathi Krishnamurthi, 2024; Yamamoto et al., 2020), further corroborating the present findings. Additionally, Hidri et al. (2022) demonstrated that *S. fruticosa* harbors a taxonomically diverse rhizospheric microbiome, encompassing genera such as *Glutamicibacter* sp. and *Pseudomonas* sp., whose members exert beneficial effects on host plants by modulating antioxidant defense mechanisms and enhancing soil biological activity, thereby alleviating salinity-induced physiological impairment.

Inoculation with isolate SA1, identified as *Bacillus simplex* PX957145, yielded significant improvements in tomato growth parameters under salt stress conditions, including marked increases in fresh and dry biomass as well as root elongation. These outcomes are in agreement with the observations reported by Nawaz et al. (2020) and Arminjon and Lefort (2025). This growth-promoting effect is further substantiated by Patani et al. (2023), who demonstrated that several *Bacillus* spp. significantly elevate soluble sugar concentrations in tomato plants subjected to salinity stress, a physiological response associated with osmotic adjustment and stress tolerance.

The identification of the *S. xylosus* PQ123789 strain as a halotolerant rhizobacterium associated with *S. fruticosa* provided further confirmation of its ability to promote growth in both wheat and tomato. These findings are consistent with those of Romero et al. (2016), Kumar and Naik (2021) and Lohith Kumar et al. (2023), the latter having demonstrated siderophore production capacity in this species alongside significant stimulation of plant growth. Complementary evidence reported by Nagaraju et al. (2021) further indicates that inoculation with *S. xylosus* enhances enzymatic activity and elevates proline accumulation in chickpea leaves, thereby improving crop productivity under salt stress conditions. The plant growth-promoting traits of *S. xylosus* make it a promising candidate for biofertilizer development. However, its deliberate release into agricultural environments raises biosafety concerns distinct from its traditional use in fermented meats. Unlike controlled food fermentation, field inoculation involves an open system exposing soil microbiota, crops, and potentially immunocompromised individuals to high bacterial loads (Chouhan et al., 2023). A primary risk is the species' capacity to serve as a reservoir of antibiotic resistance (AMR) genes. This is exemplified by the recent identification of a multidrug-resistant *S. xylosus* strain on avocados, carrying resistance genes to multiple antibiotic classes (Tenea & Angamarca, 2025). Such findings highlight the potential for environmental dissemination of AMR via the biofertilizer itself. Furthermore, the opportunistic pathogenicity of *S. xylosus*, demonstrated by its role in a severe respiratory outbreak in immunosuppressed rats (Detmer et al., 1991), cannot be ignored in an agricultural context involving potential human or animal exposure. These concerns are reflected in the European Food Safety Authority's decision not to grant *S. xylosus* "Qualified Presumption of Safety" status, despite its history in food fermentation (Seitter, 2016). A safe agronomic application therefore requires rigorous, strain-level genomic screening for AMR genes, virulence factors, and toxigenic potential prior to any field deployment.

The capacity of the genus *Pseudomonas* spp. to alleviate salt stress in crop plants is well established in the literature (Ahmad et al., 2013; Nakbanpote et al., 2014). In the present study, isolate TA3 (*Pseudomonas* sp. PX957002) inoculated onto wheat demonstrated a competitive advantage over indigenous soil microflora under saline conditions, resulting in measurable growth stimulation Fig. 7. These properties are in line with previously documented outcomes for *P. fluorescens* and *P. syringae* (Ahmad et al., 2013; Dalli et al., 2026; Nakbanpote et al., 2014). Supporting these observations, Mehrabi et al. (2024) reported

an approximately 18% increase in foliar protein content in inoculated plants, a finding that corroborates the results obtained in the present study and underscores the role of *Pseudomonas* spp. in enhancing nitrogen metabolism under osmotic stress.

The TA3 (*Pseudomonas* sp. PX957002) strain behaved as a halotolerant plant growth-promoting rhizobacterium (PGPR). Inoculation with TA3 (*Pseudomonas* sp. PX957002) alleviated the deleterious effects of salinity on wheat by improving germination rates (+ 30%), doubling dry biomass production (0.62 g vs. 0.34 g), and substantially increasing the seedling vigor index (SLVI: 8519 vs. 2432). As a catalase-positive strain, TA3 (*Pseudomonas* sp. PX957002) likely contributes to salinity stress mitigation by reducing the accumulation of reactive oxygen species (ROS), particularly hydrogen peroxide, while simultaneously stimulating antioxidant defense systems. These findings are in agreement with those reported by Hossain and Dietz, 2016.

In contrast, the SF6 (*Staphylococcus xylosus* PQ123789) and SA1 (*Bacillus simplex* PX957145) strains exacerbated the negative effects of salinity on wheat. Seed germination declined drastically (20%), and seedling development was almost completely suppressed. Under stress conditions, these strains may act as aggressive competitors for water and nutrients at the expense of the seed, or alternatively may produce phytotoxic compounds in response to salt stress, as previously suggested by Saraf et al., 2014.

With respect to the underlying mechanisms, isolates SF6 (*S. xylosus* PQ123789), TA3 (*Pseudomonas* sp. PX957002), and SA1 (*B. simplex* PX957145) were found to operate through multiple complementary stimulatory pathways that collectively attenuate the physiological impacts of salt stress. These include hydrogen cyanide (HCN) production, indole-3-acetic acid (IAA) biosynthesis, and biological nitrogen fixation, mechanisms whose relevance under saline conditions has been previously substantiated by Sharma et al. (2021). In addition, all three isolates exhibited antifungal activity against phytopathogenic fungi, mediated either through the induction of systemic plant defenses or the biosynthesis of bioactive antimicrobial compounds, as previously described by Elshahat et al. (2016). The concurrence of these functional traits across all tested strains reinforces their potential as multifunctional bioinoculants for use in salt-affected agricultural systems.

When the three-strain consortium (TA3 + SF6 + SA1) was applied, the measured parameters (SLVI: 4230; SWVI: 17) returned to values close to or slightly higher than those of the control treatment. This observation suggests the occurrence of microbial competition within the rhizosphere. Although TA3 (*Pseudomonas* sp. PX957002) retained part of its protective effect, a significant proportion of its metabolic activity was likely diverted toward competition with SF6 (*Staphylococcus xylosus* PQ123789) and SA1 (*Bacillus simplex* PX957145). Consequently, the biostimulant effect was diluted, although TA3 (*Pseudomonas* sp. PX957002) still partially buffered the harmful impact of the two other strains Fig. 7.

Observations by Mokrani et al. (2020), whose work similarly highlighted the benefits of multi-strain consortia relative to monospecific inoculants. Furthermore, our findings for tomato are concordant with data from Damodaran et al. (2023), confirming the capacity of the consortium to enhance growth in saline environments.

In tomato, salinity was identified as the major regulating factor ($p < 0.001$). Bacterial inoculation produced a significant effect at 100 mM NaCl, doubling the germination success compared with the control treatment (60% vs. 30%). However, at 150 mM NaCl, salinity exceeded the physiological tolerance threshold of tomato plants, thereby abolishing any beneficial bacterial effect.

A comparison between wheat and tomato responses revealed that wheat tolerated the basal salinity level of saline soil more effectively, whereas tomato exhibited a rapid decline in performance at 150 mM NaCl.

ANOVA analysis further highlighted that, under non-saline conditions (0 mM NaCl), TA3 (*Pseudomonas* sp. PX957002) did not significantly improve tomato biomass production. However, under moderate salt stress (50 mM NaCl), control plants lost 78% of their fresh weight, whereas TA3 (*Pseudomonas* sp. PX957002)-inoculated plants exhibited only a 29% reduction. These results indicate that TA3 (*Pseudomonas* sp. PX957002) acts primarily as an osmotic stress alleviator rather than a constitutive growth promoter. Several studies have demonstrated that bacteria belonging to the genus *Pseudomonas* spp. mitigate salinity-induced osmotic stress by enhancing compatible osmolyte accumulation, improving rhizospheric water retention, maintaining ionic homeostasis, and stimulating antioxidant defenses, thereby preserving cellular turgor and plant water balance Peng et al., 2023; Zboralski and Filion, 2023.

Under non-saline conditions (0 mM NaCl), the consortium (TA3 + SF6 + SA1) displayed a remarkable synergistic effect, tripling tomato fresh weight compared with the control treatment (0.815 g vs. 0.286 g). However, as salinity increased to 50 or 100 mM NaCl, the consortium became less effective than TA3 (*Pseudomonas* sp. PX957002) alone. This behavior may be explained by the fact that, under optimal conditions, the bacterial consortium efficiently occupies complementary ecological niches and stimulates plant growth through multiple mechanisms. Under saline stress, however, competition for resources intensifies, and the presence of SF6 (*Staphylococcus xylosus* PQ123789) and SA1 (*Bacillus simplex* PX957145) may either divert metabolic resources or interfere with the protective activity of TA3 (*Pseudomonas* sp. PX957002), as proposed by Saraf et al., 2014.

Nevertheless, consortium efficacy proved highly dependent on the conditions of our assays. Under controlled culture conditions (in tomato), the consortium induced notable growth stimulation. Conversely, on wheat in non-sterile saline soil, consortium efficacy in promoting plant growth was reduced relative to the tomato assay, suggesting limitations in its competitiveness against indigenous soil microflora and species-specificity to the tested plant. Therefore, additional field trials should be conducted on multiple plant varieties and species, comparing single-strain and consortium inoculation strategies.

5. Conclusion

To better capitalize on these observations, in-depth characterization of additional growth-promotion parameters is warranted. Among the tested isolates, strain SA1 (*Bacillus simplex* PX957145) demonstrated superior efficacy for tomato, whereas TA3 (*Pseudomonas* sp. PX957002) distinguished itself for wheat.

Under optimal conditions (0 mM NaCl), the bacterial consortium SA1 (*Bacillus simplex* PX957145) + TA3 (*Pseudomonas* sp. PX957002) + SF6 (*Staphylococcus xylosus* PQ123789) efficiently partitions ecological niches and promotes plant growth through multiple axes validating the concept of multi-strain microbial synergy. Under salt stress, however, competition for resources intensifies dramatically, and the presence of strains SF6 (*Staphylococcus xylosus* PQ123789) and SA1 (*Bacillus simplex* PX957145) either depletes energy reserves or impairs the protective activity of strain TA3 (*Pseudomonas* sp. PX957002).

The conducted investigations confirm that isolation of plant growth-promoting bacteria from halophytic plants constitutes an effective strategy for improving plant growth under salinity conditions. However, the efficacy of the isolates is strongly dependent on the plant species and cultivation conditions. This variability in response underscores the crucial importance of validating inoculants across diverse edaphic contexts. Furthermore, although our study demonstrates a significant effect of inoculation with TA3 (*Pseudomonas* sp. PX957002) and the consortium on wheat growth under saline conditions, the absence of genotypically contrasting lines (tolerant vs. sensitive) constitutes a recognizable limitation of our experimental design. Future work should therefore include such lines in order to determine whether the beneficial effect of the inoculant is amplified in naturally sensitive genotypes or, conversely, limited in already tolerant genotypes.

Declarations

Competing Interests

The authors declare that they have no competing financial or non-financial interests that are directly or indirectly related to this work.

Author Contributions

Conceptualization: Y.D.; Supervision: Y.D.; Methodology: Y.D., A.G., D.D.; Formal analysis: Y.D., A.G., D.D.; Data curation: Y.D., A.G., D.D.; Writing—original draft preparation: Y.D., A.G., D.D.; Writing—review and editing: Y.D. All authors have read and agreed to the published version of the manuscript.

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Figures

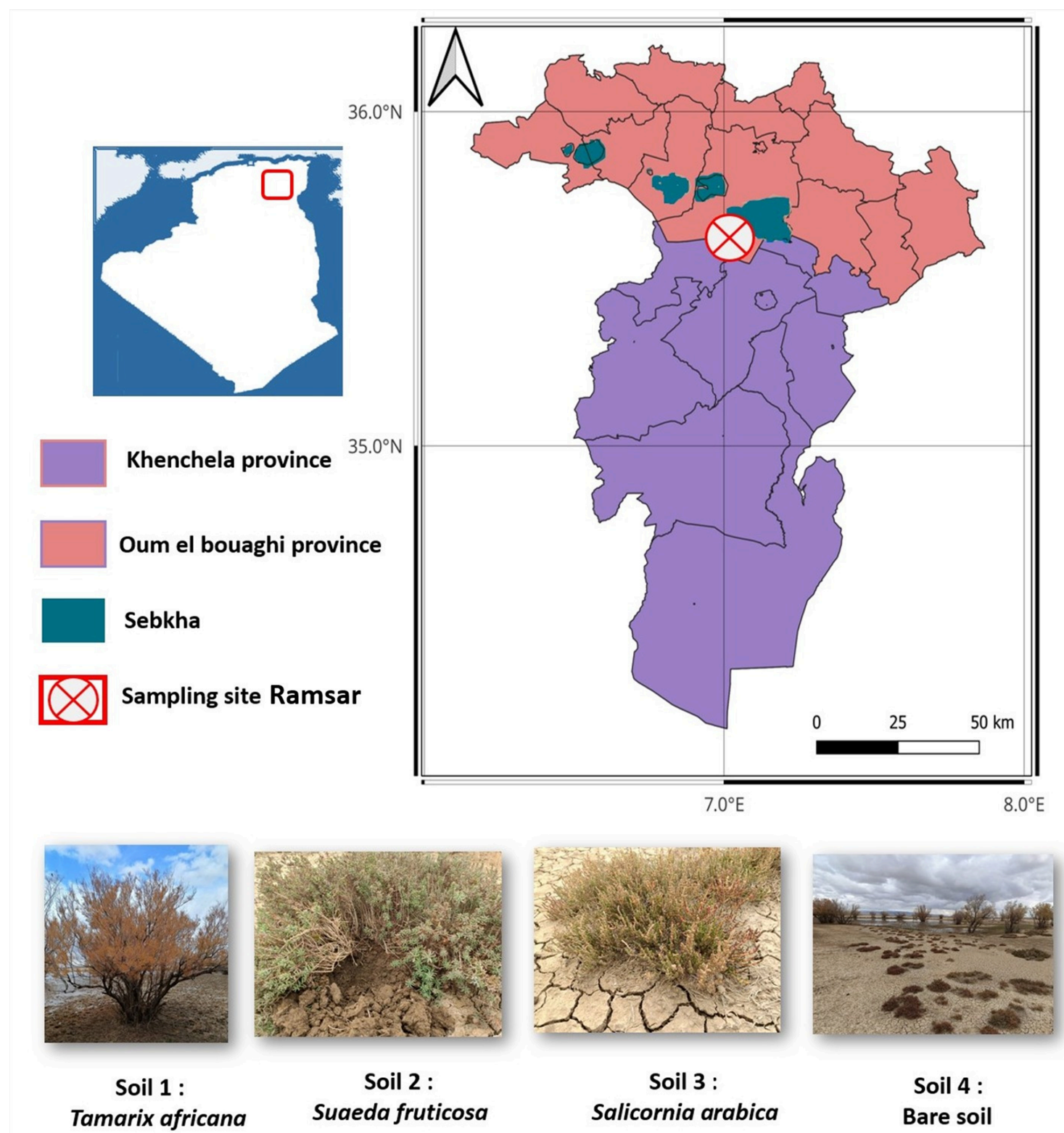


Figure 1

Geographic location of the sampling site.

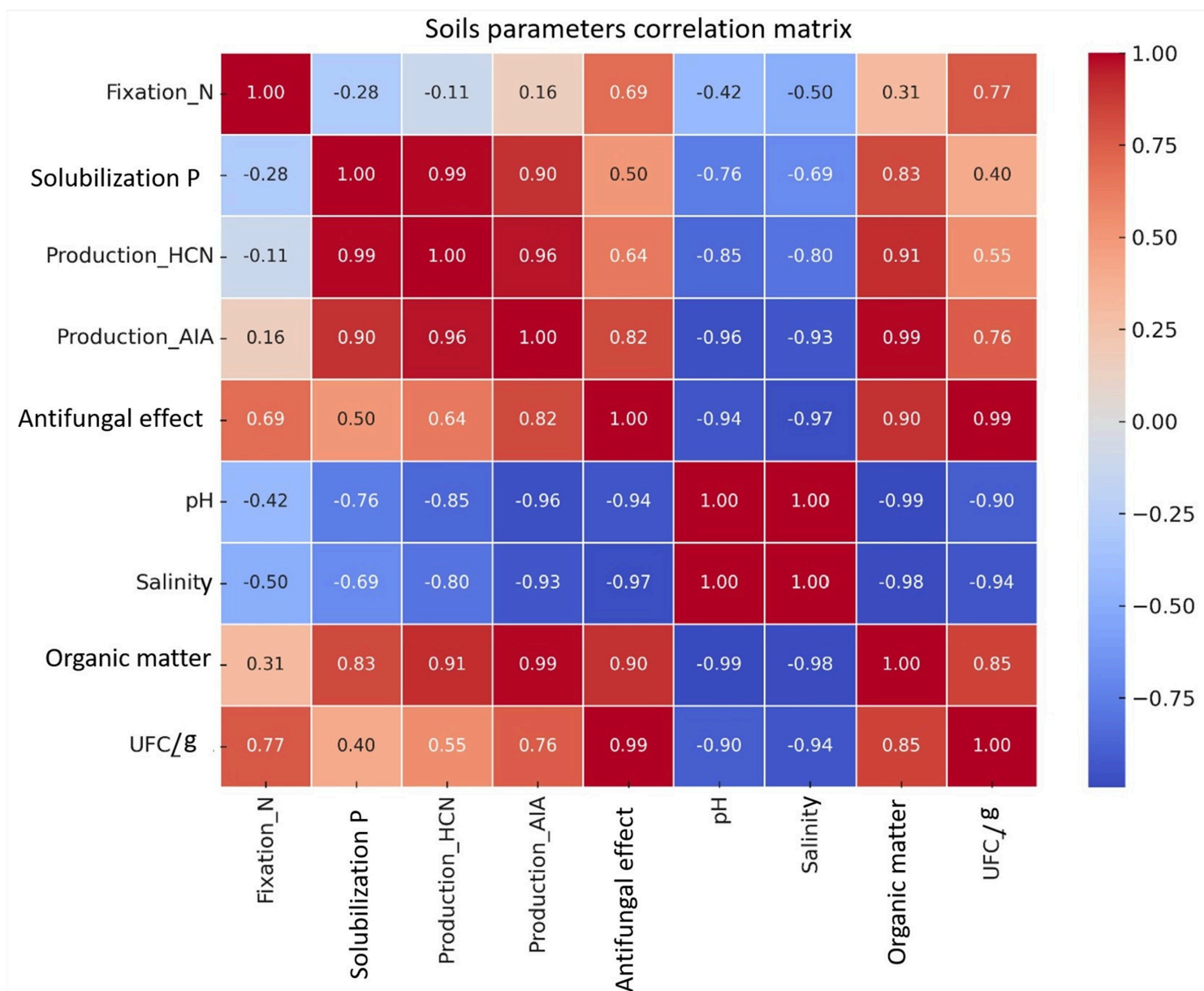


Figure 2

Linking soil physicochemical properties to bacterial Plant Growth-Promoting (PGP) traits a correlation analysis.

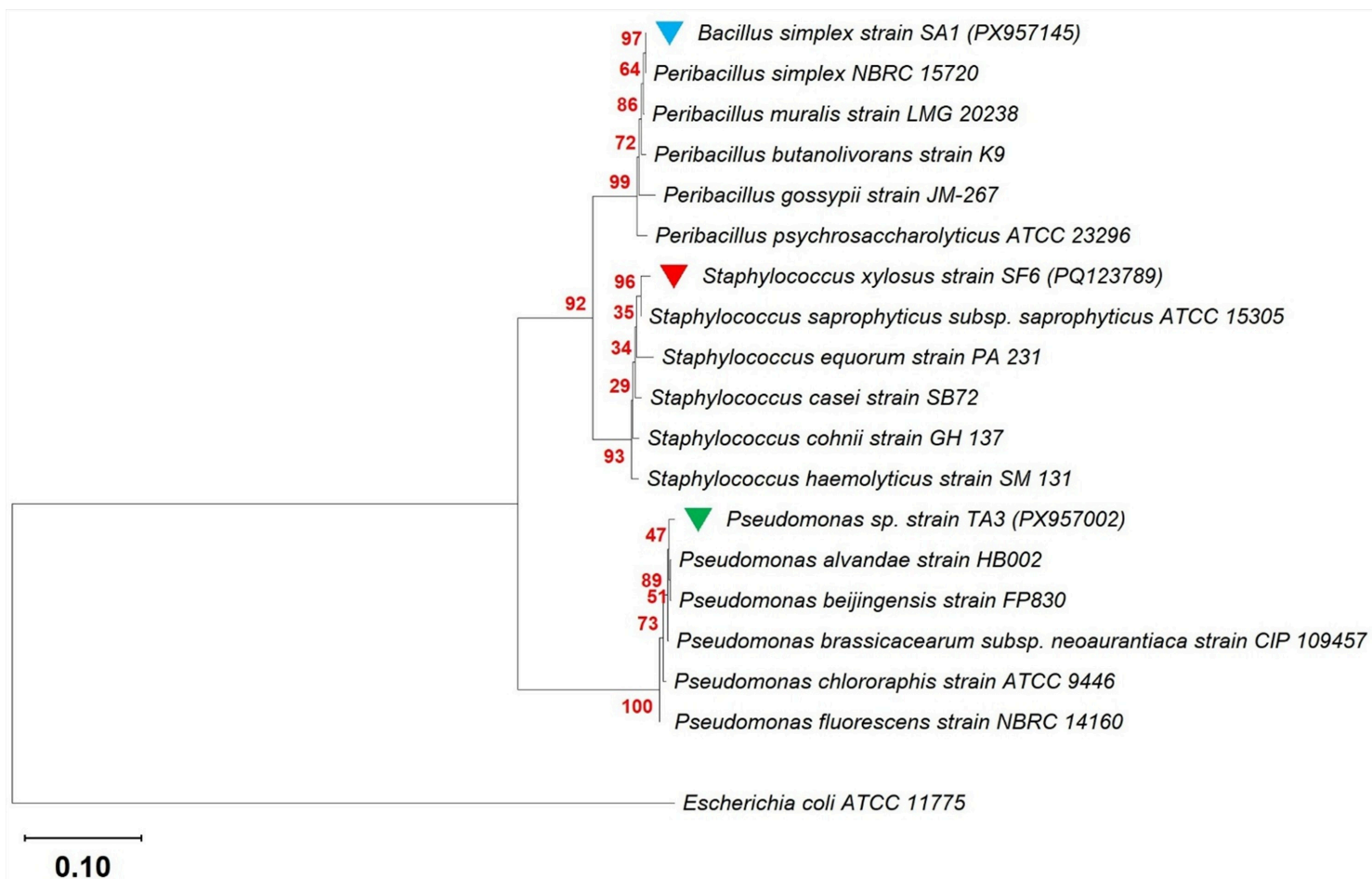


Figure 3

Neighbor-joining phylogenetic tree based on 16S rRNA gene sequences, showing the relationship between inoculated strains SF6, TA3, and SA1 and their closest phylogenetic relatives.

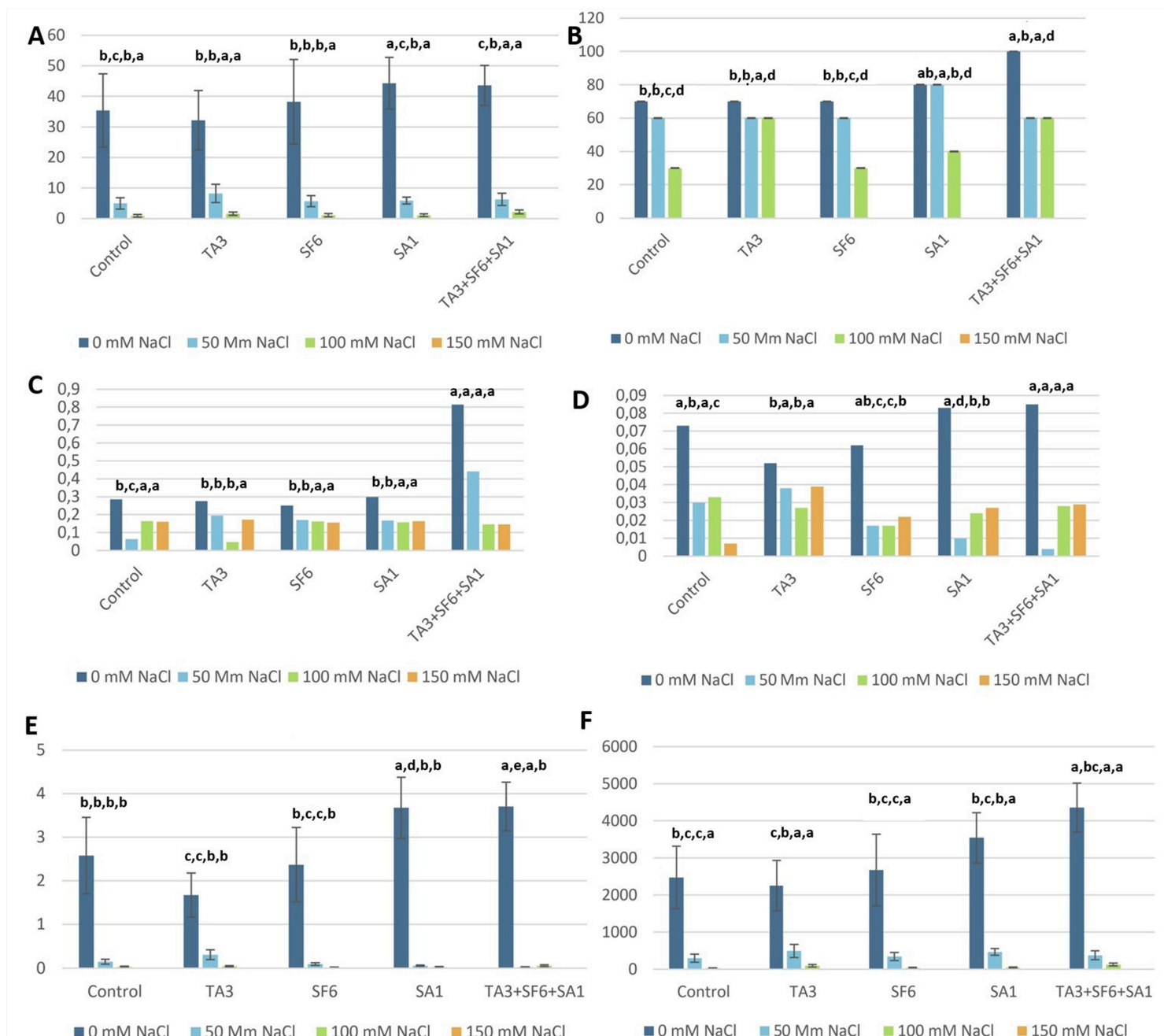


Figure 4
Effect of selected rhizobacteria on growth attributes of tomato under saline condition **(A)** Root length, **(B)** Percentage of seed germination, **(C)** Fresh weight, **(D)** Dry weight, **(E)** SWVI, **(F)** SLVI.

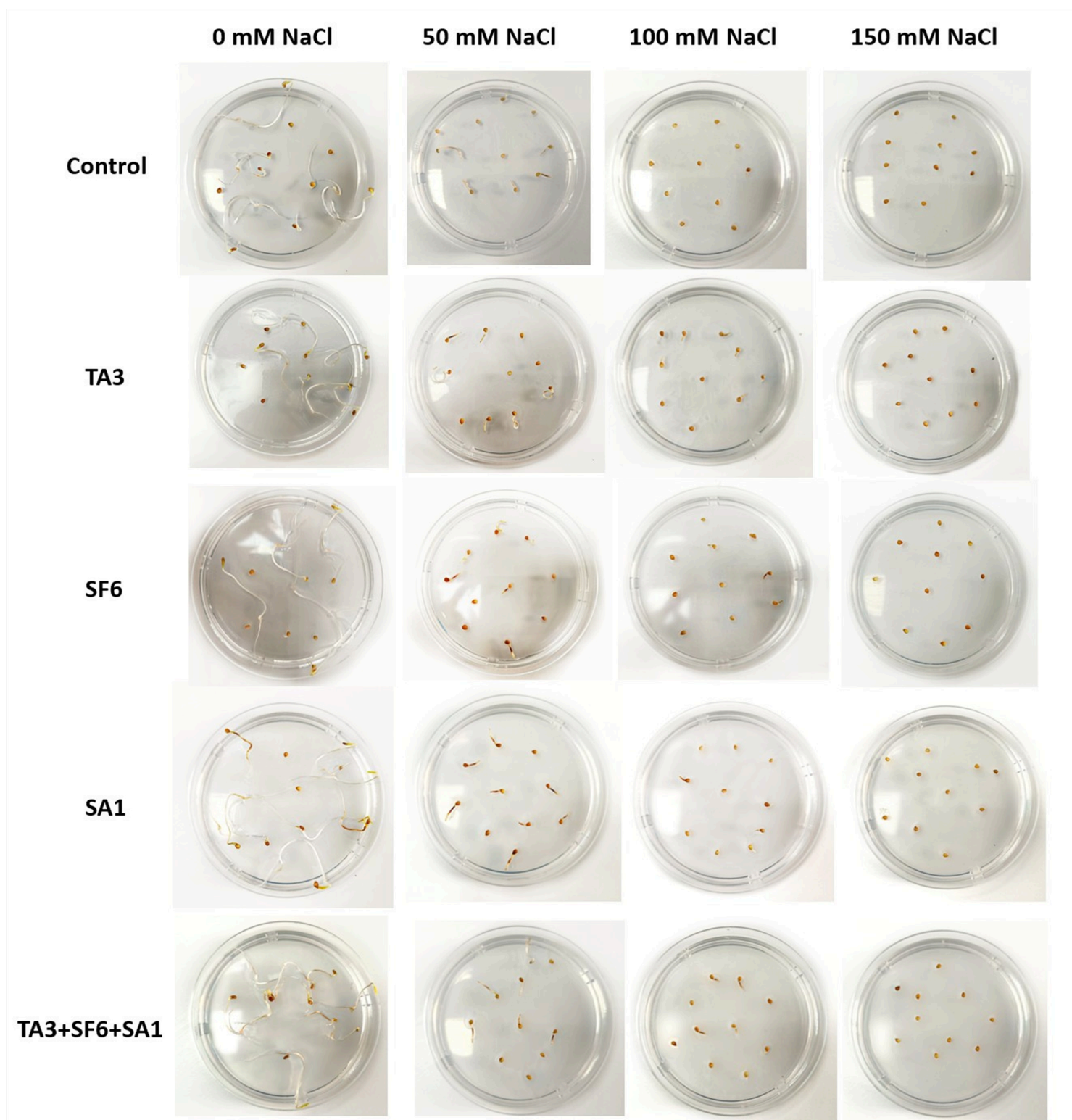


Figure 5

In vitro effect of bacterial isolates on tomato seedlings growth exposed to different concentration of NaCl.

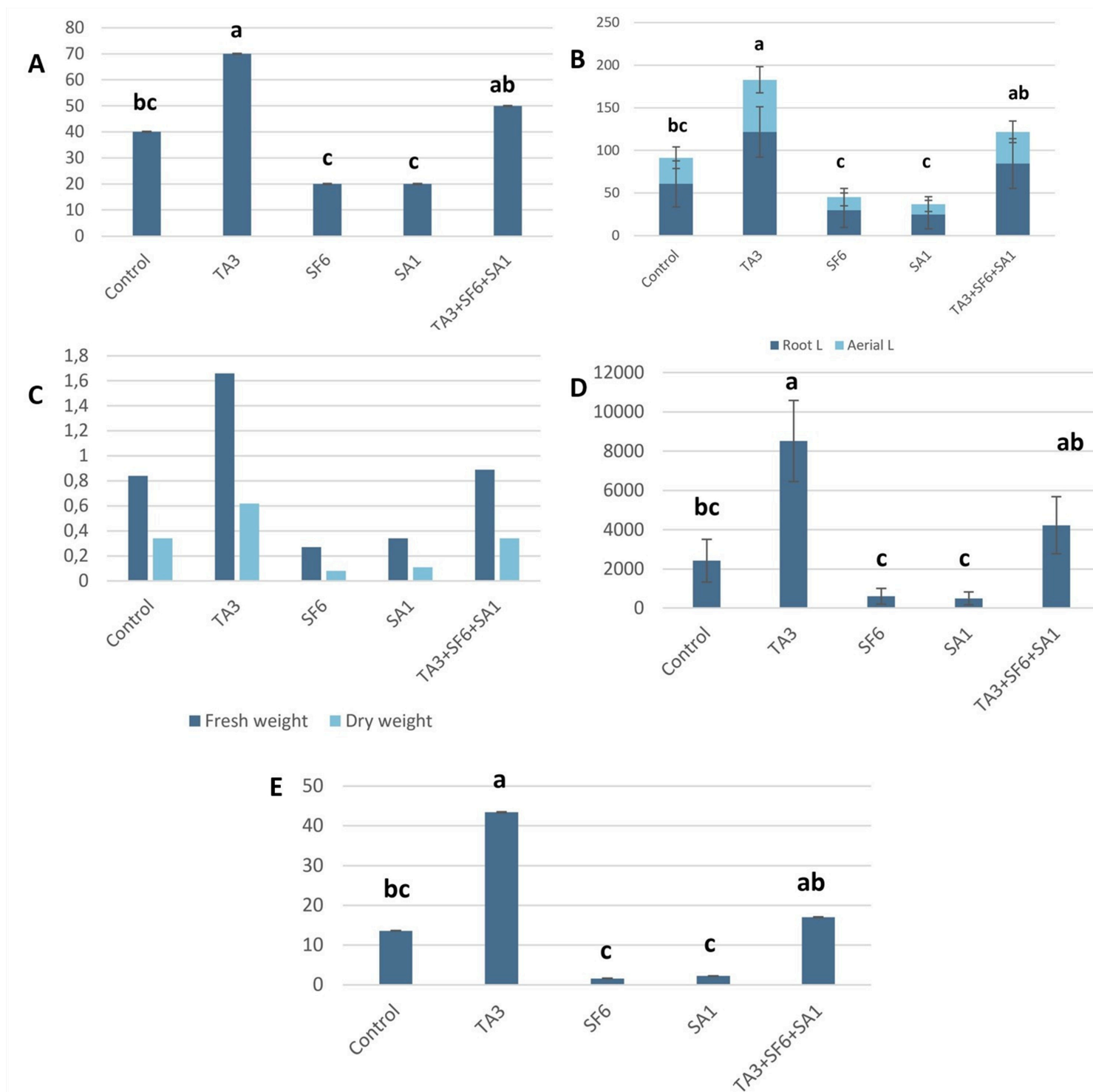


Figure 6
Effect of selected rhizobacteria on growth attributes of wheat under saline soil (A) Percentage of seeds germination, (B) Aerial and root length, (C) Fresh and dry weight, (D) SLVI, (E) SWVI.



Figure 7

In planta evaluation of rhizobacterial isolates for their ability to enhance wheat seed proliferation.

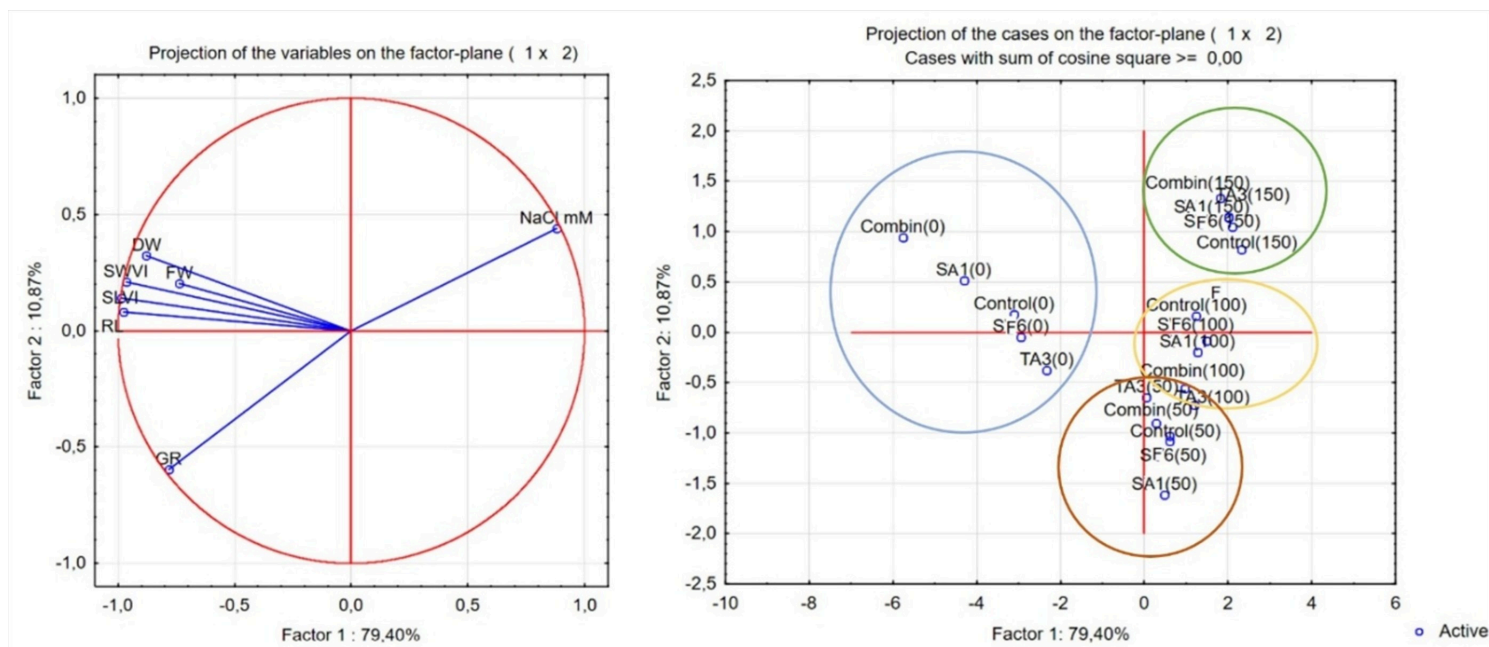


Figure 8

Principal Component Analysis (PCA) of tomato growth parameters in response to bacterial inoculation under different NaCl concentration.