

Article

Rhizosphere Bacterial Isolation from Indigenous Plants in Arid and Semi-Arid Algerian Soils: Implications for Plant Growth Enhancement

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Abstract: The Mediterranean area is one of the geographic zones most affected by land degradation and desertification and these conditions appear to be even more exacerbated by climate change. Based on this idea, this work aimed to isolate, identify, characterize, and select bacterial strains able to tolerate salinity and drought, which could possibly be used in agriculture as plant biofertilizers. The sampling of rhizosphere soil was performed in two Algerian regions, Ghardaïa and Djelfa (arid and semi-arid zones, respectively) in six provinces, targeting fourteen native plant species, known for their therapeutic use. A total of 288 bacterial strains were isolated, identified, and characterized for their growth at different temperatures and salt tolerance. Based on these capabilities, 95 isolates were selected. These strains underwent further evaluation for their plant-beneficial traits, including siderophore synthesis, auxin production, and phosphate solubilization. Additionally, we assessed their impact on tomato, cucumber, and sorghum seed germination. In a final screening step, nine bacterial strains were tested for their potential plant growth-promoting activity on tomato plants grown in semi-controlled conditions. Our results demonstrated that three strains (*Bacillus simplex* AH24, *Microbacterium arborescens* PU10, and *Microbacterium paludicola* AEA23) showed plant growth promotion activities on tomato.

Keywords: biofertilizer; rhizosphere; desert area; arid zones; semi-arid zones; PGPB; rhizobacteria

1. Introduction

Algeria, the largest country in Africa, spans an extensive land area of approximately 2.38 million km² (World Bank, 2021, available online: <https://www.worldbank.org/en/publication/wdr2021> (accessed on 2 July 2023)). Within this vast territory, three climate types coexist: an arid climate associated with the Sahara Desert, a Mediterranean climate in proximity to the northern mountain and coastal zones, and a semi-arid climate occurring in the highlands. Arid and semi-arid sites occupy 87% of the territory, consisting of 2.38 million Km² [1]. In the north-central desert of Algeria, 80% of these regions are represented by hypersaline zones, posing additional challenges to agricultural productivity [2]. According

to FAO estimates, only 3.6% of the country can be exploited for agriculture (available online: <http://www.fao.org/3/a-bc290e.pdf> (accessed on 12 July 2023)). On the other hand, Algeria is characterized by quite a rich biodiversity, with approximately 16,000 known species overall, of which 3164 are plant species [3]. Moreover, several of these plant species are still in use today as medicinal plants. Very recently, Hemmami et al. [4] reviewed different endemic plant species, growing spontaneously in the Saharan and Algerian areas currently used by indigenous people in an ethnomedicine context.

Algeria is an integral part of the biodiversity hotspot of the Mediterranean region [5]. In the broader context of the Mediterranean basin, desertification has become a pressing environmental issue. It is estimated that approximately 75% of the Mediterranean region is affected by desertification processes, leading to the degradation of land resources and a loss of productivity [6]. These desertification processes have profound economic implications, particularly in terms of agricultural productivity, with a significant impact on local economies and livelihoods. It is estimated that desertification in the Mediterranean basin is occurring following high soil erosion rates ($>2 \text{ t ha}^{-1}$) leading to dramatic economic losses primarily attributed to declines in agricultural production [6,7]. The combined effects of climate change, land use practices, and unsustainable agricultural practices exacerbate the vulnerability of the region to desertification with a dramatic impact on biodiversity, plant productivity, and human health. To address the challenges posed by desertification and mitigate the economic losses associated with agricultural crop production, exploring alternative strategies is imperative. The utilization of beneficial microorganisms, such as plant growth-promoting bacteria (PGPB), holds promise in enhancing soil fertility, nutrient availability, and plant growth even in arid and semi-arid regions [8–10]. The mechanisms used by PGPB in order to support plant growth under drought conditions were recently reviewed by Fadji et al. [11]. By harnessing the potential of these biofertilizers, it is possible to improve crop yields, mitigate the adverse effects of desertification, and promote sustainable agricultural development in the Mediterranean basin.

In this context, the selection of bacteria from the rhizosphere of native plants in desert areas could represent a valuable resource in the quest to enhance plant tolerance to salinity and drought stress, thereby increasing agricultural productivity. In fact, native plant species in arid and semi-arid zones have evolved unique physiological and biochemical adaptations to survive in harsh environmental conditions, including limited water availability and high soil salinity [12,13]. The rhizosphere, the region of soil surrounding plant roots, harbors an immense diversity of microorganisms that have co-evolved with native plants and developed mechanisms involved in the enhancement of plant resilience to these abiotic stresses [14]. Several papers reported that when compared to microorganisms found in non-arid soils, those from a desert area are more adapted to live in extreme environments and are efficient in increasing soil fertility [15–17] and plant growth [18].

In a previous work [19], we described the bacterial communities living in the rhizosphere of 14 plant species, native to the desert areas of north-central Algeria and well known for their importance in an ethno-medicine context, using a metabarcoding approach. The decision to focus on plants with these specific features was firstly related to the significant value that indigenous medicinal plants hold in terms of biodiversity and traditional knowledge. Moreover, medicinal plants contain active molecules that have therapeutic use and whose production can be modulated by the associated microbiota. Finally, focusing on indigenous medicinal plants may contribute to the conservation of local plants and bacterial biodiversity by raising awareness about the importance of preserving these species, their habitat, and their associated microflora.

Based on the idea that the culturable fraction of this microbiota may represent a precious and rich reservoir of microbial biodiversity with the potential to improve plant performance in challenging environments, we proceeded to the characterization of the culturable fraction of the previously characterized whole microbiota.

2. Materials and Methods

2.1. Soil Sampling

Soil sampling was performed in September 2018 in a semi-arid and arid region of Algeria (Djelfa and Ghardaïa, respectively) as described in [19] (Figure 1). The Ghardaïa region is located in the South of Algeria and is characterized by hot (mean temperature 36.8 °C) and dry summer; the Djelfa zone is hot during summer but very cold in winter and the range of temperature during night and day is very wide (climatic data such as temperature, humidity, and rainfall are reported in [19], Supplementary Materials Figures S1 and S2).

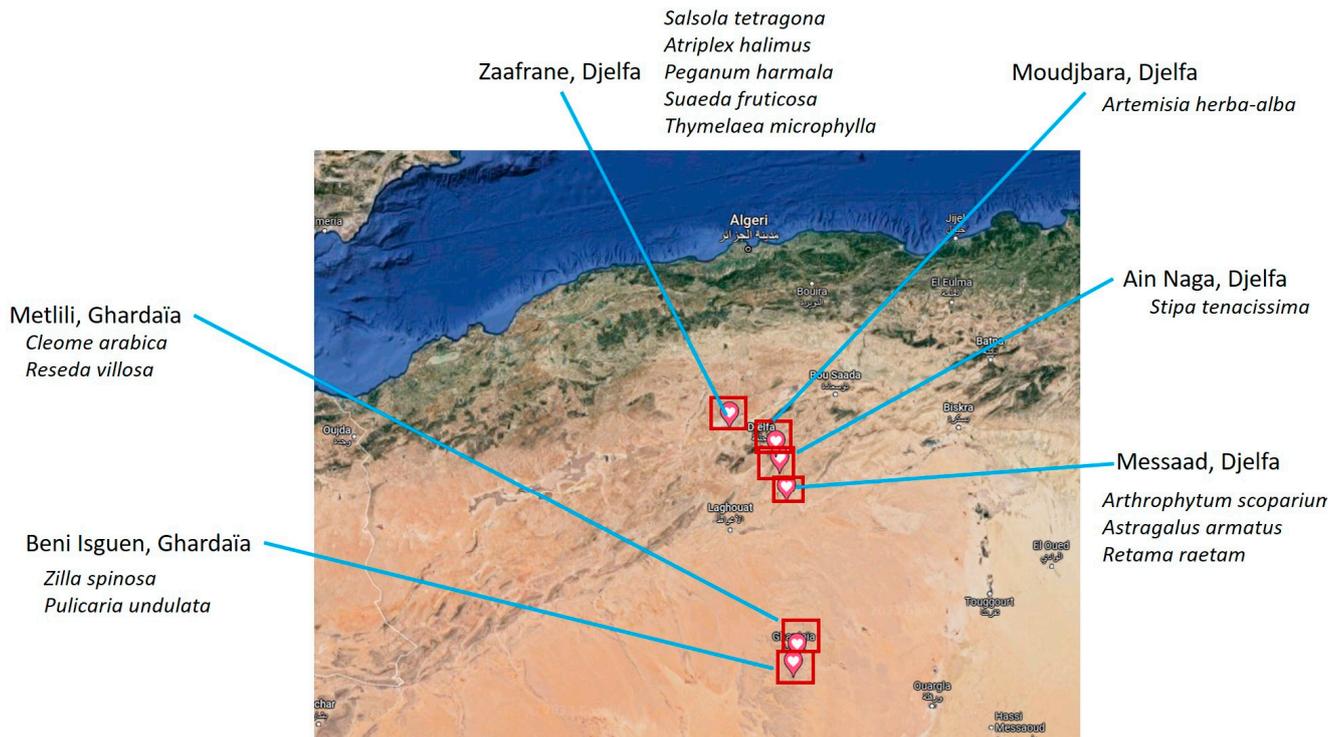


Figure 1. Map of the sampling sites. Fourteen plant species were collected from two sites in the arid region of Ghardaïa and from four sites in the semi-arid region of Djelfa. Plant species sampled at each site are reported.

Soil samples were collected in proximity of 14 native plant species (listed in Table 1), four of them (*Cleome arabica*, *Reseda villosa*, *Zilla spinosa*, and *Pulicaria undulata*) in the Ghardaïa region and the other ten (*Arthrophytum scoparium*, *Astragalus armatus*, *Retama raetam*, *Stipa tenacissima*, *Artemisia herba-alba*, *Salsola tetragona*, *Atriplex halimus*, *Peganum harmala*, *Suaeda fruticosa*, and *Thymelaea microphylla*) in Djelfa. More in detail, five soil pits for plants were sampled in proximity to the roots, after removing the surface layer (5 cm) perpendicularly all along the roots to a depth ranging from 5 to 20 cm. The excess soil close to the roots was gently removed and the soil that remained attached to the roots was considered as rhizosphere and sampled by sterile gloves. Soil samples were then stored at 4 °C until taken to laboratory. Half of the available rhizosphere soil was stored at −80 °C for the metabarcoding characterization of the microbiota (data published by [19]) and the other half was quickly processed for isolation of the culturable fraction. Chemical and physical characterization of the soils were performed as described in [20] and the data are reported in [19] (Supplementary Materials, Table S2). Information regarding the 14 plant species such as the local name, their classification, and their traditional use is reported in [19] (Supplementary Materials Table S1).

Table 1. Number of species and biodiversity indices calculated according to the plant species (CA, *Cleome arabica*; AH, *Atriplex halimus*; PU, *Pulicaria undulata*; RV, *Reseda villosa*; ZS, *Zilla spinose*; AHA, *Artemisia herba-alba*; AS, *Arthrophytum scoparium*; AA, *Astragalus armatus*; PH, *Peganum harmala*; RR, *Retama raetam*; SF, *Suaeda fruticose*; SAT, *Salsola tetragona*; ST, *Stipa tenacissima*; TM, *Thymelaea microphylla*).

	Number of Species (S)	Simpson's Dominance (D)	Simpson's Biodiversity (1-D)	Simpson's Biodiversity (1/D)	Shannon's (H')	Evenness EH = H/ln(S)
CA	11	0.240	0.760	4.172	0.833	0.347
AH	11	0.128	0.872	7.806	0.961	0.401
PU	13	0.116	0.884	8.647	1.030	0.402
RV	13	0.129	0.871	7.737	1.014	0.395
ZS	14	0.088	0.912	11.308	1.100	0.417
AHA	14	0.088	0.912	11.308	1.100	0.417
AS	10	0.164	0.836	6.080	0.876	0.380
AA	8	0.191	0.809	5.232	0.799	0.384
PH	13	0.095	0.905	10.526	1.068	0.416
RR	14	0.091	0.909	10.939	1.096	0.415
SF	12	0.107	0.893	9.308	1.021	0.411
SAT	10	0.130	0.870	7.681	0.938	0.407
ST	14	0.140	0.860	7.118	1.019	0.386
TM	6	0.225	0.775	4.446	0.708	0.395

2.2. Isolation of Culturable Bacteria

Culturable bacteria were extracted and isolated from the different rhizosphere soils collected as previously described. Ten grams of soil were added to $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ buffer (0.1M, Sigma-Aldrich, Burlington, MA, USA) and shaken at 180 rpm for 1 h at room temperature and left to stand for 30 min. Serial dilutions (up to 10^{-4}) in magnesium buffer were prepared and 100 μL of dilutions 10^{-2} , 10^{-3} , 10^{-4} were plated in triplicate on 10% TSA (Biolife) containing cycloheximide (ICN, 100 $\mu\text{g}/\text{mL}$). Plates were incubated at 28 °C and read after three and seven days.

The collected colonies were named with the abbreviated sample name followed by the number. The isolated bacterial colonies were maintained at -80 °C in glycerol. All isolated strains were subjected to microscopic observation to define the morphology of the colonies and were distinguished into the two main bacterial categories thanks to Gram staining.

2.3. Identification of Bacterial Strains

Bacterial strain identification was performed using mass spectrometry MALDI (Matrix-Assisted Laser Desorption/Ionization) and TOF/TOF (UltrafleXtreme, Bruker, Billerica, MA, USA), as reported by Novello et al. [21]. A fresh bacterial colony grown on TSA was spotted in triplicate on an MTP 384 target plate (Bruker Daltonics, Milan, Italy). Initially, the spot was covered with 70% Formic acid (Sigma Aldrich, Burlington, MA, USA) and then with HCCA (alpha-cyano-4-hydroxycinnamic acid) (Bruker, Milan, Italy). The target plate was left to dry at room temperature until crystallization of sample. The mass spectra obtained for each bacterial strain were analyzed using the Biotyper software v. 2.0 (Bruker Daltonics, Milan, Italy). The identification threshold was higher than 2, as recommended by Bruker for high-confidence species identification.

2.4. Growth Temperature Assessment and Evaluation of Salt Tolerance

In order to determine the optimal range of growth temperature, the strains were swabbed on TSA and incubated at four temperatures: 4, 28, 37, and 45 °C for 24/48 h.

The minimal inhibitory concentration (MIC) of sodium chloride was determined by microplate assay, as reported by Novello et al. [21]. In detail, NaCl (Fluka) was dissolved in Tryptic Soy Broth (TSB, Sigma, Burlington, MA, USA) until a concentration of 28.8%. Starting from this concentration, 1:2 dilutions were carried out in the plate and then 100 μL of bacterial strain was introduced into each well at a concentration of 10^6 CFU/mL. In

addition, a negative control, containing TSB and salt, and a positive control, containing TSB with bacterial strain, were placed in each microplate. The plates were incubated for 48 h at 28 °C and the well was considered positive when the presence of bacterial pellet was evident.

2.5. Determination of Plant-Beneficial Physiological Traits

The isolated bacterial strains were tested for the production of indole-3 acetic acid (IAA) following the method described by [22]. The strains were inoculated on the nitrocellulose disk at the center of the plates containing 10% TSA added with tryptophan (5 mM, Sigma-Aldrich, Burlington, MA, USA). After 72 h of incubation at 28 °C, the disk was removed and dipped in Salkowsky's reagent (FeCl_3 0.5 M in 35% HClO_4). The production of IAA was evidenced by the formation of a pink-red halo around the colony. The ability to produce siderophores was evaluated on universal medium CAS (Chrome Azurol S) [23]. The bacterial strains were inoculated in the center of the plate and incubated at 28 °C. The reading was carried out after 7 days by measuring the diameter of the colonies and the pink/orange halo; the value used for data processing was obtained by calculating the ratio between the diameter of the halo and the colony.

Phosphate solubilization activity was evaluated on two media, one containing tricalcium phosphate (TCP), and the other one containing dicalcium phosphate (DCP). The plates were incubated for 15 days at 28 °C. TCP solubilization was indicated by the growth of the colony, while DCP solubilization was identified by a clarification halo around the colony. Each experiment was performed in triplicate.

2.6. Seed Sterilization and Germination

Thirty seeds for each of the three plant species (tomato, *Solanum lycopersicum* var. Ciliegino; sorghum *Sorghum vulgare*; cucumber, *Cucumis sativus* var. Marketmore) were washed in sterile deionized water (3 times for 5 min) and then sterilized for 5 min in a 30% *v/v* of sodium hypochlorite solution. After eliminating the excess NaClO by washing three times with sterile deionized water, three seeds were put on TSA plates and incubated at 28 °C for 72 h to verify the sterility of the seeds. The remaining seeds were inoculated with the bacteria by dipping them for 30 min in a bacterial suspension containing 10^8 CFU/mL of each bacterial strain. Bacterized seeds were then placed in 15 cm Petri dishes containing wet filter paper. Negative control, represented by uninoculated seeds was set up for each plant species. The plates were wrapped in aluminum foil to avoid exposure to light and incubated at 25 °C.

Germinated seeds were counted after 3, 5, and 7 days of incubation, and the length of each root was measured at 7 days. Cucumber embryonic roots were also analyzed by WinRhizo Pro software (Regent Instruments Inc., Québec City, QC, Canada) and the following parameters were evaluated: total length, surface area, volume, fork and tip numbers, and root branching (the ratio between tip numbers and total length of roots).

2.7. Greenhouse Experiment

For the greenhouse experiment, *Solanum lycopersicum* seeds (var. Ciliegino) were sterilized as indicated before, germinated, and inoculated with each of the selected bacterial strains.

The pots were set up with a layer of 4/5 mm quartz sand, central layer of non-sterile soil, and a surface layer of 2/3 mm quartz sand. After 20 days from sowing, the seedlings were re-inoculated with the bacterial suspension containing 10^8 CFU/mL and after 60 days they were harvested. Plants were rinsed under tap water to remove any soil residue from roots. For each plant the following parameters were measured: root and shoot lengths, total plant fresh weight, and the fresh and dry weights of root, shoot and leaves. The fresh roots were analyzed by WinRhizo Pro, and total length, surface area, volume, fork and tip numbers, and root branching (the ratio between tip number and total length of roots) were recorded. Finally, to obtain the dry weights, fresh roots, shoots, and leaves were placed in an oven at 60 °C until completely dry, then they were weighed.

2.8. Statistical Analysis

Data obtained for all experiments were analyzed using ANOVA. Statistical analyses were performed with the software STATVIEW 4.5 (Abacus Concepts, Berkeley, CA, USA); data were compared using one-way ANOVA, followed by a post hoc Fisher's Protected Least Significant Difference (PLSD) test ($p \leq 0.05$). A Principal component analysis, based on the relative abundance of the culturable bacterial species from arid and semi-arid regions, was performed by R (v. 3.5.1) (R Core Team, Vienna, Austria, 2018) using FactoMineR and Factoextra packages. Biodiversity indices were calculated according to the plant species.

3. Results

3.1. Screening of the Isolated Bacterial Strains

A total of 288 bacterial strains were isolated from the different rhizospheric soils. In detail, 19 bacterial strains from *Astragalus armatus* (referred to as AA), 22 from *Atriplex halimus* (AH), 20 from *Peganum harmala* (PH), 22 from *Stipa tenacissima* (ST), 22 from *Cleome arabica* (CA), 21 from *Artemisia herba-alba* (AEA), 19 from *Salsola tetragona* (SAT), 20 from *Pulicaria undulata* (PU), 18 from *Thymalalea microphylla* (TM), 20 from *Reseda villosa* (RV), 22 from *Suaeda fruticosa* (SF), 23 from *Arthrophytum scoparium* (AS), 21 from *Zilla spinosa* (ZS), and 19 from *Retama raetam* (RR) (Tables S1–S15). These bacteria were then tested for their capability to grow at different temperatures and for their salt tolerance through MIC determination. While all these bacterial strains were able to grow at 28 °C, only 31% grew at 4 °C, 96.5% at 37 °C, and 71.5% at 45 °C. Interestingly, the percentage of bacterial strains able to develop colonies at 45 °C was high among bacteria isolated from *R. villosa* and *A. alimus* (95.4% of the strains isolated from these plant species). Similarly, the capability to grow at 4 °C was particularly spread among bacteria isolated from *A. armatus* and *A. scoparium* (58% and 65.2% of the strains isolated from these plant species, respectively). Regarding salt tolerance, the majority of the strains (75.6%) showed a 14% MIC value, while 23.6% of the bacterial isolates showed a 28% MIC value. The highest number of bacterial strains tolerant to 28% of salt was found in the rhizosphere of *T. microphylla* (81% of the strains isolated from this plant species) (Supplementary Materials Tables S1–S14).

3.2. Bacterial Culturable Fraction Profiling

All isolated bacterial strains were identified using mass spectrometry MALDI-TOF.

The bacterial isolates belonged to 60 species. Among them, *Bacillus* sp. was common in the rhizosphere of all the considered plant species. On the contrary, about 50% of the bacterial species (29 out of 60) were found to be non-dominant, mutually exclusive of each plant species (Table S15). The identification of each bacterial strain reported in Table S1–S14 (Supplementary Materials) was used in order to verify a possible relation between bacterial species, plant species, sampling sites, and climatic zones (arid and semi-arid).

Biodiversity indices were calculated according to the plant species (Table 1). The highest number of bacterial species and Shannon's biodiversity index was recorded in the rhizosphere of *A. herba-alba* (14 and 1.100, respectively), *R. retam* (14 and 1.096, respectively), *Z. spinosa* (14 and 1.082, respectively), and *S. tenacissima* (14 and 1.019, respectively), while the lowest one was observed in the rhizosphere of *T. microphylla* (6 and 0.708, respectively).

Based on PCA analysis, Moudjbara (semi-arid) was the most separated site on the two dimensions, and it was characterized by the presence of *B. endophyticus*, *B. idriensis*, *B. indicus*, *Cellulosimicrobium* sp., *M. paludicola*, *P. aurescens*, *P. brassicacearum*, *P. chlororaphis*, *P. corrugata*, and *V. paradoxus* (Figure 2). The Ain Naga (semi-arid) site was mainly characterized by *Bacillus* sp. and *P. glucanolyticus*. The species typically associated with Beni Isguen (arid) were *Arthobacter* sp., *M. timonae*, *M. arborescens*, and *P. oxydans* (Figure 2). The bacterial species *B. cereus*, *B. atropheus*, *M. luteus*, and *S. warneri* were associated with Methlili (arid). On the contrary, Messâad and Zahâfrane were weakly separated according to the first two dimensions of the PCA (Figure 2).

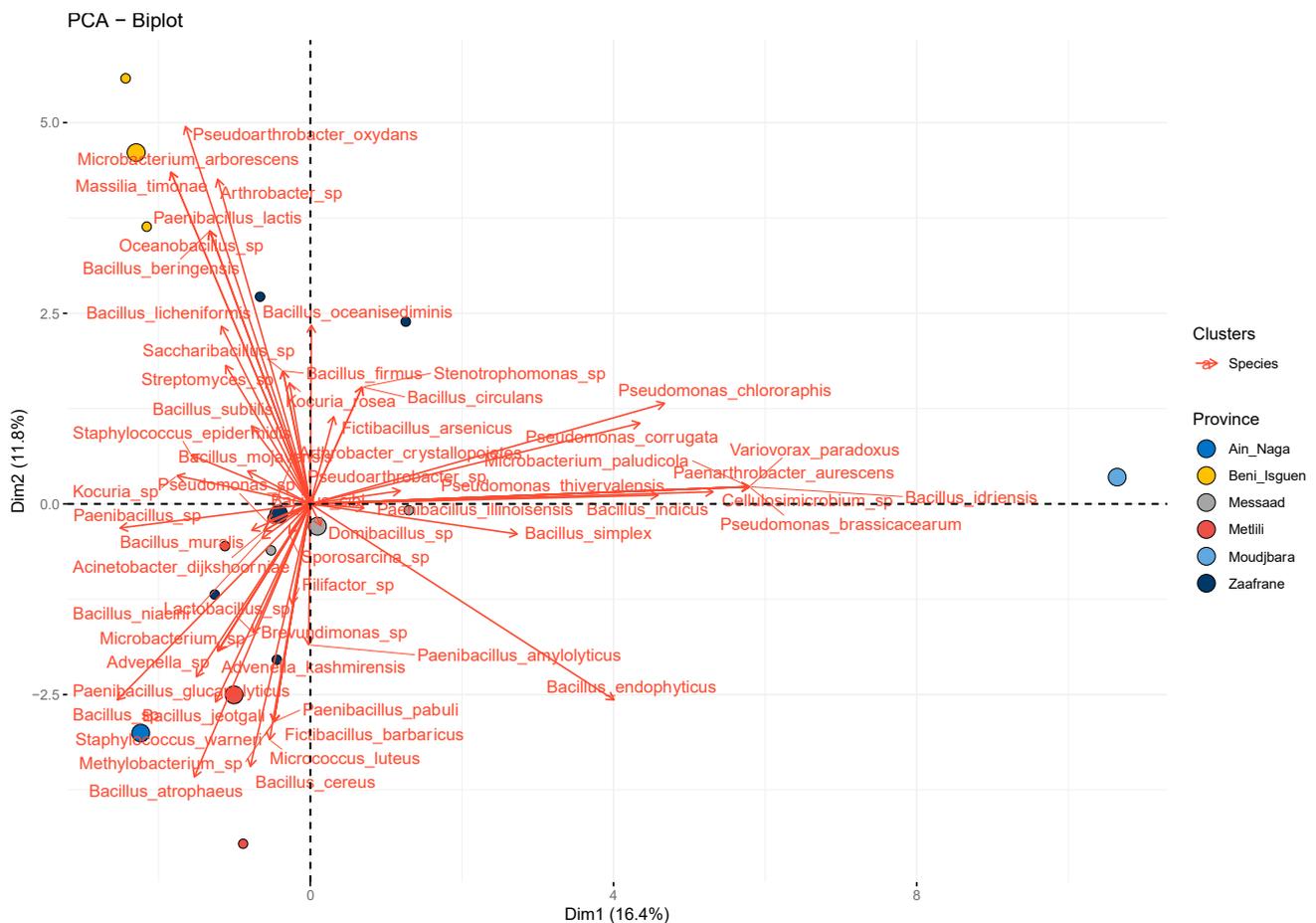


Figure 2. PCA analysis at species level based on the sampling sites (Ain Naga, Béni Isguen, Messaad, Metlili, Moudjbara, Zaâfrane). A total of 28.2% of variability was explained by dimensions 1 and 2. Each bigger dot represents the mean value for each considered parameter while each little dot represents each considered sample.

Based on PCA analysis, arid and semi-arid sites clustered separately according to the two dimensions. The species *Arthobacter* sp., *M. timonae*, *M. arborescens*, and *P. oxydans* were associated with the arid zone, while *B. endophyticus*, *B. idriensis*, *B. indicus*, *B. simplex*, *Cellulosimicrobium* sp., *M. paludicola*, *P. aurescens*, *P. brassicearum*, *P. chlororaphis*, *P. corrugata*, and *V. paradoxus* were related to the semi-arid zone (Figure 3).

3.3. Plant Growth-Promoting Trait Characterization

Based on the identification, the temperature growth test, and the determination of tolerance to NaCl, 95 bacterial strains out of the initial 288 were selected. In addition, bacterial strains not identified at the species level as well as those belonging to species known to be human or plant pathogens were discarded; therefore, only one bacterial strain from the same plant and belonging to the same species was chosen. The selected strains were subjected to the characterization of plant-beneficial traits and used as inoculants in the seed germination assay.

The plant-beneficial physiological activities considered were phosphate solubilization, siderophore production, and synthesis of auxin. Among the 95 strains, only one (*Bacillus mojavensis* ST-12, isolated from the *Stipa tenacissima* rhizosphere) showed the capabilities of releasing siderophores, solubilizing phosphate and producing IAA. Only 20% of the selected strains (19 strains out of 95) were siderophore producers. The percentage of strains able to solubilize phosphate (36 out of 95, 37.9%) was slightly higher. On the contrary, the capability of strains to synthesize IAA was less widespread (14 out of 95 strains, 14.7%) (Table S16, Supplementary Materials).

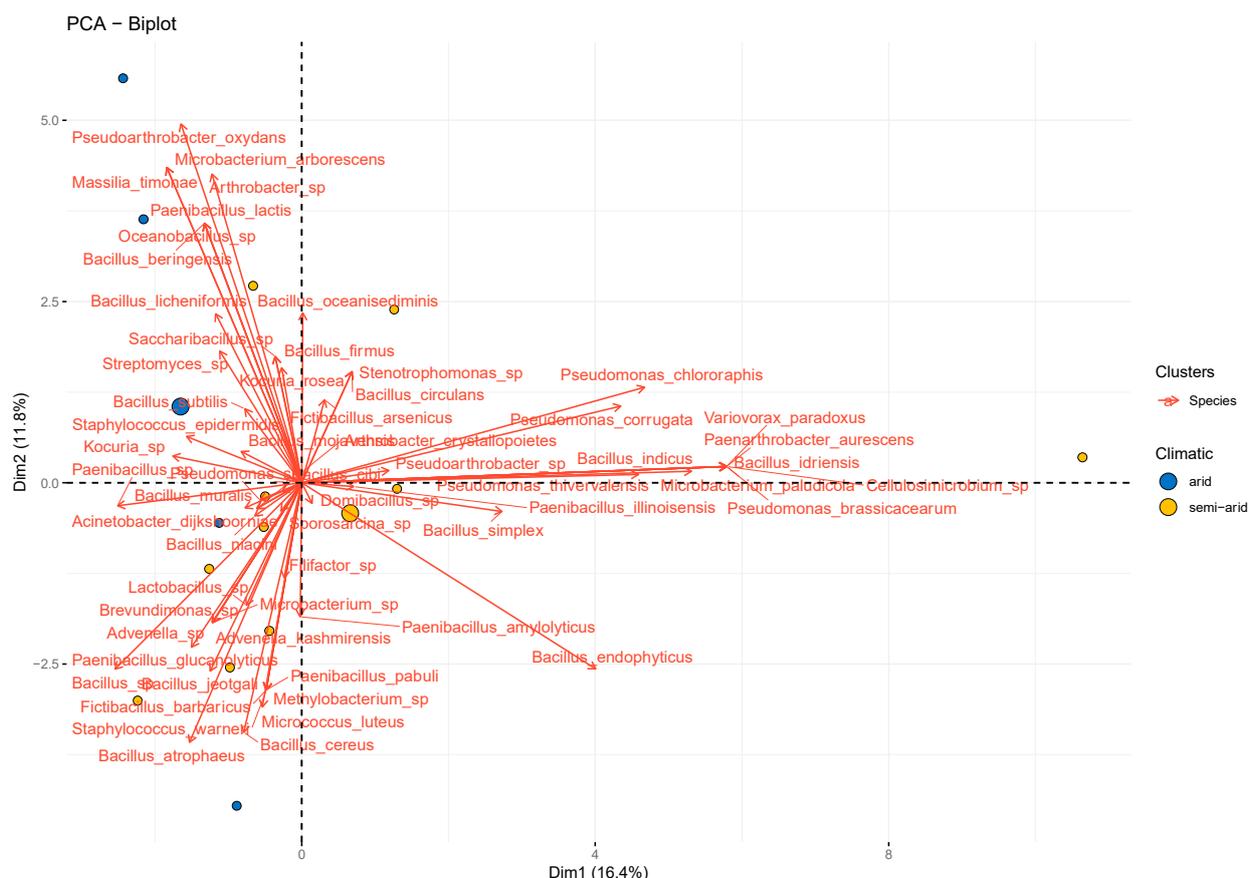


Figure 3. PCA analysis at species level based on the climatic zones (arid vs. semi-arid). A total of 28.2% of variability was explained by dimensions 1 and 2. Each bigger dot represents the mean value for each considered parameter while each little dot represents each considered sample.

Interestingly, no siderophore producers were isolated from *Artemisia herba-alba* and *Cleome Arabica*, no phosphate solubilizing strains were isolated from *Artemisia herba-alba*, *Sueda fruticosa* and *Peganum harmala*, and no strain that was able to produce IAA was isolated from *Reseda villosa*, *Thymalea microphylla*, *Zilla spinosa*, *Peganum harmala*, *Pulicaria undulata*, *Retama raetam*, and *Arthrophytum scoparium* (Table S16, Supplementary Materials).

3.4. Effects on Seed Germination

The plate test on the germination of tomato, sorghum, and cucumber seeds was carried out by inoculating the seeds with the 95 selected strains.

The percentage of germination on cucumber and sorghum seeds, at 7 days, was significantly modified by 13 (seven strains increased this value, while six decreased it) and 46 (25 strains increased this parameter, while 21 decreased it) bacterial strains, respectively. As far as tomato seeds are concerned, no strain showed a positive effect when compared to the control, with 42.1% (40 out of 95 strains) of the tested strains reducing the seed germination percentage.

The results on the length of the tap root showed that 6 out of the 95 tested strains (6.32%) enhanced the germination of sorghum seeds, but not the root growth. Of these, the AS15 strain (isolated from *A. scoparium* and identified as *Bacillus subtilis*) had the same effect on cucumber seeds, and the ST10 strain (isolated from *S. tenacissima* and identified as *Bacillus subtilis*) induced similar effects on tomato seeds.

All data regarding the percentage of seed germination and length of the tap root are reported in Supplementary Materials (Table S17); the data obtained for WinRhizo Pro analysis of cucumber seeds are reported in Table S18 (Supplementary Materials).

3.5. Selection of Possible PGP Bacterial Strains and Their Effects on Plant Growth

Nine strains (*Bacillus mojavensis* SF1, *Bacillus endophyticus* ST4, *Bacillus subtilis* ZS11, *Bacillus simplex* AH24, *Pseudomonas* sp. SAT5, *Microbacterium arborescens* PU10, *Bacillus muralis* AA14, *Microbacterium paludicola* AEA23, and *Bacillus mojavensis* ST12) were selected based on the germination assay and the physiological analysis results. These strains were tested for their impact on tomato plant growth under greenhouse conditions. The summary of the physiological characteristics of these nine strains is reported in Table 2.

Table 2. Physiological traits of the nine selected bacterial strains (*B. mojavensis* SF1, *B. endophyticus* ST4, *B. subtilis* ZS11, *B. simplex* AH24, *Pseudomonas* sp. SAT5, *M. arborescens* PU10, *B. muralis* AA14, *M. paludicola* AEA23, and *B. mojavensis* ST12): growth at different temperatures (4, 28, 37 and 45 °C), evaluation of salt tolerance by MIC measurement, IAA and siderophore production, and phosphate solubilization capability on di-calcium phosphate and tri-calcium phosphate.

Bacterial strain	Growth at				MIC NaCl%	IAA	Siderophores	DCP	TCP
	4 °C	28 °C	37 °C	45 °C					
SF1	+	+	+	+	14.4	–	+	–	+
ST4	+	+	+	+	28.8	+	–	+	+
ZS11	–	+	+	+	14.4	–	+	+	+
AH24	–	+	+	+	14.4	+	–	–	+
SAT5	–	+	+	–	14.4	–	–	+	+
PU10	+	+	+	+	14.4	–	–	+	+
AA14	+	+	+	+	14.4	+	–	–	+
AEA23	–	+	+	+	14.4	+	–	–	–
ST12	–	+	+	+	28.8	+	+	–	+

Plant biomass was significantly increased by the strains AH24, PU10, and AEA23 (by 58.3, 61.1, and 77.0%, respectively) (Figure 4A). The beneficial effect of the bacterial strains was more evident on the roots (six out of the nine selected strains induced a significant increase) than on the shoots (only three out of the nine selected strains induced a significant increase). In detail, plants inoculated with SF1, ZS11, AH24, PU10, AEA23, and ST12 showed higher root fresh weight compared to the control (by 51.4, 52.3, 57.4, 81.9, 64.4, and 44.7%, respectively) (Figure 4B). Enhancement of the shoot fresh weight was observed in plants inoculated with AH24, PU10, and AEA23 (by 57.9, 57.3, and 79.8%, respectively) (Figure 4C). Similarly, these three bacterial isolates were able to significantly increase the fresh leaf weight (AH24 by 66.2%, PU10 by 62.5%, and AEA23 by 94%) (Figure 4D), root, shoot, and leaf dry weight (Table 3). On the contrary, bacterial strains did not induce any significant variation on the shoot length (Figure 4E).

Table 3. Effects of the nine selected bacterial strains (*B. mojavensis* SF1, *B. endophyticus* ST4, *B. subtilis* ZS11, *B. simplex* AH24, *Pseudomonas* sp. SAT5, *M. arborescens* PU10, *B. muralis* AA14, *M. paludicola* AEA23, and *B. mojavensis* ST12) on tomato growth parameters compared to uninoculated controls (C): root, shoot, and leaf dry weight. Different letters after values indicate significant differences among the treatments at $p \leq 0.05$.

Treatments	Root Dry Weight (g)	Shoot Dry Weight (g)	Leaf Dry Weight (g)
C	0.06 ± 0.01 d	0.20 ± 0.04 c	0.16 ± 0.03 c
SF1	0.08 ± 0.01 abcd	0.26 ± 0.05 abc	0.19 ± 0.03 bc
ST4	0.06 ± 0.01 cd	0.17 ± 0.02 c	0.14 ± 0.01 c
ZS11	0.09 ± 0.01 abcd	0.25 ± 0.04 abc	0.18 ± 0.03 bc
AH24	0.09 ± 0.01 abcd	0.31 ± 0.03 ab	0.24 ± 0.02 ab
SAT5	0.08 ± 0.01 bcd	0.22 ± 0.03 bc	0.16 ± 0.01 c
PU10	0.11 ± 0.01 a	0.30 ± 0.04 ab	0.24 ± 0.02 ab
AA14	0.07 ± 0.00 cd	0.22 ± 0.03 bc	0.18 ± 0.03 bc
AEA23	0.10 ± 0.01 ab	0.33 ± 0.04 a	0.27 ± 0.02 a
ST12	0.09 ± 0.01 abc	0.26 ± 0.02 abc	0.21 ± 0.01 abc

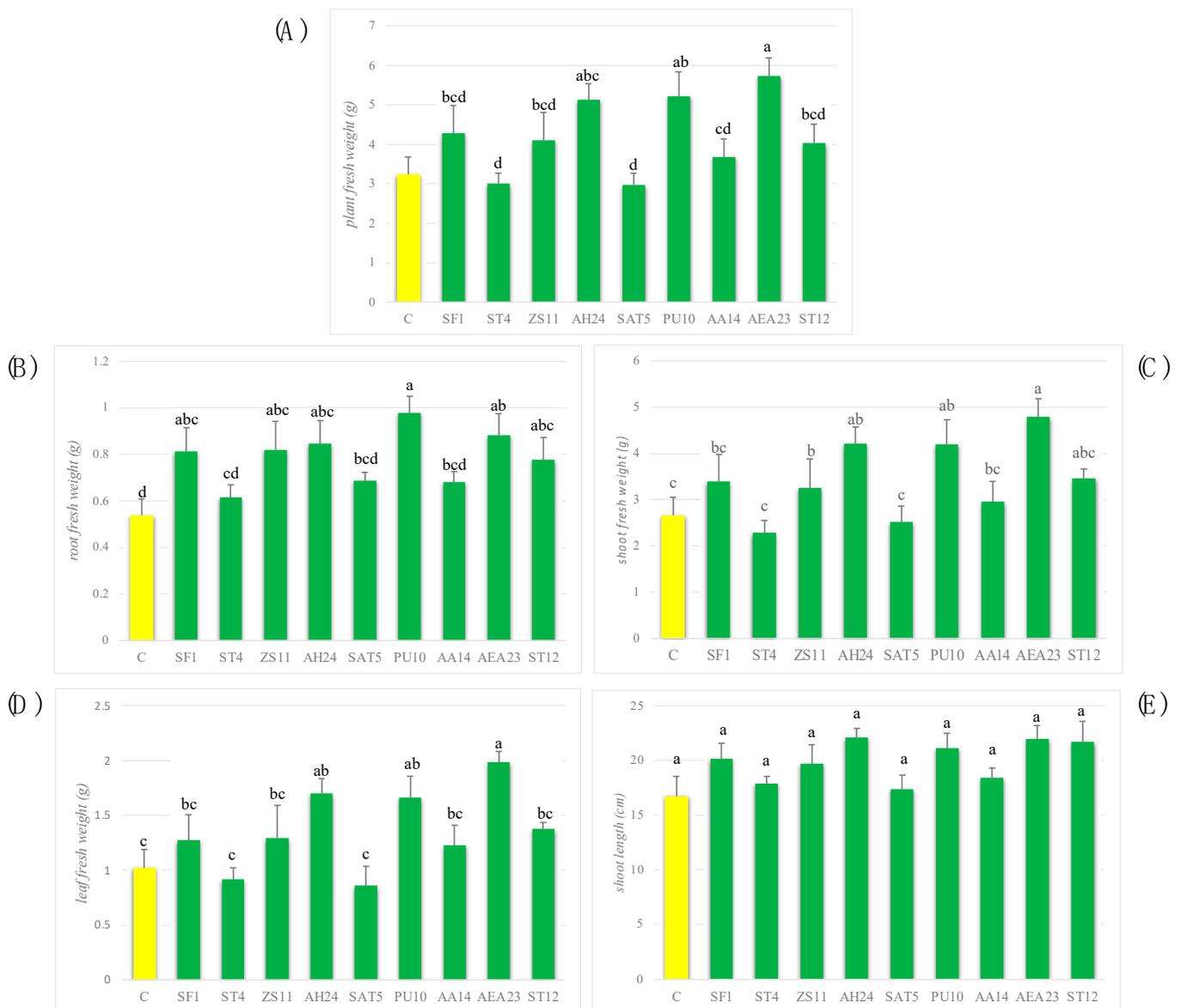


Figure 4. Effects of the nine selected bacterial strains (*B. mojavensis* SF1, *B. endophyticus* ST4, *B. subtilis* ZS11, *B. simplex* AH24, *Pseudomonas* sp. SAT5, *M. arborescens* PU10, *B. muralis* AA14, *M. paludicola* AEA23, and *B. mojavensis* ST12) on tomato growth parameters: plant (A), root fresh (B) and shoot (C) weight, leaf fresh weight (D) and shoot length (E). Yellow bars are the uninoculated controls, while green bars are the different bacterial treatments. Different letters among the treatments indicate significant differences at $p \leq 0.05$.

Seven out of the nine bacterial strains showed an impact on root architecture. Except for SF1, all the bacterial isolates significantly enhanced the length of the tap root (Figure 5A). The root systems of plants inoculated with strains SF1, ZS11, AH24, SAT5, PU10, AEA23, and ST12 were characterized by longer roots, with a higher surface area and tip number compared to the control (Figure 5B–D). The best-performing strain was AEA23, causing an increase of 128% in the root length, 99% in the root surface, and 121% in the tip number compared to the control plants (Figure 5B–D).

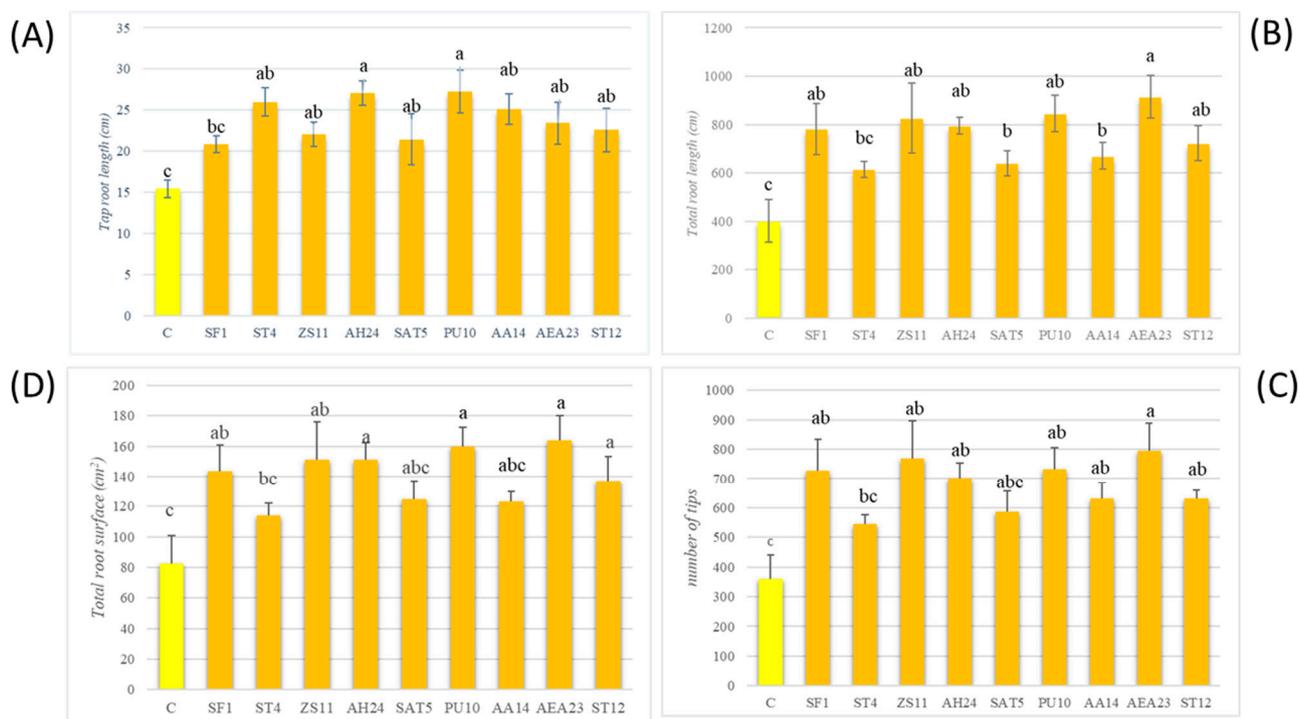


Figure 5. Effects of the nine selected bacterial strains (*B. mojavensis* SF1, *B. endophyticus* ST4, *B. subtilis* ZS11, *B. simplex* AH24, *Pseudomonas* sp. SAT5, *M. arborescens* PU10, *B. muralis* AA14, *M. paludicola* AEA23, and *B. mojavensis* ST12) on tomato root architecture parameters: tap root length (A), total root length (B), total root surface (C), and number of tips (D). Yellow bars are the uninoculated controls, while orange bars are the different bacterial treatments. Different letters among the treatments indicate significant differences at $p \leq 0.05$.

4. Discussion

The knowledge of the intricate interactions between microorganisms and native plant species in arid and semi-arid areas, where desertification processes are occurring leading to significant economic and agricultural losses, provides a comprehensive framework for implementing effective strategies to combat desertification, enhance agricultural productivity, and promote sustainable development. In a previous paper [19], we revealed the biodiversity of soil bacterial communities associated with native plants in the desert areas of north-central Algeria, highlighting the influence of arid and semi-arid climatic zones on the composition and diversity of these bacterial communities.

The present work aimed to isolate, identify, and characterize bacterial strains associated with the rhizosphere of fourteen native plant species able to tolerate salinity and drought stresses typical of Algeria's arid and semi-arid zones. The basic idea of the experimental plan was to specifically focus on the rhizosphere bacteria associated with native plant species growing in arid and semi-arid environments of the Algerian Sahara Desert in order to select bacteria well-adapted to these environments and also able to promote plant growth under salinity and drought stress conditions. Moreover, the identification of culturable bacterial strains from the rhizosphere provides valuable insights into the specific microbial communities that interact with plants and contribute to their adaptation and survival in desert environments.

As explained in the Results section, 288 bacterial strains were isolated from the rhizosphere of 14 plant species native to the central-north desert of Algeria, four of them growing in arid zones and the remaining in semi-arid zones. Bacterial identification using mass spectrometry MALDI-TOF revealed the presence of diverse bacterial species, with *Bacillus* sp. being commonly found as dominant in the rhizosphere of all the plant species. While *Bacillus* sp. commonly represents a large proportion of the soil, rhizosphere, and

endo-rhizosphere bacterial community, our results are consistent with previous studies conducted in other arid regions, such as the Sahara Desert and Middle East [24,25]. *Bacillus* spp. are well-documented for their ubiquitous nature and their ability to colonize various plant hosts and produce plant-beneficial metabolites, as well as antibiotics, enhance nutrient availability (especially phosphate), and improve plant tolerance to abiotic stresses [26–28]. They are able to survive under arid, high-temperature conditions and other environmental stresses thanks to their ability to form endospores, and thus resist changes in environmental conditions. Therefore, the abundance of *Bacillus* spp. in the rhizosphere of native plant species in Algeria suggests their potential role as key drivers in the plant–microbe interactions in these challenging environments. On the other hand, approximately 50% of the culturable bacterial species turned out to be non-dominant and mutually exclusive to specific plant species, indicating that different native plant species in arid and semi-arid zones may recruit specific bacterial communities and improve their adaptation and survival under harsh conditions, potentially indicating plant–microbe co-evolution and niche-specific adaptations [29,30]. Although the ecological role of species occurring at low frequency is poorly known, these bacterial species are supposed to contribute to plant–microbe interactions through unique metabolic capabilities. According to a recent study performed on *Zea mays* and *Sophora davidii* intercropping systems in China, significant changes in rare bacterial species are recognized as the best predictor of rhizosphere elements circling, multifunctionality, above-ground yield, and trade-offs [31]. This possibility was also reported in studies exploring the rhizosphere microbiome of native plant species in arid ecosystems [32–34].

All the isolated strains were tested for their capability to grow at different temperatures and for their tolerance against salinity. It is well known that soil temperature affects bacterial survival, activity, and growth and selects those members of the bacterial community that are more adapted to the prevailing temperature regime. Desert soil is typically subjected to large fluctuations in daily and annual temperatures. As an example, the Djelfa region is characterized by very cold weather in winter and hot weather in summer, with wide temperature excursions over the day. The higher percentage of bacterial strains capable of growing at 42 °C, particularly in the rhizosphere of *R. villosa* and *A. alimus*, is consistent with the findings of studies of bacterial communities in hot desert soils [35–37]. On the other hand, the highest proportion of bacterial strains able to grow at 4 °C was found in the rhizosphere of *A. armatus* (58%) and *A. scoparium* (65%), sampled in the Messâad province of the Djelfa region. It is possible to suppose that these bacteria have developed mechanisms to withstand and thrive under extreme temperature conditions. Similarly, the high percentage of bacterial strains tolerant to salinity, especially in the rhizosphere of *T. microphylla* (81% of the isolated strains tolerant to 28% of salt), is consistent with previous studies highlighting the role of halotolerant bacteria in arid and saline environments [18,38–40]. However, to the best of our knowledge, no published paper deals with the bacterial community associated with this important plant with antioxidant and anti-inflammatory properties [41]; therefore, a comparison is impossible. Our findings support the idea that bacterial communities in arid and semi-arid zones possess unique adaptive traits to cope with challenging environmental conditions with an inestimable value, especially in the biotechnology field (enzymes, biomolecules, compatible solutes). The variation in temperature and salt tolerance among the isolated bacterial strains reflects their adaptation to the specific environmental conditions of the arid and semi-arid zones.

Biodiversity indices varied among the different plant species. A higher number of bacterial species and Shannon’s biodiversity index were measured in the rhizosphere of *Artemisia herba-alba*, *R. retam*, *Z. spinosa*, and *S. tenacissima*. Conversely, the rhizosphere of *T. microphylla* showed lower bacterial species richness and biodiversity indices and highest dominance. These variations in microbial diversity among plant species may be attributed to plant-specific root exudates and interactions with soil bacteria. The differences in microbial diversity among plant species highlight the importance of plant-specific interactions in shaping the composition and functioning of rhizosphere microbiomes, as

well as the importance of the soil and climatic characteristics of the different geographic zones [19,42,43].

Principal Component Analysis (PCA) provided insights into the clustering patterns of bacterial species in relation to sampling sites and climatic zones. The semi-arid site of Moudjbara exhibited distinct bacterial species composition, including *Bacillus endophyticus*, *Bacillus idriensis*, *Bacillus indicus*, *Cellulosimicrobium* sp., *Microbacterium paludicola*, *Pae-narthrobacter aureescens*, *Pseudomonas brassicacearum*, *Pseudomonas chlororaphis*, *Pseudomonas corrugata*, and *Variovorax paradoxus*. In contrast, the arid site of Beni Isguen was characterized by *Arthobacter* sp., *Massilia timonae*, *Microbacterium arborescens*, and *Pseudoarthrobacter oxydans*. These results indicate site-specific microbial assemblages in arid and semi-arid zones [32,44–46], reflecting the influence of local environmental factors, such as soil properties, climatic conditions, and plant species on shaping microbial communities.

Most of the selected bacterial strains, especially *Bacillus simplex* AH24, *Microbacterium arborescens* PU10, and *Microbacterium paludicola* AEA23, exhibited plant growth-promoting traits and significantly enhanced tomato plant growth under greenhouse conditions.

The representatives of the *Microbacteriaceae* family are typical soil bacteria with non-motile, non-spore-forming, rod-shaped, Gram-positive cells. While some members of the genus *Microbacterium* (*Microbacterium album* and *deserti*) were isolated from desert soil [46], no data in the literature about the isolation of *Microbacterium paludicola* [47] and *M. arborescens* [48] from arid or semi-arid climatic zones are available. However, the strain JZ37 of *Microbacterium* spp. isolated from plants collected in the Saudi Arabia Desert demonstrated a capability to assist plant growth and enhance salt tolerance in *A. thaliana* [18].

The observed positive effects on root parameters, such as increased fresh weight, length, surface area, and tip number, suggest that plants inoculated with these bacterial strains improve root architecture parameters and consequently nutrient acquisition efficiency. In particular, long root systems are indicative of roots with a high capability to explore the soil for resources, such as water and nutrients. When roots have a higher total length, it typically means that they are extending deeper into the soil and exploring a larger volume of soil space [49]. This exploration activity is crucial for plants to efficiently acquire essential resources for their growth and development, especially in arid and semi-arid climates.

This study provides insights into the microbial diversity, adaptation, and plant growth-promoting potential of bacterial strains isolated from the rhizosphere soil of native plant species in arid and semi-arid zones of Algeria. The identification of plant-specific microbial communities and the selection of bacterial strains with biofertilizer activity offer promising opportunities for developing sustainable agricultural practices in arid and semi-arid regions. Moreover, this study expanded the information already provided by our previous work focused on the whole microbiota and performed by metabarcoding analysis [19]. According to the analysis of the culturome, we found variations in bacterial species composition and diversity based on the climatic zones and plant species.

Finally, the identification of plant growth-promoting bacterial strains from the rhizosphere offers promising prospects for developing biofertilizers and improving agricultural practices in arid and semi-arid regions, contributing to enhanced plant productivity and sustainable food production. Elucidating the role of bacteria in this kind of environmental context could help contribute to the development of sustainable agricultural practices promoting ecosystem resilience, mitigating the impacts of climate change, and addressing the economic losses associated with desertification in the Mediterranean basin. Harnessing the capabilities of these bacteria through their application as biofertilizers offers a promising approach to enhancing crop productivity and ensuring food security in arid and semi-arid regions, where the impacts of salinity and drought stress on agriculture are particularly pronounced [8,50–52].

5. Conclusions

The present work is part of a wider project aimed at characterizing the microbiota associated with the native plants of the Algerian desert areas, which are known for their medicinal use and high tolerance to drought, aridity, and salinity. Based on the idea that the culturable fraction of these microbiota may represent a precious source of bacterial strains able to favor plant growth, especially under stressful conditions, we identified 288 bacterial isolates. According to their physiological activities and their effects on seed germination, nine of these strains were selected and used as inoculants in tomato plants. Through our comprehensive analysis, we identified three promising strains: *Bacillus simplex* AH24, *Microbacterium arborescens* PU10, and *Microbacterium paludicola* AEA23, which showed significant plant growth-promotion activities. These results can open new future perspectives. In fact, due to climate change, both soil salinization and drought are dramatically increasing; in this scenario, the availability of halotolerant bacteria able to support plant growth and boost plant yield, especially under such stressful conditions, offers relevant opportunities to be exploited in agriculture. Of course, further research is required to explore the full potential of these bacterial strains and their possible application in sustainable agriculture practices for combating land degradation and desertification. Considering the results obtained in this work, our next step will be to assess the efficacy of the three selected strains on tomato plants exposed to drought stress.

Supplementary Materials: The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/pr11102907/s1>, Table S1: Identification, growth temperature ranges, and NaCl tolerance (evaluated as Minimum Inhibitory Concentration, MIC) of bacterial strains isolated from *Cleome arabica*. N.D. indicates not determined (the species is a potential human opportunistic pathogen or belongs to *Streptomyces* genus which is difficult to dissolve in a liquid growth medium), Table S2: Identification, growth, temperature ranges, and NaCl tolerance (evaluated as Minimum Inhibitory Concentration, MIC) of bacterial strains isolated from *Pulicaria undulata*. N.D. indicates not determined (the species is a potential human opportunistic pathogen or belongs to *Streptomyces* genus which is difficult to dissolve in a liquid growth medium), Table S3: Identification, growth, temperature ranges, and NaCl tolerance (evaluated as Minimum Inhibitory Concentration, MIC) of bacterial strains isolated from *Reseda villosa*. N.D. indicates not determined (the species is a potential human opportunistic pathogen or belongs to *Streptomyces* genus which is difficult to dissolve in a liquid growth medium), Table S4: Identification, growth temperature ranges, and NaCl tolerance (evaluated as Minimum Inhibitory Concentration, MIC) of bacterial strains isolated from *Zilla spinosa*. N.D. indicates not determined (the species is a potential human opportunistic pathogen or belongs to *Streptomyces* genus which is difficult to dissolve in a liquid growth medium), Table S5: Identification, growth temperature ranges, and NaCl tolerance (evaluated as Minimum Inhibitory Concentration, MIC) of bacterial strains isolated from *Artemisia herba-alba*, Table S6: Identification, growth temperature ranges, and NaCl tolerance (evaluated as Minimum Inhibitory Concentration, MIC) of bacterial strains isolated from *Arthrophytum scoparium*. N.D. indicates not determined (the species is a potential human opportunistic pathogen or belongs to *Streptomyces* genus which is difficult to dissolve in a liquid growth medium), Table S7: Identification, growth temperature ranges, and NaCl tolerance (evaluated as Minimum Inhibitory Concentration, MIC) of bacterial strains isolated from *Astragalus armatus*. N.D. indicates not determined (the species is a potential human opportunistic pathogen or belongs to *Streptomyces* genus which is difficult to dissolve in a liquid growth medium), Table S8: Identification, growth temperature ranges, and NaCl tolerance (evaluated as Minimum Inhibitory Concentration, MIC) of bacterial strains isolated from *Atriplex halimus*. N.D. indicates not determined (the species is a potential human opportunistic pathogen or belongs to *Streptomyces* genus which is difficult to dissolve in a liquid growth medium), Table S9: Identification, growth temperature ranges, and NaCl tolerance (evaluated as Minimum Inhibitory Concentration, MIC) of bacterial strains isolated from *Peganum harmala*. N.D. indicates not determined (the species is a potential human opportunistic pathogen or belongs to *Streptomyces* genus which is difficult to dissolve in a liquid growth medium), Table S10: Identification, growth temperature ranges, and NaCl tolerance (evaluated as Minimum Inhibitory Concentration, MIC) of bacterial strains isolated from *Retama raetam*. N.D. indicates not determined (the species is a potential human opportunistic pathogen or belongs to *Streptomyces* genus which is difficult to dissolve in a liquid growth medium), Table S11:

Identification, growth temperature ranges, and NaCl tolerance (evaluated as Minimum Inhibitory Concentration, MIC) of bacterial strains isolated from *Suaeda fruticosa*, Table S12: Identification, growth temperature ranges, and NaCl tolerance (evaluated as Minimum Inhibitory Concentration, MIC) of bacterial strains isolated from *Salsola tetragona*, Table S13: Identification, growth temperature ranges, and NaCl tolerance (evaluated as Minimum Inhibitory Concentration, MIC) of bacterial strains isolated from *Stipa tenacissima*, Table S14: Identification, growth temperature ranges, and NaCl tolerance (evaluated as Minimum Inhibitory Concentration, MIC) of bacterial strains isolated from *Thymelaea microphylla*. N.D. indicates not determined (the species is a potential human opportunistic pathogen or belongs to *Streptomyces* genus which is difficult to dissolve in a liquid growth medium), Table S15: Frequency (%) of the bacterial species isolated from the rhizosphere of *Cleome arabica* (CA), *Pulicaria undulata* (PU), *Reseda villosa* (RV), *Zilla spinosa* (ZS), *Artemisia herba halba* (AHA), *Arthrophytum scoparium* (AS), *Astragalus armatus* (AA), *Atriplex halimus* (AH), *Peganum harmala* (PH), *Retama raetam* (RR), *Suaeda fruticosa* (SF), *Salsola tetragona* (SAT), *Stipa tenacissima* (ST) and *Thymelaea microphylla* (TM). A= arid SA = Semi-arid. Table S16: Plant-beneficial physiological traits of the 95 selected bacterial strains: siderophore synthesis on CAS medium, phosphate solubilization activity on di- and tri-calcium phosphate (DCP and TCP medium, respectively), and indole-acetic acid (IAA) production, Table S17: Percentage of seed germination and length roots of cucumber, tomato, and sorghum inoculated or not with the 95 selected bacterial strains. Different letters indicate significant differences between the control (C) and the treatments at $p \leq 0.05$. * ND: not determined (germinated but undeveloped seed). Table S18. Root architecture of inoculated or not cucumber seeds at 7 days of growth described by the determination of total root length, total surface area, total root volume, number of tips, and root branching performed through WinRhizo analysis. Different letters indicate significant differences between the control (C) and the treatments at $p \leq 0.05$.

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