

# ENDOPHYTIC BACTERIA OF COMMON TAMARISK (*TAMARIX GALLICA* L.) AND ALKALI SEEPWEED (*SUAEDA FRUTICOSA* (L.) FORSSK.) AS POTENTIAL BIOCONTROL AND PLANT GROWTH-PROMOTING AGENTS IN ARID ENVIRONMENTS

BAKELLI, A.<sup>1,2,3\*</sup> – AMRANI, S.<sup>1</sup> – BOURI, M.<sup>3</sup> – KALAYCI, S.<sup>3</sup> – SAHIN, F.<sup>3</sup>

<sup>1</sup>*Laboratoire de Biologie et de Physiologie des Organismes, Faculté des Sciences Biologiques, Université des Sciences et de la Technologie Houari Boumediene (USTHB), BP32 El-Alia, 16111 Bab Ezzouar, Algiers, Algeria*

<sup>2</sup>*Département de Biologie, Faculté des Sciences de la Nature et de la Vie et Sciences de la Terre, Université de Ghardaia, BP 455, Ghardaïa 47000, Algeria*

<sup>3</sup>*Yeditepe University, Faculty of Engineering, Department of Genetics and Bioengineering, Kayisdagi St., 34755 Istanbul, Turkey*

\*Corresponding author

e-mail: bakelliaissa@gmail.com; phone: +213-21-24-72-17; fax: +213-21-24-79-50

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**Abstract.** In this study, fifty-two endophytic halotolerant bacteria from *Tamarix gallica* L. and *Suaeda fruticosa* (L.) Forssk. growing in the M'Zab valley (Southern Algeria) were isolated to test their ability to reduce salt stress in barley (*Hordeum vulgare* L.), and tomato (*Solanum lycopersicum* L.) seeds. Identification by Gas Chromatography (GC) and matrix-assisted laser desorption ionization-time of flight (MALDI-TOF) confirmed the presence of strains belonging mainly to the genera *Bacillus* followed by *Pseudomonas*, *Pantoea*, *Klebsiella* and *Providencia*. All isolates were characterized for their antagonistic activities against some plant pathogenic bacteria and fungi. In addition, isolates were screened for their *in vitro* plant growth promotion (PGP) abilities, including ACC-deaminase activity, inorganic phosphate solubilization and production of indole-3-acetic acid (IAA), siderophores and lytic enzyme activity. Four isolates that expressed interesting PGP capabilities were tested for their efficacy in modulating salt stress and promoting the growth of barley and tomato seedlings in Petri dish experiments. The strain TE7 showed interesting performances under salt stress for tomato seedlings while strains SE5 and SE19 produced positive results for barley seedlings under normal conditions. The results of this study suggest that endophytic bacteria from natural plants in saline habitats present an interesting source for the isolation of salt-tolerant PGP bacteria that can be used in plant cultivation under normal and saline conditions.

**Keywords:** endophytes, halophytes, PGPB, Algeria, crops, seed enhancement

## Introduction

Soil salinity and drought are among the most prevalent abiotic stresses limiting production and yield, especially in arid and semi-arid regions, causing a decrease in cultivable areas and threatening food balance (Shrivastava and Kumar, 2015).

Plants that grow in regions facing prolonged exposure to different abiotic stresses may have been adapted and developed specific physiological and molecular stress responses that allow them to grow and thrive (Ma et al., 2020). Furthermore, these plants may also select particular root-associated bacteria capable of helping the plants to cope with unfavourable growth conditions (Oleńska et al., 2020). These adapted bacteria can possess potential Plant Growth-Promoting (PGP) abilities which make them very attractive for research purposes as they can support plant growth, health and resistance to

different abiotic stresses (salt, drought, etc.) (Souza et al., 2015; Compant et al., 2019; Leontidou et al., 2020). The PGP endophytic bacteria associated with different plant species have been largely documented in many areas of the world (Souza et al., 2015; ALKahtani et al., 2020). Many of them have been identified from various genera of which *Pseudomonas* and *Bacillus* are most extensively studied (Santoyo et al., 2016). The plant-associated bacteria can directly or indirectly influence the plant growth by fixing atmospheric nitrogen, solubilizing various minerals (P, K, etc.), producing siderophores, HCN, phytohormones as well as ACC deaminase activity which mitigate the effects of various stresses in plants (Souza et al., 2015; Leontidou et al., 2020).

Algeria's flora contains plenty of halophytic plant species of multiple interests. *Tamarix gallica* L. (*Tamaricaceae*) and *Suaeda fruticosa* (L.) Forssk. (*Amaranthaceae*) are among halophyte trees or shrubs that occur in arid and semi-arid regions (Chenchouni, 2012; Baameur et al., 2015). These plants can grow under various environmental conditions such as high temperatures, drought and especially salinity (Chekroun-Bechlaghem et al., 2019; Bencherif et al., 2019). In addition, *T. gallica* L. and *S. fruticosa* (L.) Forssk. are two salt-tolerant plants that are potentially useful in traditional medicine (Ksouri et al., 2009, 2012; Aslam and Ali, 2018; Fellah et al., 2018).

To our knowledge, there are no reports on endophytes from *T. gallica* L., and only one recent paper has been published on endophytes isolated from different species of *Suaeda* including, *S. fruticosa* (L.) Forssk. (Alishahi et al., 2020). However, most researchers have been focusing on the study of the rhizospheric bacteria associated with *S. fruticosa* (L.) Forssk. roots (Goswami et al., 2014a,b; Ullah and Bano, 2015; Aslam and Ali, 2018). Thus, these plants can be valuable for plant-associated bacteria research and the selection of potential PGP Bacteria (PGPB) for agricultural uses.

For the purpose of targeting bacterial endophytes from *T. gallica* L. and *S. fruticosa* (L.) Forssk. with a promising potential for biocontrol and plant growth enhancement, we explored the diversity of the associated bacteria isolated from an arid environment in Algeria (Ghardaïa Province). The obtained bacteria were identified by MALDI-TOF analysis, Gas Chromatographic analysis of Fatty Acid Methyl Esters (GC-FAME), evaluated for their antagonistic potential toward different plant pathogens and their PGP abilities.

## Materials and Methods

### *Sample collection*

Samples that were collected from the edges of the M'Zab valley (N 32°26'39" E 3°45'33") in Ghardaïa Province (Northern Algerian Sahara) consisted of three individual plants of *T. gallica* L. and *S. fruticosa* L. (Forssk.) each, collected aseptically in September 2018 and were put in an icebox and directly transferred to the laboratory for analysis.

### *Isolation, growth conditions and conservation of endophytic bacteria*

Parts of the root systems from the three individuals of each plant were surface sterilized by dipping in a solution of 5.25% calcium hypochlorite ( $\text{CaCl}_2\text{O}_2$ ) for 5 min, then 70% ethanol for 1 min followed by three times washing with sterilized distilled water (Coombes and Franco, 2003). The sterilization procedure was checked by spreading 100  $\mu\text{L}$  of the

last washing water on Tryptic Soy Agar (TSA) medium (GranuCult™, Merck KGaA Germany).

One gram (1g) of the sterilized roots was then macerated with 9 ml of a 0.9% physiological saline solution and subjected to serial dilutions (up to  $10^{-6}$ ). The resulting dilutions were spread in triplicate plates on TSA medium amended with 0%, 3%, 6% and 10% of NaCl (w/v) and a 0.45  $\mu\text{m}$  sterilized solution of cycloheximide (50 mg/L) (AppliChem GmbH, Darmstadt, Germany). Plates were incubated at  $30\pm 2^\circ\text{C}$  for one week and Colony-Forming Units (CFU) per gram of root weight were counted.

Based on colony morphology characteristics for example: form, elevation, margin, surface, opacity and pigmentation, only bacterial colonies were hand-picked and further purified by multiple streaking. Twenty-six endophytic bacterial colonies from plant roots were randomly selected, cultured on TSA medium and stored at  $-20^\circ\text{C}$  in 2 mL cryotubes filled with Tryptic Soy broth (TSB) (GranuCult™, Merck KGaA Germany) and 30% glycerol.

### ***Identification of endophytic bacteria***

#### *Identification by MALDI-TOF MS system*

Endophytic bacteria were identified using a matrix-assisted laser desorption ionization-time of flight mass spectrometry systems: Microflex LT (Bruker Daltonics, Bremen, Germany) equipped with a 337 nm wavelength UV laser MBT Compass software (Sauget et al., 2017). According to the manufacturer's recommendations, sample preparation was carried out on fresh bacterial cultures. Briefly, bacterial colonies were streaked on the polished steel plate MSP 96 target (BC), covered with formic acid (1  $\mu\text{L}$ , 70%) and air-dried. The samples were sprayed with 1  $\mu\text{L}$  cyano-4-hydroxycinnamic acid in an organic solution (50% acetonitrile and 2.5% trifluoroacetic acid) (Bruker Daltonics, Bremen, Germany). The target plate was then submitted to the MALDI-TOF analyzer.

The spectra were recorded by Flex Control software in linear positive mode over a mass range between 2 and 20 kDa at an acceleration voltage of 20 kV. Spectra were analyzed using Bruker Biotyper automation control, the Biotyper software (MBT Compass and Explorer 4.1.80) with the Bruker database (7311 MSP available).

#### *Identification of endophytic bacteria by Gas Chromatography (GC)*

The identification of the endophytic bacteria from both plants by Gas Chromatography was based on the analysis of Fatty Acid Methyl Esters (FAME) (Oates et al., 2017; Miura et al., 2017). To proceed with the FAME analysis, samples were processed according to the recommendations as-signed by MIDI Corporation (Sasser, 2001). Fresh bacterial cultures (24-48 h) replicas were subjected to several saponification, methylation and extraction treatments. The prepared samples were stored at  $-20^\circ\text{C}$  until they were analyzed. The FAMES were analyzed using a 6890 Plus Agilent gas chromatograph (Agilent Technologies, Palo Alto, CA, USA), including a 7683 auto-injector, a splitless inlet with Merlin Microseal septum (Merlin Instrument, Half Moon Bay, CA, USA). The system was controlled by Chemstation (Agilent) and Sherlock (MIDI) software. Fatty acid identities were determined by using the TSBA 40 method with a microbial identification system (MIDI) version Sherlock 6.1.

### ***Tolerance of endophytic bacteria to salt stress***

All isolated bacteria were tested for their ability to tolerate different salt concentrations. Bacterial isolates were inoculated on TSA plates containing 0%, 3%, 6%, 12%, 15%, 18% and 22% of NaCl, and their growth was monitored and scored as + for growth and – for no growth after ten days of culture (Ramadoss et al., 2013).

### ***Antimicrobial activity***

Bacterial strains were screened *in vitro* for their antimicrobial activity by the agar disc diffusion method (Bonev et al., 2008). The antimicrobial activity was conducted on several phytopathogenic microorganisms obtained from the collection of the Laboratory of Biology of Microbial Systems (LBSM, Ecole Normale Supérieure de Kouba, Algiers, Algeria) and the Laboratory of Microbiology (Department of Genetics and Bioengineering, Yeditepe University, Istanbul-Turkey). The phytopathogenic bacterial species used in this study were: *Agrobacterium tumefaciens*, *Pseudomonas syringae*, *Xanthomonas campestris* pv. *vesicatoria* and *Erwinia amylovora*, while the used phytopathogenic fungal strains were: *Clavibacter michiganensis* subsp. *insidiosus*, *Clavibacter flaccumfaciens*, *Aspergillus carbonarius*, *Aspergillus niger*, *Aspergillus ochraceus*, *Fusarium culmorum* and *Umbelopsis ramanniana*.

Briefly, Tryptic Soy Agar (TSA) disc (6 mm in diameter) from the fresh culture of each bacterial isolate was placed on semi-solid TSA and PDA plates streaked with the phytopathogenic bacteria and fungi, respectively. Sterile TSA agar discs were used as negative controls. The plates were incubated at 30±2°C and checked for inhibition zones after 24 h and 72 h against phytopathogenic bacteria and fungi, respectively.

### ***In vitro characterization of PGP traits***

#### ***Nitrogen fixation***

The nitrogen (N) fixation activity was tested on NFb semi-solid medium containing malic acid (Döbereiner, 1980). The bacterial strains were tested in a solid medium containing 0.5% bromothymol blue as a pH indicator. Blue-colored colonies were considered positive for nitrogen fixation. Positives tests were confirmed after inoculation on tubes containing semi-solid NFb medium incubated at 30±2°C for seven days and strains that formed a pellicle were considered positive for nitrogen fixation.

#### ***Phosphate solubilization***

For phosphate solubilization, all bacterial strains were inoculated onto plates of NBRIP (Nautiyal, 1999) and Pikovskaya (PKV) (Pikovskaya, 1948) agar medium containing 5 g.L<sup>-1</sup> tricalcium phosphate (Ca<sub>3</sub>PO<sub>4</sub>). After 72 h of incubation at 30±2°C, a clear zone around the colony indicated phosphate solubilization activity. For quantitative estimation of inorganic phosphate solubilization by the isolates, after ten days of incubation at 30±2°C in liquid Pikovskaya medium, the concentration of the soluble phosphate was estimated from the supernatant by stannous chloride method (King, 1932).

#### ***Potassium solubilization***

To test potassium solubilization capacity, the isolates were transferred to the Aleksandrov agar medium (HiMedia Laboratories GmbH, Germany) and incubated at

30±2°C for 48 h (Aleksandrov et al., 1967). Inorganic potassium solubilization activity was checked by the appearance of clear zones around the bacterial colonies.

#### *Calcium solubilization*

Bacterial strains were separately grown on Henderson's culture medium (Henderson and Duff, 1963) at 30±2°C for seven days. Calcium solubilization activity was displayed by clear halos around the bacterial colonies (Saleh et al., 2019).

#### *Siderophore production*

Siderophore production by bacterial isolates was performed according to the method described by Schwyn and Neilands (1987) by using blue indicator dye and chrome azurol S (CAS) agar. After inoculation of 1 µl of bacterial suspensions in plates containing (CAS) and incubation at 30±2°C for five days, the production of siderophores by bacteria was visualized by a color change of the medium from blue to orange/yellow color.

#### *Ammonia production*

For the ammonia production test, all bacterial strains were grown in peptone water (10 g/L peptone and 5 g/L sodium chloride) for 48 h at 30±2°C. Nessler reagent (K<sub>2</sub>HgI<sub>4</sub>; 1.4%) was added to the tubes containing suspensions in a 2:1 ratio. The development of a brown to yellow color was recorded as a positive reaction for ammonia production. A faint yellow color indicates a small amount of ammonia and deep yellow to brownish color indicates maximum ammonia production. Optical density was measured at 450 nm using a spectrophotometer (JASCO V-530, USA). The concentration of ammonia was estimated using the standard curve of ammonium sulfate (Cappuccino and Sherman, 1992).

#### *Indole-3-Acetic Acid (IAA) production*

Production of IAA was carried out by the method of Patten and Glick (2002), with some modifications. The bacterial isolates were cultured in Luria Bertani (LB) medium (Sigma Aldrich, Germany) supplemented with L-tryptophan (500 µg.mL<sup>-1</sup>). After four days of incubation at 30±2°C, bacterial suspensions were centrifuged (10000 g, 5 min at 4°C). A 2 mL of the cell-free supernatants were mixed with 4 mL of Salkowski's reagent in the ratio of 2:4 (containing 150 mL of pure H<sub>2</sub>SO<sub>4</sub>, 250 mL of H<sub>2</sub>O and 75 mL of 0.5 M FeCl<sub>3</sub> × 6H<sub>2</sub>O) and 150 µL of 10mM phosphoric acid (H<sub>3</sub>PO<sub>4</sub>) and incubated at room temperature in the dark for 25 min. The development of a pink color indicated the production of IAA, which was quantitatively estimated by the absorbance of supernatant mixtures at 530 nm compared to the standard curve of IAA (Gordon and Weber, 1951).

#### *1-aminocyclopropane-1-carboxylate (ACC) deaminase activity*

Based on the modified method of Glick et al. (1995), all bacterial strains were screened for 1-aminocyclopropane-1-carboxylate (ACC) deaminase activity on a DF (Dworkin and Foster) salts minimal medium containing 3 mM ACC as the only nitrogen source (Dworkin and Foster, 1958). ACC deaminase activity was considered positive if bacterial growth was recorded on the medium after 48 h of incubation at 30±2°C.

## ***In vitro* characterization of PGP traits**

### *Cellulase activity*

To study the cellulase production, the bacterial strains were transferred in a basal solid medium supplemented with Carboxy Methyl Cellulose (CMC; 10 g/L) as the only carbon source and incubated for 72 h at  $30\pm 2^{\circ}\text{C}$ . The cellulolytic activity of bacteria was revealed by adding Congo red (0.1%) to the medium for 30 min and then discoloring with sodium chloride (1M) solution for 15 min. The formation of clear halos around colonies indicated positive degradation of CMC (Teather and Wood, 1982).

### *Amylolytic activity*

One microliter (1  $\mu\text{L}$ ) of each bacterial suspension was transferred on plates containing nutrient agar medium enriched with 0.5% soluble starch and incubated at  $30\pm 2^{\circ}\text{C}$  for 48 h (Akpan et al., 1999). The coloring reagent solution (0.254 g iodine and 4.0 g potassium iodide in 1 L) was poured on the surface of the medium, and the apparition of a clear halo around colonies denotes a positive amylolytic activity of the strains (Raj et al., 2009).

### *Protease activity*

Protease activity was tested on Skim Milk Agar medium (Loper and Schroth, 1986). After incubation at  $30\pm 2^{\circ}\text{C}$  for 24 h, plates were examined for the development of clear halos around the bacterial colonies.

## ***In vitro* characterization of PGP traits**

Barley (*Hordeum vulgare*; cultivar Yakamoz) and tomato (*Solanum lycopersicum* L.; cultivar Aicha) seeds were used in the experiments. Seeds were immersed in 70% ethanol for 1 min, then surface sterilized by 2% sodium hypochlorite (NaOCl) solution for 3 min, followed by rinsing and continuous agitation with sterile distilled water.

Active fresh suspensions of bacterial isolates cultured in TSB medium for 24 h at  $30\pm 2^{\circ}\text{C}$  under shaking conditions (150 rpm) were prepared. After centrifugation at 15000 g for 10 min, approximately  $10^8$  CFU/mL in 3 mL of Phosphate Buffered Saline (PBS) solution were prepared, added to the seeds and gently agitated for 10 minutes. Twenty pre-germinated seeds were placed in Petri dishes (90 mm diameter) within sterile Whatman filter papers; the experiments were realized in three replicates and performed in controlled environmental conditions with three replicates without any bacterial isolates were used as a negative control group. The Petri dishes were maintained in growth room conditions with a temperature of  $25\pm 2^{\circ}\text{C}$ , 16 h light/8 h dark photoperiod and relative humidity of 70%.

The germination rate was determined after three days for barley seeds and five days for tomato seeds. The growth morpho-physiological parameters: stem and root lengths and fresh and dry weights were determined after ten days for both germinated seeds.

### ***Statistical analysis***

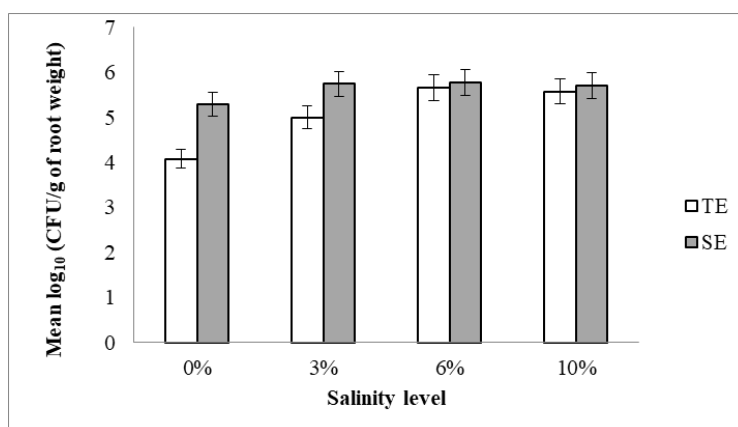
The bacterial diversity was performed in triplicates, the mean and Standard Deviation (SD) were calculated using Microsoft Excel 2016. All data of tomato and barley growth parameters (in triplicate) obtained from plant growth promotion assay were analyzed by one-way ANOVA at  $P = 0.05$  followed by Tukey's test. In addition, a Principal Component Analysis (PCA) was performed with R v3.5.1 using the Rcmdr package and

FactoMine R function. This analysis was carried out with the objective of the selection of the best bacterial strain in terms of growth promotion for tomato and barley cultured in the presence and absence of salt stress.

## Results

### *Density of endophytic bacteria*

The diversity of cultivable endophytic bacteria associated with *S. fruticosa* (L.) Forssk. and *T. gallica* L. was assessed on TSA supplemented with different concentrations of NaCl (0, 3, 6 and 10%) (Fig. 1). Overall, with and without NaCl supplementation, bacterial growth was noticed in all the Petri-dishes. Several colonies were observed in all the salt levels for samples from *S. fruticosa* (L.) Forssk. compared to those from *T. gallica* L. We noticed important bacterial populations in the roots of both studied plants with a high abundance of the halotolerant population, whereas bacteria that can grow in high salinity concentrations, in our case 10% NaCl were detected.



**Figure 1.** Cultivable bacterial endophytes abundance from the roots of *Tamarix gallica* L. and *Suaeda fruticosa* (L.) Forssk. on TSA medium without and with different NaCl concentrations. Error bars represent the standard deviation ( $n = 3$ ). TE: *Tamarix* Endophytes, SE: *Suaeda* Endophytes

### *Identification of endophytic bacteria*

In order to identify the fifty-two endophytic isolates from *T. gallica* L. (26 isolates) and *S. fruticosa* (L.) Forssk. (26 isolates), we used the MALDI-TOF as well as GC analyses. All the isolates showed characteristic MALDI-TOF MS spectra after comparison to the reference database however, several gave a No Reliable Identification (NRI) by GC analysis (Table 1).

In this study, the MALDI-TOF generated more secure results at genus and species levels with (5.8%) and (94.2%), respectively. Strains collected from *T. gallica* L. identified by MALDI-TOF were assigned to *Pseudomonas* sp. (1 strain), and the remaining twenty-five strains (25) were identified as *Bacillus* species (17 *B. cereus*, 6 *B. subtilis*, 1 *B. megaterium* and 1 *Bacillus* sp.). The twenty-six endophytes isolated from the roots of *S. fruticosa* (L.) Forssk. were affiliated to five genera with *Bacillus* as the predominant genus (20 strains), followed by *Providencia* and *Pantoea* with two strains each and only one strain of *Pseudomonas aeruginosa* and *Klebsiella aerogenes* each.

**Table 1.** MALDI-TOF, GC (FAME-MIDI) and salt tolerance of endophytic bacterial isolates from *Tamarix gallica* L. and *Suaeda fruticosa* (L.) Forssk

Plant	Strain code	MALDI-TOF species match	Score	GC (FAME-MIDI) species match	SI	Phylum	Salt tolerance (% NaCl)							
							0	3	6	9	12	15	18	22
<i>Tamarix gallica</i> L.	TE1	<i>Bacillus subtilis</i>	2.13	<i>Bacillus subtilis</i>	0.820	Firmicutes	+	+	+	+	+	+	+	+
	TE2	<i>Bacillus subtilis</i>	2.06	NRI	-	Firmicutes	+	+	+	+	+	+	+	+
	TE3	<i>Bacillus subtilis</i>	2.17	<i>Bacillus subtilis</i>	0.731	Firmicutes	+	+	+	+	+	+	+	+
	TE4	<i>Bacillus subtilis</i>	2.07	<i>Bacillus subtilis</i>	0.825	Firmicutes	+	+	+	+	+	+	+	+
	TE5	<i>Bacillus subtilis</i>	2.02	<i>Bacillus subtilis</i>	0.821	Firmicutes	+	+	+	+	+	+	+	+
	TE6	<i>Bacillus subtilis</i>	2.20	NRI	-	Firmicutes	+	+	+	+	+	+	+	+
	TE7	<i>Pseudomonas</i> sp.	1.98	NRI	-	Gammaproteobacteria	+	+	+	+	+	+	+	-
	TE8	<i>Bacillus cereus</i>	2.16	NRI	-	Firmicutes	+	+	+	+	+	+	-	-
	TE9	<i>Bacillus cereus</i>	2.05	NRI	-	Firmicutes	+	+	+	+	+	+	+	-
	TE10	<i>Bacillus cereus</i>	2.02	NRI	-	Firmicutes	+	+	+	+	+	+	+	+
	TE11	<i>Bacillus cereus</i>	2.17	NRI	-	Firmicutes	-	+	+	+	+	+	+	+
	TE12	<i>Bacillus cereus</i>	2.24	NRI	-	Firmicutes	-	+	+	+	+	+	+	+
	TE13	<i>Bacillus megaterium</i>	2.42	NRI	-	Firmicutes	-	+	+	+	+	+	+	+
	TE14	<i>Bacillus cereus</i>	2.10	NRI	-	Firmicutes	-	+	+	+	+	+	+	+
	TE15	<i>Bacillus cereus</i>	2.21	NRI	-	Firmicutes	-	+	+	+	+	+	+	+
	TE16	<i>Bacillus cereus</i>	2.18	NRI	-	Firmicutes	-	+	+	+	+	+	+	+
	TE17	<i>Bacillus cereus</i>	2.35	NRI	-	Firmicutes	-	+	+	+	+	+	+	+
	TE18	<i>Bacillus cereus</i>	2.05	NRI	-	Firmicutes	-	+	+	+	+	+	+	+
	TE19	<i>Bacillus cereus</i>	2.24	NRI	-	Firmicutes	-	+	+	+	+	+	+	+
	TE20	<i>Bacillus cereus</i>	2.12	NRI	-	Firmicutes	-	+	+	+	+	+	+	+
	TE21	<i>Bacillus cereus</i>	2.21	NRI	-	Firmicutes	-	+	+	+	+	+	+	+
	TE22	<i>Bacillus cereus</i>	2.21	NRI	-	Firmicutes	-	+	+	+	+	+	+	+
	TE23	<i>Bacillus cereus</i>	2.18	NRI	-	Firmicutes	-	+	+	+	+	+	+	+
	TE24	<i>Bacillus cereus</i>	2.01	NRI	-	Firmicutes	-	+	+	+	+	+	+	+
	TE25	<i>Bacillus</i> sp.	1.98	NRI	-	Firmicutes	-	+	+	+	+	+	+	+
	TE26	<i>Bacillus cereus</i>	2.14	NRI	-	Firmicutes	-	+	+	+	+	+	+	+



Plant	Strain code	MALDI-TOF species match	Score	GC (FAME-MIDI) species match	SI	Phylum	Salt tolerance (% NaCl)							
							0	3	6	9	12	15	18	22
<i>Suaeda fruticosa</i> (L.) Forssk.	SE1	<i>Bacillus cereus</i>	2.08	<i>Bacillus cereus</i>	0.655	Firmicutes	+	+	+	+	-	-	-	-
	SE2	<i>Bacillus cereus</i>	2.04	<i>Bacillus cereus</i>	0.656	Firmicutes	+	+	+	+	-	-	-	-
	SE3	<i>Bacillus cereus</i>	2.01	<i>Bacillus cereus</i>	0.657	Firmicutes	+	+	+	+	-	-	-	-
	SE4	<i>Bacillus cereus</i>	2.08	<i>Bacillus cereus</i>	0.658	Firmicutes	+	+	+	+	-	-	-	-
	SE5	<i>Providencia rettgeri</i>	2.47	NRI	-	Gammaproteobacteria	+	+	+	+	-	-	-	-
	SE6	<i>Klebsiella aerogenes</i>	2.34	NRI	-	Gammaproteobacteria	+	+	+	+	+	+	-	-
	SE7	<i>Bacillus subtilis</i>	2.26	<i>Bacillus subtilis</i>	0.872	Firmicutes	+	+	+	+	+	+	-	-
	SE8	<i>Bacillus subtilis</i>	2.09	<i>Bacillus subtilis</i>	0.745	Firmicutes	+	+	+	+	+	+	-	-
	SE9	<i>Bacillus subtilis</i>	2.17	<i>Bacillus pumilis</i>	0.659	Firmicutes	+	+	+	+	+	-	-	-
	SE10	<i>Bacillus subtilis</i>	2.09	NRI	-	Firmicutes	+	+	+	+	-	-	-	-
	SE11	<i>Bacillus subtilis</i>	2.23	<i>Bacillus subtilis</i>	0.765	Firmicutes	+	+	+	+	-	-	-	-
	SE12	<i>Providencia rettgeri</i>	2.47	NRI	-	Gammaproteobacteria	+	+	+	+	-	-	-	-
	SE13	<i>Bacillus subtilis</i>	2.17	<i>Bacillus subtilis</i>	0.789	Firmicutes	+	+	+	+	+	-	-	-
	SE14	<i>Bacillus subtilis</i>	2.08	NRI	-	Firmicutes	+	+	+	+	+	-	-	-
	SE15	<i>Bacillus subtilis</i>	2.18	<i>Bacillus subtilis</i>	0.890	Firmicutes	+	+	+	+	+	-	-	-
	SE16	<i>Bacillus subtilis</i>	2.20	<i>Bacillus subtilis</i>	0.856	Firmicutes	+	+	+	+	+	+	+	+
	SE17	<i>Bacillus subtilis</i>	2.05	<i>Bacillus subtilis</i>	0.842	Firmicutes	+	+	+	+	+	+	+	+
	SE18	<i>Bacillus</i> sp.	1.70	<i>Bacillus</i> sp.	0.481	Firmicutes	+	+	+	+	-	-	-	-
	SE19	<i>Pantoea agglomerans</i>	2.20	NRI	-	Gammaproteobacteria	+	+	+	+	-	-	-	-
	SE20	<i>Pantoea agglomerans</i>	2.34	NRI	-	Gammaproteobacteria	+	+	+	+	+	-	-	-
	SE21	<i>Bacillus licheniformis</i>	2.08	<i>Bacillus</i> sp.	0.459	Firmicutes	+	+	+	+	+	+	+	+
	SE22	<i>Bacillus subtilis</i>	2.34	<i>Bacillus subtilis</i>	0.931	Firmicutes	+	+	+	+	+	+	+	+
	SE23	<i>Bacillus subtilis</i>	2.19	<i>Bacillus subtilis</i>	0.889	Firmicutes	+	+	+	+	+	+	+	+
	SE24	<i>Bacillus subtilis</i>	2.06	<i>Bacillus subtilis</i>	0.890	Firmicutes	+	+	+	+	+	+	+	+
	SE25	<i>Pseudomonas aeruginosa</i>	2.39	<i>Pseudomonas aeruginosa</i>	0.721	Gammaproteobacteria	+	+	+	+	+	-	-	-
	SE26	<i>Bacillus subtilis</i>	2.20	<i>Bacillus subtilis</i>	0.892	Firmicutes	+	+	+	+	+	+	+	+

NRI: No Reliable Identification, -: No growth, +: Growth. SI: Similarity index. For MALDI-TOF: +++, score value 2.300–3.000, highly probable species identification; ++, score value 2.000–2.299, secure genus identification and probable species identification; +, score value 1.700–1.999, probable genus identification, - score value < 1.700 no reliable species identification. For GC- FAME: samples with an SI of 0.500 or higher and with a separation of 0.100 between the first and second choice are considered good library comparisons

Furthermore, isolates affiliated with the genera *Bacillus* were assigned to four species with *Bacillus subtilis* (14 strains) as the predominant, followed by four *B. cereus* strains (4) and one strain of *B. licheniformis* and *Bacillus* sp. each.

### ***Salt tolerance profile of endophytic bacteria***

All the endophytic bacterial isolates were tested for their growth at different salt concentrations (0, 3, 6, 9, 12, 15, 18% and 22% NaCl). The obtained results show that twenty-six strains from the roots of *S. fruticosa* (L.) Forssk. were found to be 100% halotolerant, whereas the strains associated with *T. gallica* L. were divided into 38.46% of halotolerant bacteria (10 strains) and 61.54% of halophiles (16 strains) which were able to grow only at NaCl concentrations  $\geq 3\%$ .

### ***Antimicrobial properties of endophytic bacteria***

The antagonistic activity of the fifty-two strains selected from the endophytic bacterial population associated with *T. gallica* L. and *S. fruticosa* (L.) Forssk. were tested against some phytopathogenic bacteria and fungi (Table 2).

Six strains associated with *T. gallica* L. showed moderate to high antibacterial activities against all the tested phytopathogenic bacteria, while no antagonistic activity was recorded within isolates from *S. fruticosa* (L.) Forssk except for SE23 and SE25. The anti-bacterial activity was limited to the *Bacillus* genus only, whereas the antifungal activity was also detected in other genera such as *Providencia*, *Klebsiella* and *Pseudomonas* associated with *S. fruticosa* (L.) Forssk. Further, no antifungal activities were observed within the strains isolated from *T. gallica* L.

### ***Plant growth-promoting traits of endophytic bacteria***

The PGP traits of the fifty-two endophytic bacteria were screened according to their nitrogen fixation ability, phosphate, potassium and calcium solubilization, siderophore, ammonia and IAA production, and ACC deaminase activity (Table 3).

The results showed that seven strains (7/52) were able to fix atmospheric nitrogen with one strain from *T. gallica* L. and six strains from *S. fruticosa* (L.) Forssk.

Phosphate solubilization ability was measured in solid and liquid media. Based on NBRIP and PKV solid media results, one strain for both media from *T. gallica* L. and 13 strains from *S. fruticosa* (L.) Forssk. were phosphate solubilizers. For the quantification results, the phosphate solubilization activity was limited within endophytic bacteria associated with *T. gallica* L. to only *Pseudomonas* sp. TE7 with  $23 \mu\text{g.mL}^{-1}$  while 13 strains gave positive results with the maximum, was obtained by strain *Providencia rettgeri* SE12.

The potassium solubilization ability was detected for only one strain from *T. gallica* L. (TE7) and SE12, SE20 and SE21, three strains isolated from *S. fruticosa* (L.) Forssk. On the other hand, two strains (SE19 and SE20) that were isolated from the roots of *S. fruticosa* (L.) Forssk were able to solubilize calcium.

Furthermore, a total of ten isolates out of fifty-two were able to produce siderophores with the majority (9/10) isolated from *T. gallica* L. For ammonia production, all the bacterial strains showed positive ammonia production. Variable quantities that ranged from  $6.56 \mu\text{g.mL}^{-1}$  to  $122.01 \mu\text{g.mL}^{-1}$  and the maximum given by strain SE13 were obtained.

**Table 2.** Antimicrobial activity of endophytic bacterial isolates from *Tamarix gallica* L. and *Suaeda fruticosa* (L.) Forssk.

Plant	Strain code	Species	Bacteria						Fungi				
			GRAM-			GRAM+			Ac	An	Ao	Fc	Ur
			Ag	Ps	Xv	Ea	Cf	Cm					
<i>Tamarix gallica</i> L.	TE1	<i>Bacillus subtilis</i>	28	22	14	12	22	30	0	0	0	0	0
	TE2	<i>Bacillus subtilis</i>	28	22	15	12	22	30	0	0	0	0	0
	TE3	<i>Bacillus subtilis</i>	30	22	14	12	22	30	0	0	0	0	0
	TE4	<i>Bacillus subtilis</i>	26	22	14	12	22	30	0	0	0	0	0
	TE5	<i>Bacillus subtilis</i>	28	22	14	12	22	30	0	0	0	0	0
	TE6	<i>Bacillus subtilis</i>	24	22	14	12	22	30	0	0	0	0	0
	TE7	<i>Pseudomonas</i> sp.	0	0	0	0	0	0	0	0	0	0	0
	TE8	<i>Bacillus cereus</i>	0	0	0	0	0	0	0	0	0	0	0
	TE9	<i>Bacillus cereus</i>	0	0	0	0	0	0	0	0	0	0	0
	TE10	<i>Bacillus cereus</i>	0	0	0	0	0	0	0	0	0	0	0
	TE11	<i>Bacillus cereus</i>	12	0	0	0	0	0	0	0	0	0	0
	TE12	<i>Bacillus cereus</i>	13	0	0	0	0	0	0	0	0	0	0
	TE13	<i>Bacillus megaterium</i>	15	0	0	0	0	0	0	0	0	0	0
	TE14	<i>Bacillus cereus</i>	16	0	0	0	0	0	0	0	0	0	0
	TE15	<i>Bacillus cereus</i>	14	0	0	0	0	0	0	0	0	0	0
	TE16	<i>Bacillus cereus</i>	14	0	0	0	0	0	0	0	0	0	0
	TE17	<i>Bacillus cereus</i>	13	0	0	0	0	0	0	0	0	0	0
	TE18	<i>Bacillus cereus</i>	13	0	0	0	0	0	0	0	0	0	0
	TE19	<i>Bacillus cereus</i>	14	0	0	0	0	0	0	0	0	0	0
	TE20	<i>Bacillus cereus</i>	0	0	0	0	0	0	0	0	0	0	0
	TE21	<i>Bacillus cereus</i>	12	0	0	0	0	0	0	0	0	0	0
	TE22	<i>Bacillus cereus</i>	12	0	0	0	0	0	0	0	0	0	0
	TE23	<i>Bacillus cereus</i>	13	0	0	0	0	0	0	0	0	0	0
	TE24	<i>Bacillus cereus</i>	15	0	0	0	0	0	0	0	0	0	0
	TE25	<i>Bacillus</i> sp.	14	0	0	0	0	0	0	0	0	0	0
	TE26	<i>Bacillus cereus</i>	15	0	0	0	0	0	0	0	0	0	0

Plant	Strain code	Species	Bacteria						Fungi				
			GRAM-			GRAM+			Ac	An	Ao	Fc	Ur
			Ag	Ps	Xv	Ea	Cf	Cm					
<i>Suaeda fruticosa</i> (L.) Forssk.	SE1	<i>Bacillus cereus</i>	0	0	0	0	0	0	0	0	0	0	12
	SE2	<i>Bacillus cereus</i>	0	0	0	0	0	0	0	0	0	0	12
	SE3	<i>Bacillus cereus</i>	0	0	0	0	0	0	0	0	0	0	12
	SE4	<i>Bacillus cereus</i>	0	0	0	0	0	0	0	0	0	0	12
	SE5	<i>Providencia rettgeri</i>	0	0	0	0	0	0	0	0	20	14	20
	SE6	<i>Klebsiella aerogenes</i>	0	0	0	0	0	0	0	0	22	24	25
	SE7	<i>Bacillus subtilis</i>	0	0	0	0	0	0	0	0	25	20	34
	SE8	<i>Bacillus subtilis</i>	0	0	0	0	0	0	0	0	22	0	26
	SE9	<i>Bacillus subtilis</i>	0	0	0	0	0	0	0	0	30	20	30
	SE10	<i>Bacillus subtilis</i>	0	0	0	0	0	0	0	0	21	0	25
	SE11	<i>Bacillus subtilis</i>	0	0	0	0	0	0	0	0	20	26	29
	SE12	<i>Providencia rettgeri</i>	0	0	0	0	0	0	0	0	19	0	0
	SE13	<i>Bacillus subtilis</i>	0	0	0	0	0	0	0	0	20	0	0
	SE14	<i>Bacillus subtilis</i>	0	0	0	0	0	0	0	0	0	0	0
	SE15	<i>Bacillus subtilis</i>	0	0	0	0	0	0	0	0	18	20	25
	SE16	<i>Bacillus subtilis</i>	0	0	0	0	0	0	0	0	24	25	26
	SE17	<i>Bacillus subtilis</i>	0	0	0	0	0	0	0	0	20	0	32
	SE18	<i>Bacillus</i> sp.	0	0	0	0	0	0	0	0	0	0	0
	SE19	<i>Pantoea agglomerans</i>	0	0	0	0	0	0	0	0	0	0	0
	SE20	<i>Pantoea agglomerans</i>	0	0	0	0	0	0	0	0	0	0	0
	SE21	<i>Bacillus licheniformis</i>	0	0	0	0	0	0	0	0	0	0	15
	SE22	<i>Bacillus subtilis</i>	0	0	0	0	0	0	0	0	0	0	18
	SE23	<i>Bacillus subtilis</i>	22	0	0	0	0	0	0	0	0	0	24
	SE24	<i>Bacillus subtilis</i>	0	0	0	0	0	0	0	0	0	0	20
	SE25	<i>Pseudomonas aeruginosa</i>	18	0	0	0	0	0	22	22	25	16	27
	SE26	<i>Bacillus subtilis</i>	0	0	0	0	0	0	0	0	0	0	0

Ag: *Agrobacterium tumefaciens*, Ps: *Pseudomonas syringae*, Xv: *Xanthomonas campestris* pv. *vesicatoria*, Ea: *Erwinia amylovora*, Cm: *Clavibacter michiganensis*, Cm: *Clavibacter michiganensis* subsp. *insidiosus*, Cf: *Clavibacter flaccumfaciens*, Ac: *Aspergillus carbonarius*, An: *Aspergillus niger*, Ao: *Aspergillus ochraceus*. Fc: *Fusarium culmorum*, Ur: *Umbelopsis ramanniana*. Inhibition zone values are given including the disc (10 mm). <10 mm: Low activity, 10-20 mm: Medium activity, 20-30: High activity, >30: very strong activity

**Table 3.** Plant growth promotion traits of endophytic bacterial isolates from *Tamarix gallica* L. and *Suaeda fruticosa* (L.) Forssk.

Plant	Strain code	Species	PGP traits										Enzymatic activity			
			N <sub>2</sub> -fixation	Phosphate solubilization			Potassium solubilization	Calcium solubilization	Siderophore production	Amonia production (µg.ml <sup>-1</sup> )	IAA (µg.ml <sup>-1</sup> )	ACC deaminase	Cellulase	Amylase	Protease	
			NR	BIP	PKV	PKV (µg.ml <sup>-1</sup> )										
<i>Tamarix gallica</i> L.	TE1	<i>Bacillus subtilis</i>	-	-	-	0	-	-	-	-	84.87	0.0	-	-	+	+
	TE2	<i>Bacillus subtilis</i>	-	-	-	0	-	-	-	-	61.30	0.0	-	-	+	+
	TE3	<i>Bacillus subtilis</i>	-	-	-	0	-	-	-	-	54.87	0.0	-	-	+	+
	TE4	<i>Bacillus subtilis</i>	-	-	-	0	-	-	-	-	59.16	0.0	-	-	+	+
	TE5	<i>Bacillus subtilis</i>	-	-	-	0	-	-	-	-	20.84	0.0	-	-	+	+
	TE6	<i>Bacillus subtilis</i>	-	-	-	0	-	-	-	-	68.44	0.0	-	-	+	+
	TE7	<i>Pseudomonas</i> sp.	+	+	+	23	+	-	+	+	54.87	53.5	+	-	-	-
	TE8	<i>Bacillus cereus</i>	-	-	-	0	-	-	-	-	79.87	0.0	-	-	+	+
	TE9	<i>Bacillus cereus</i>	-	-	-	0	-	-	-	-	25.59	0.0	-	-	+	+
	TE10	<i>Bacillus cereus</i>	-	-	-	0	-	-	-	-	47.73	0.0	-	-	+	+
	TE11	<i>Bacillus cereus</i>	-	-	-	0	-	-	-	-	53.44	0.0	-	-	+	+
	TE12	<i>Bacillus cereus</i>	-	-	-	0	-	-	-	-	75.59	0.0	-	-	+	+
	TE13	<i>Bacillus megaterium</i>	-	-	-	0	-	-	-	-	19.87	0.8	-	-	+	+
	TE14	<i>Bacillus cereus</i>	-	-	-	0	-	-	-	-	27.73	7.2	-	-	+	+
	TE15	<i>Bacillus cereus</i>	-	-	-	0	-	-	-	-	44.16	3.1	-	-	+	+
	TE16	<i>Bacillus cereus</i>	-	-	-	0	-	-	-	-	45.59	0.0	-	-	+	+
	TE17	<i>Bacillus cereus</i>	-	-	-	0	-	-	-	-	18.44	0.0	-	-	+	+
	TE18	<i>Bacillus cereus</i>	-	-	-	0	-	-	-	-	47.73	0.0	-	-	+	+
	TE19	<i>Bacillus cereus</i>	-	-	-	0	-	-	-	+	64.16	1.9	-	-	+	+
	TE20	<i>Bacillus cereus</i>	-	-	-	0	-	-	-	+	75.59	3.2	-	-	+	+
	TE21	<i>Bacillus cereus</i>	-	-	-	0	-	-	-	+	36.30	2.8	-	-	+	+
	TE22	<i>Bacillus cereus</i>	-	-	-	0	-	-	-	+	42.01	6.8	-	-	+	+
	TE23	<i>Bacillus cereus</i>	-	-	-	0	-	-	-	+	59.16	4.5	-	-	+	+
	TE24	<i>Bacillus cereus</i>	-	-	-	0	-	-	-	+	51.30	74.5	-	-	+	+
	TE25	<i>Bacillus</i> sp.	-	-	-	0	-	-	-	+	44.87	0.8	-	-	+	+
	TE26	<i>Bacillus cereus</i>	-	-	-	0	-	-	-	+	47.73	0.2	-	-	+	+

Plant	Strain code	Species	PGP traits									Enzymatic activity			
			N <sub>2</sub> -fixation	Phosphate solubilization			Potassium solubilization	Calcium solubilization	Siderophores	Amonia production (µg.ml <sup>-1</sup> )	IAA (µg.ml <sup>-1</sup> )	ACC deaminase	Cellulase	Amylase	Protease
				NRBIP	PKV	PKV (µg.ml <sup>-1</sup> )									
<i>Suaeda fruticosa</i> (L.) Forssk.	SE1	<i>Bacillus cereus</i>	-	-	-	0.0	-	-	-	64.09	0.0	-	-	+	+
	SE2	<i>Bacillus cereus</i>	-	-	-	0.0	-	-	-	106.30	0.0	-	-	+	+
	SE3	<i>Bacillus cereus</i>	-	-	-	0.0	-	-	-	100.59	0.0	-	-	+	+
	SE4	<i>Bacillus cereus</i>	-	-	-	0.0	-	-	-	100.59	0.0	-	-	+	+
	SE5	<i>Providencia rettgeri</i>	+	+	+	31	-	-	+	114.87	174.5	+	-	+	+
	SE6	<i>Klebsiella aerogenes</i>	+	+	+	12	-	-	-	42.01	101.7	-	-	+	+
	SE7	<i>Bacillus subtilis</i>	-	+	+	16	-	-	-	64.87	0.0	-	-	+	+
	SE8	<i>Bacillus subtilis</i>	-	+	+	13	-	-	-	47.01	0.0	-	-	+	+
	SE9	<i>Bacillus subtilis</i>	-	+	+	15	-	-	-	53.44	0.0	-	-	+	+
	SE10	<i>Bacillus subtilis</i>	-	+	-	0.0	-	-	-	89.87	0.0	-	+	+	+
	SE11	<i>Bacillus subtilis</i>	-	+	-	0.0	-	-	-	84.87	0.0	-	+	+	+
	SE12	<i>Providencia rettgeri</i>	+	+	+	35	+	-	-	99.16	39.9	+	+	+	+
	SE13	<i>Bacillus subtilis</i>	-	+	+	0.5	-	-	-	122.01	30.8	+	-	+	-
	SE14	<i>Bacillus subtilis</i>	-	+	+	12	-	-	-	6.56	33.5	+	+	+	+
	SE15	<i>Bacillus subtilis</i>	-	+	-	0.0	-	-	-	20.84	0.0	-	+	+	+
	SE16	<i>Bacillus subtilis</i>	-	+	-	0.0	-	-	-	13.70	0.0	-	+	+	+
	SE17	<i>Bacillus subtilis</i>	-	+	-	0.0	-	-	-	7.73	0.0	-	+	+	+
	SE18	<i>Bacillus</i> sp.	-	+	-	0.0	-	-	-	108.44	0.0	-	+	+	+
	SE19	<i>Pantoea agglomerans</i>	-	+	+	29	-	+	-	106.30	0.0	+	+	+	-
	SE20	<i>Pantoea agglomerans</i>	+	+	+	14	+	+	-	55.59	0.0	+	+	+	-
	SE21	<i>Bacillus licheniformis</i>	+	+	-	0.0	+	-	-	41.30	0.0	-	+	+	+
	SE22	<i>Bacillus subtilis</i>	-	+	-	0.0	-	-	-	59.16	49.9	-	-	+	+
	SE23	<i>Bacillus subtilis</i>	-	+	+	14	-	-	-	54.87	0.0	-	-	+	+
	SE24	<i>Bacillus subtilis</i>	-	+	+	12	-	-	-	61.30	0.0	-	-	+	+
	SE25	<i>Pseudomonas aeruginosa</i>	-	+	+	18	-	-	-	84.87	0.0	+	-	+	+
	SE26	<i>Bacillus subtilis</i>	+	-	-	0.0	-	-	-	77.01	0.0	-	+	+	+

PKV: Pikovskaya medium, NBRIP: National Botanical Research Institute's phosphate growth medium, +: Positive reaction, -: Negative reaction

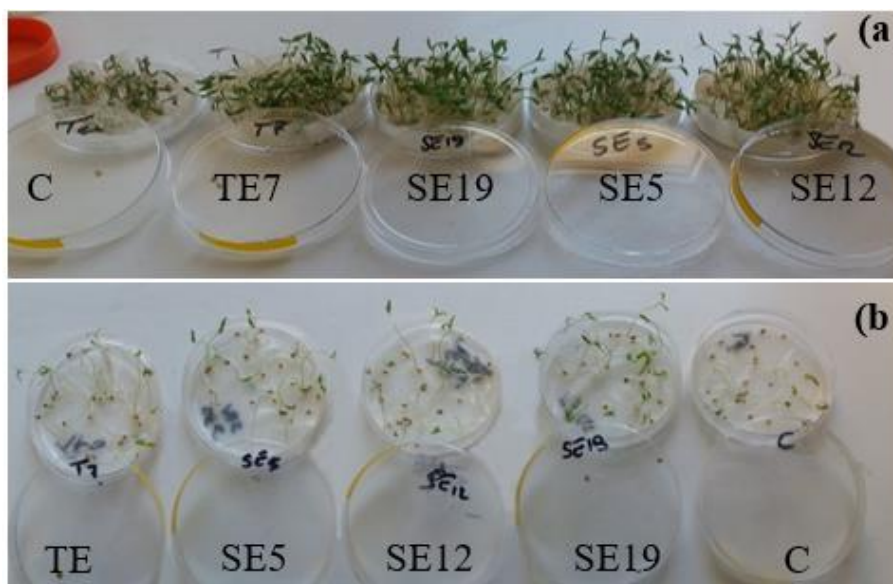
The results presented in *Table 3* show that seventeen strains were IAA producers with 12 strains were isolated from *T. gallica* L. and six strains from *S. fruticosa* (L.) Forssk. The recorded range of concentrations of IAA were  $0.2 \mu\text{g.mL}^{-1}$  to  $174.5 \mu\text{g.mL}^{-1}$  with the strain SE5 as the biggest producer. Out of fifty-two (52) tested strains, only one strain *Pseudomonas* sp. TE7 obtained from the roots of *T. gallica* L. and seven strains (SE5, SE12, SE13, SE14, SE19, SE20, and SE25) isolated from *S. fruticosa* (L.) Forssk. roots had positive ACC deaminase activity.

### ***Plant growth-promoting traits of endophytic bacteria***

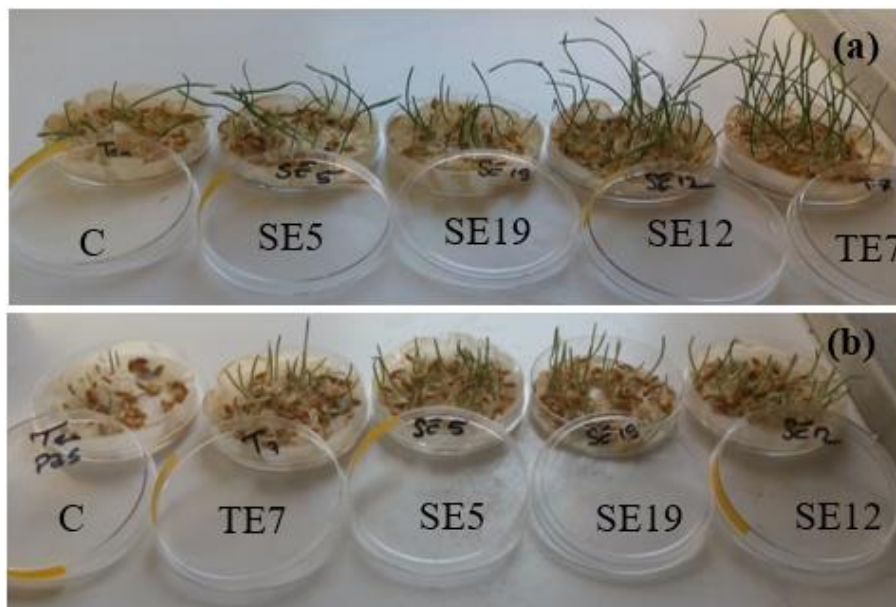
Each of the cellulase, amylase and protease production by the fifty-two strains was verified in specific media with the overview of the results is shown in *Table 3*. No cellulose degradation activity was noticed for the strains isolated from *T. gallica* L. while several strains (12) isolated from *S. fruticosa* (L.) Forssk. were positive for cellulase activity. The majority of the strains had positive amylase activity, with only the strain TE7 did not show any amylase production. For protease activity, most of the strains were positive with only the strain (TE7) that was isolated from *T. gallica* L. and the three strains SE13, SE19 and SE20 that were isolated from *S. fruticosa* (L.) Forssk. were negative producers of proteases (*Table 3*).

### ***Petri-dishes experiment***

From the endophytes collection of our study, we selected four strains, namely: TE7, SE5, SE12 and SE19 that showed salt tolerance and ACC-deaminase activity and many of the PGP traits for a Petri dish experiments for both tomato and barley plants under salt stress control (*Figs. 2 and 3*).



**Figure 2.** Morphological appearance of tomato seedlings ten days after sowing. (a) Uninoculated seeds without salt stress (C), Inoculated seeds with TE7, SE19, SE5 and SE12. (b) Tomato seeds subjected to salt stress (150 Mm NaCl), (C) Uninoculated seeds, seeds inoculated with strains TE7, SE5, SE12 and SE19



**Figure 3.** Morphological appearance of barley seedlings ten days after sowing. (a) Uninoculated seeds without stress (C), Inoculated seeds with SE5, SE19, SE12 and TE7. (b) Barley seeds subjected to salt stress (150 Mm NaCl), (C) Uninoculated seeds, seeds inoculated with strains TE7, SE5, SE19 and SE12

A reduction in the germination percentage, fresh and dry weights and seedling lengths of tomato and barley plants were observed at 150 mM of salt concentration compared to the control groups (Figs. 4 and 5). It seems that tomato and barley plants were affected by salt. The tomato and barley seeds that were inoculated by the four selected bacterial endophytes showed growth promotion for both plants by increasing germination percentage, dry weight and seedling lengths at 0 and 150 mM NaCl compared to the uninoculated seeds.

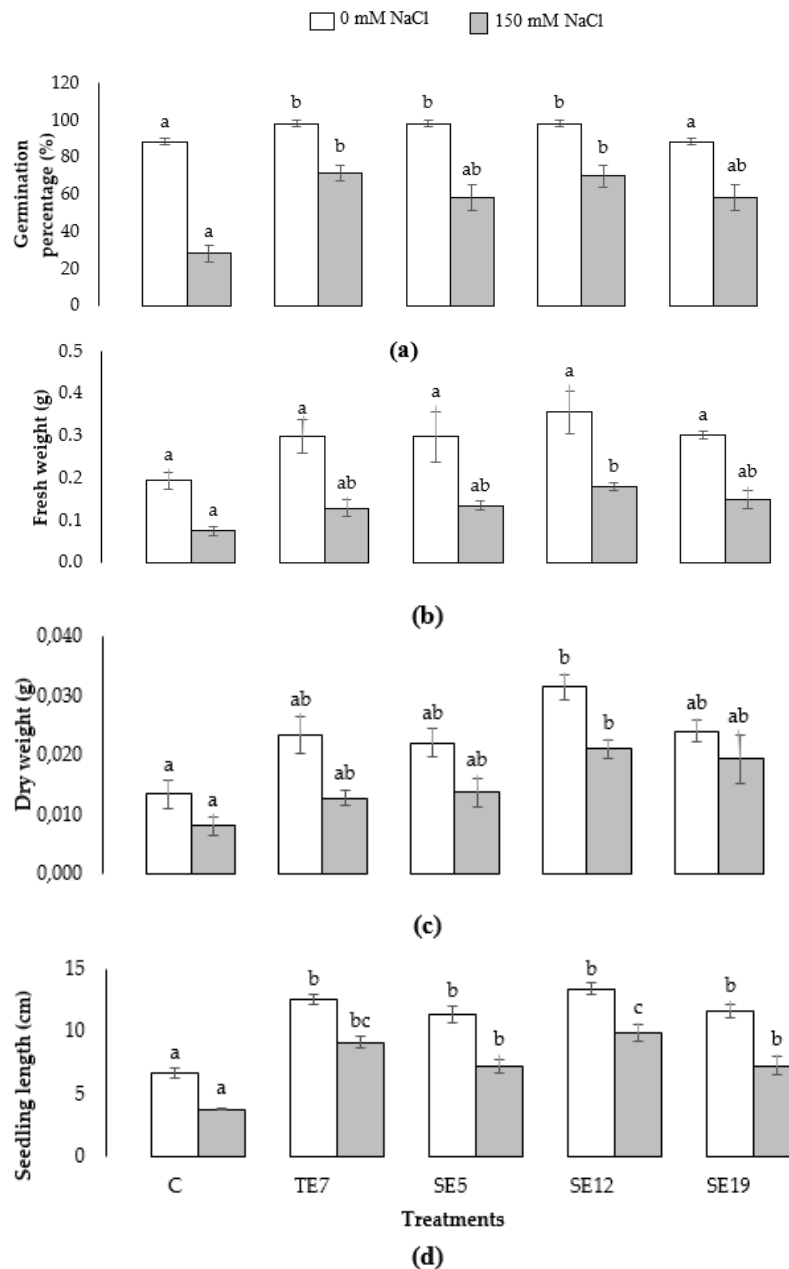
The germination percentage of the inoculated tomato seeds by the strains TE7, SE5 and SE12 were significantly higher than the control and the other tested strain SE19 in 0 Mm NaCl concentration. There was no significant difference in the fresh weight of the inoculated and uninoculated tomato seeds compared to the dry weight. The seedling lengths of the inoculated tomato seeds by all four strains were significantly higher than the control group.

On the other hand, the inoculation with TE7, SE5 and SE12 significantly ameliorated the germination percentage, fresh and dry weights as well as seedling lengths for barley seeds under normal conditions (0 mM NaCl) whereas, all the strains used as bioinoculants showed a positive impact on all the recorded parameters under 150 mM of NaCl.

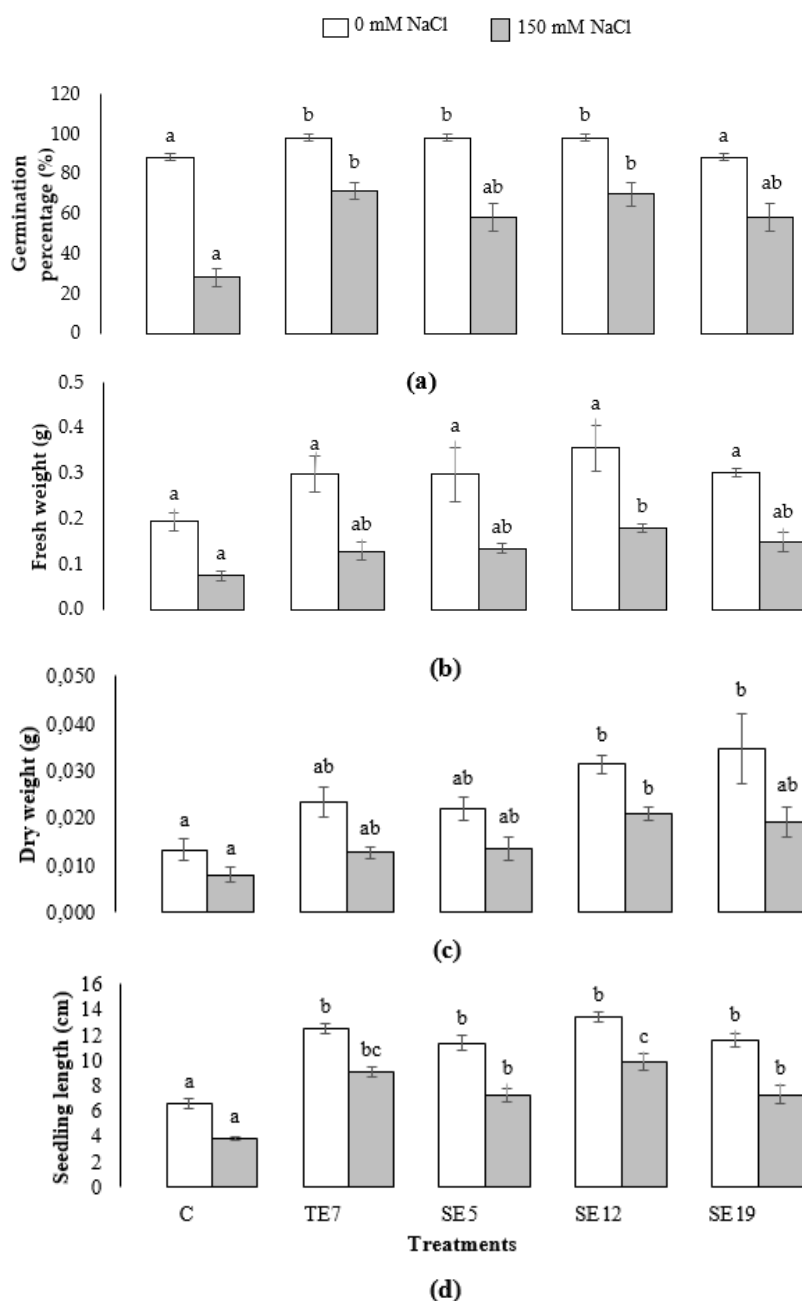
In order to determine the best bacterial strains that promoted tomato and barley growth under normal and saline conditions, we conducted a PC analysis. As shown in Fig. 6a, the first axis (Dim 1) and a second axis (Dim 2) accounted for 96.83% of the total variability exhibited by the analyzed parameters taken into consideration (germination percentage, fresh and dry weight and seedling length). Four clusters were obtained with the two clusters of SE5 and TE7 (cluster 1) and SE12 and SE19 (cluster 2) showed notable plant growth-promoting ability in the absence of salt stress (0 mM NaCl). In the presence of 150 mM of NaCl, the strain TE7 clustered in a group with the control group of non-saline condition (negative control, cluster 3). Thus, the strain SE7 seemed to efficiently



mitigate salt stress permitting plant performance to be equivalent to those obtained in non-saline control conditions. The remaining fourth cluster was composed of the four strains that were used as bioinoculants under the tested salt stress condition (150 mM of NaCl). The inoculation with the four strains TE7, SE5, SE12 and SE19 exhibited performances closer to those expected by the positive control group (cluster 4) making the results less interesting.

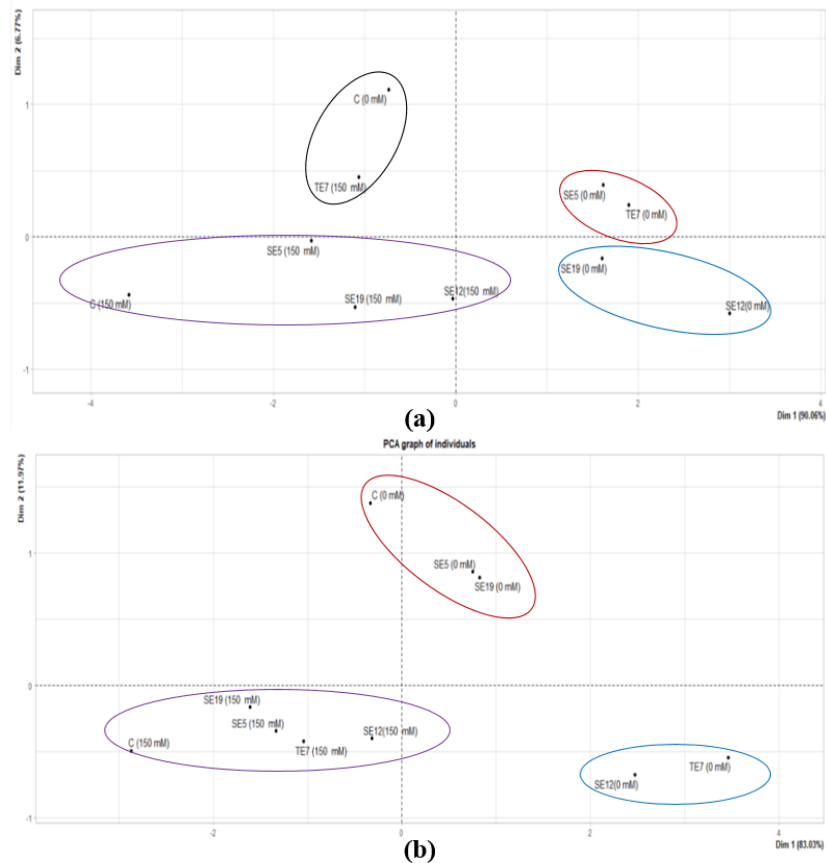


**Figure 4.** Effect of seed bacterization with endophytic bacteria on plant growth promotion of tomato. (a) Germination percentage, (b) Fresh weight, (c) Dry weight, (d) Seedling length. Evaluation was made 10 days after sowing. Bars represent mean  $\pm$  SD of three replicates. Different letters on bars indicate significant differences between white columns treatments (0mM NaCl) or grey columns treatments (150 mM NaCl), Tukey test at  $P= 0.05$



**Figure 5.** Effect of seed bacterization with endophytic bacteria on plant growth promotion of barley. (a) Germination percentage, (b) Fresh weight, (c) Dry weight, (d) Seedling length. Evaluation was made 10 days after sowing. Bars represent mean ± SD of three replicates. Different letters on bars indicate significant differences between white columns treatments (0mM NaCl) or grey columns treatments (150 mM NaCl), Tukey test at P= 0.05

For the barley Petri-dish experiment and the results obtained in Fig. 6b, the two axes (Dim 1 and Dim 2) accounted for 90.00% of the total variability exhibited by the analyzed parameters taken into consideration (germination percentage, fresh and dry weight and seedling growth).



**Figure 6.** Principal Component Analysis (PCA) plots (PC1 and PC2) to show the efficacy of endophytic bacterial strains (TE7, SE5, SE12 and SE19) for their growth-promoting effect on tomato (a) and barley (b) seedlings in the absence (0 Mm NaCl) and presence of salt stress (150 Mm NaCl)

This time, we distinguished only three clusters. The first cluster was formed by the strains SE5 and SE19 and the negative control while the second cluster was formed only by strains TE7 and SE12 in which both of them were grown in the absence of salt stress (0 mM NaCl). These two strains performed close to that of negative control which make them the most interesting ones. The last cluster was formed by all the tested strains under 150 mM of NaCl that exhibited growth performances closer to the positive control group.

## Discussion

Many researchers have been studying the bacterial communities from the endosphere of naturally occurring plants which can potentially have PGP traits that can be useful to plants that are grown under plant-limiting environments like saline soils or even when facing biological attackers (Etesami and Beattie, 2018; Fadiji and Babalola, 2020).

Two halophytes that thrive in arid rangelands of Algeria and are known for their medicinal properties are *T. gallica* L. (*Tamaricaceae*) and *S. fruticosa* (L.) Forssk. (*Amaranthaceae*). From literature in Algeria, these two plants have never been the subject of research on their associate endophytes which make them good candidates for searching

for bacteria that can promote plant growth under plant-limiting environments as the case for saline conditions.

Many researchers are seeking to develop fast and efficient methods with moderate cost that can be applied to identify and classify microorganisms, including bacteria. One of the methods of choice is the matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) that is based on analyzing proteins for the identification and is used in clinical studies (Croxatto et al., 2012; Sauget et al., 2017). Further, The MIDI MIS technique is based on fatty acid methyl ester analysis by gas chromatography (GC-FAME) and has been used to identify microorganisms isolated from clinical and environmental samples (Slabbinck et al., 2009). When comparing the two different methods for taxonomic identification, it seems that MALDI-TOF gave a better identification percentage when compared to GC FAME MIDI identification. This latter gave only four reliable identifications similar to those obtained by MALDI-TOF of the twenty-six isolates of *T. gallica* L. In addition, more than 70% of the isolates were identified by GC FAME MIDI with only two differences from the MALDI-TOF results. The MIDI MIS system and MALDI-TOF analysis are two methods used for the identification of bacterial species by many authors (Adams et al., 2005; Fykse et al., 2015). In our case, the difference between the MIDI MIS and MALDI-TOF results can be explained by the difference in the databases as suggested by Fykse et al. (2015). We did not perform the 16S rDNA sequencing method, which is time-consuming and more laborious compared to the other two methods. Still, we stipulate that MALDI -TOF results can be accurate, as suggested by Fykse et al. (2015) and Dybwad et al. (2012). From the roots of the studied plants in our study, endophytes were isolated with a total of twenty-six per plant. The obtained identification-based results showed the dominance of *Bacillus* genera in both studied plants. Many researchers reported the dominance of *Bacillus* as endophytes in many plant species (maize, cacao, vanilla orchids and rice (White et al., 2014; Bodhankar et al., 2017; Lu et al., 2021). *Bacillus* species have been reported to have PGP traits that help the plants, especially under limiting environments and bio-control of plant diseases (Yu et al., 2011; Lim et al., 2013; Chowdhury et al., 2015).

In addition to *Bacillus*, other minor species were isolated and belonged to *Pseudomonas*, *Pantoea*, *Klebsiella* and *Providencia* that have also been implicated in the positive effects on many host plants (Naz et al., 2016; Pavlova et al., 2017; Lu et al., 2021). The use of halotolerant bacteria and halophiles is one of the strategies used by the scientific community for crops grown in stress conditions (Dodd and Perez-Alfocea, 2012; Etesami and Beattie, 2018).

Antagonistic activity of the isolated endophytes tested was tested toward different phytopathogenic bacteria and fungi. All the endophytes isolated from *T. gallica* L. showed no antifungal activity against all the tested fungi, while the majority (23/26 strains) of endophytes isolated from *S. fruticosa* (L.) Forssk. showed antifungal activity against at least one tested-fungi. Six *Bacillus subtilis* strains (TE1, TE2, TE3, TE4, TE5 and TE6) out of the twenty-six isolated from the roots of *T. gallica* L. have shown antibacterial activity towards all the tested bacterial phytopathogens whereas, only *Bacillus subtilis* SE23 and *Pseudomonas aeruginosa* SE25 presented antibacterial activity for *Agrobacterium tumefaciens* only for isolated from *S. fruticosa* (L.) Forssk. Many *Bacillus* strains are known for their antimicrobial activities as reported by many authors (Chowdhury et al., 2015; Foldes et al., 2000; Lu et al., 2021). Foldes et al. (2000) showed that from the results of 25 *Bacillus* tested isolates; only one (4%) has shown activity towards phytopathogens.

In general, PGPB's help the plants to have the efficient acquisition of nutrients through nitrogen fixation activity increased availability of minerals such as phosphate and potassium, or production of siderophores (iron acquisition). *Pseudomonas* sp. TE7, *Providencia rettgeri* SE5 and SE12, *Klebsiella aerogenes* SE6, *Pantoea agglomerans* SE20 and *Bacillus licheniformis* SE21 are the only isolates to fix nitrogen. Members of the genera *Klebsiella*, *Bacillus* and *Pseudomonas* are capable of fixing atmospheric nitrogen as reported in James (2000).

As *Pseudomonas* sp. TE7, *Providencia rettgeri* SE5 and SE12 as well as *Pantoea agglomerans* SE19 were found to possess many PGPB activities we chose them for *in vitro* pot experiments. Our results were in accordance with those reported by Li et al. (2020) from which *Providencia rettgeri* P2 had IAA, nitrogen fixation, P-solubilization and biocontrol activity.

Many bacteria producing ACC-deaminase are able to facilitate plant growth under salt stress. They are able to promote root elongation and plant growth by lowering ethylene levels in the roots, as stated by Suárez et al. (2008). Our results revealed that a number of the tested strains were ACC deaminase positive, from which TE7, SE5 and SE12 had an interesting ACC deaminase activity. The plants inoculated with these bacteria ameliorated their germination rate in the presence and absence of NaCl for both tomato and barley. These results are in accordance with the work of Damodaran et al. (2013) who reported that endophytes improved germination of plants when facing salt stress. One of the mechanisms that can explain this effect is the production of auxins such as IAA by our strains. This could have triggered the activity of specific enzymes that promoted early germination (Chiwocha et al., 2005). The present study that compared inoculated and uninoculated seeds with PGP endophytes under normal (0 mM NaCl) and salt stress conditions (150 mM NaCl) showed maximum seedling lengths. Furthermore, the inoculation with SE12 gave the best results followed by TE7 and SE9, whereas a minimum was recorded in the control group. Several authors obtained similar results for maize by Shaharouna et al. (2006) and tomato by Neelam and Meenu (2010) and Masmoudi et al. (2021).

## Conclusion

Bacteria act as important components in agriculture. They can promote plant growth potential by controlling external abiotic adverse effects. The current study highlights the beneficial plant-microbe interactions between the tested bacterial halophytes on tomato and barley seeds exposed to one salt level. The tested strains had multiple PGP properties that helped alleviate salt stress on the used plants, making them attractive for further field experiments with the use of the four strains alone or as a small synthetic community as bioinoculants.

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