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Authors: Abdul Aziz Eida (Conceptualization) (Formal analysis) (Investigation) (Methodology) (Validation) (Visualization) (Writing - original draft) (Writing - review and editing), Hanin S. Alzubaidy (Formal analysis) (Investigation), Axel de Zélicourt (Conceptualization) (Formal analysis) (Methodology) (Writing - review and editing), Lukáš Synek (Formal analysis) (Investigation) (Visualization) (Writing - review and editing), Wiam Alsharif (Formal analysis) (Investigation), Feras F. Lafi (Investigation) (Methodology), Heribert Hirt (Conceptualization) (Funding acquisition) (Project administration) (Supervision) (Writing - original draft) (Writing - review and editing), Maged M. Saad (Conceptualization) (Formal analysis) (Investigation) (Project administration) (Supervision) (Writing - original draft) (Writing - review and editing)

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Phylogenetically diverse endophytic bacteria from desert plants induce transcriptional changes of tissue-specific ion transporters and salinity stress in *Arabidopsis thaliana*

Abdul Aziz Eida[1], Hanin S. Alzubaidy[1], Axel de Zélicourt[1,#a], Lukáš Synek[1], Wiam Alsharif[1], Feras F. Lafi[1,#b], Heribert Hirt[1*], and Maged M. Saad[1]

[1] King Abdullah University of Science and Technology (KAUST), Desert Agriculture Initiative, Biological and Environmental Sciences and Engineering Division (BESE), Thuwal 6900-23955, Kingdom of Saudi Arabia.
[#a] Present address: Institute of Plant Sciences Paris-Saclay (IPS2), UMR 9213/UMR1403, CNRS, INRA, Université Paris-Sud, Université d'Evry, Université Paris-Diderot, Sorbonne Paris-Cité, Bâtiment 630, 91405 Orsay, France.
[#b] Present address: College of Natural and Health Sciences, Zayed University, Abu Dhabi campus, United Arab Emirates.

*** Corresponding author:**
heribert.hirt@kaust.edu.sa (HH)

Highlights

- Taxonomically diverse bacteria from Jizan desert plants promoted salinity stress tolerance in *Arabidopsis thaliana*.
- Bacterial isolates enhanced the shoot and root biomass and induced variable changes in the root system architecture.
- Plants exhibited common effects of reduced Na^+/K^+ shoot ratios and tissue-specific expression of ion transporters.
- Lower Na^+ and higher K^+ contents in shoots may be a result of increased root expression of *HKT1* and *SKOR*.
- Bacterial isolates displayed different plant growth promoting traits, tolerance to abiotic stresses and colonization abilities.

Abstract

Salinity severely hampers crop productivity worldwide and plant growth promoting bacteria could serve as a sustainable solution to improve plant growth under salt stress. However, the molecular mechanisms underlying salt stress tolerance promotion by beneficial bacteria remain unclear. In this work, six bacterial isolates from four different desert plant species were screened for their biochemical plant growth promoting traits and salinity stress tolerance

promotion of the unknown host plant *Arabidopsis thaliana*. Five of the isolates induced variable root phenotypes but could all increase plant shoot and root weight under salinity stress. Inoculation of Arabidopsis with five isolates under salinity stress resulted in tissue-specific transcriptional changes of ion transporters and reduced Na^+/K^+ shoot ratios. The work provides first insights into the possible mechanisms and the commonality by which phylogenetically diverse bacteria from different desert plants induce salinity stress tolerance in Arabidopsis. The bacterial isolates provide new tools for studying abiotic stress tolerance mechanisms in plants and a promising agricultural solution for increasing crop yields in semi-arid regions.

Key words: plant growth promoting bacteria; volatile compound; desert bacteria; sodium; potassium; ion transporters.

1. Introduction

According to the United Nations Organization, the current world population of 7.6 billion is expected to increase beyond 9.8 billion by the year 2050 [1]. The ever-increasing human population, reduction in arable land and emerging effects of climate change-associated abiotic stresses pose serious threats for agricultural sustainability and global food security. Abiotic stresses, such as drought, extreme temperatures, UV radiation, nutrient deficiency or inaccessibility and soil salinity, account for more than 50% of crop yield losses [2, 3]. Affecting approx. 20% of irrigated land, soil salinity is one of the major abiotic stresses that adversely affects plant growth and crop yields, especially in arid and semi-arid regions [4].

Saline soils with high concentrations of soluble salts can have a severe impact on plant growth and development through osmotic (higher external osmotic pressure) and ionic (accumulation of sodium ions/Na^+) effects [5]. Osmotic effects occur as a result of excessive amounts of Na^+ and chloride ions/Cl^- outside the roots, which decrease the water potential of the soil solution, thereby hampering the plant's ability to take up water and nutrients. Ionic effects occur due to the accumulation of Na^+ in cells that compete with potassium ions/K^+, which are important cofactors for enzymes and binding tRNA to ribosomes for protein synthesis [6]. The accumulation of Na^+ can have severe effects on plants, leading to an imbalance in cellular homeostasis, oxidative stress, nutrient deficiency, inhibition of photosynthesis, protein synthesis and enzymatic activity and eventually early leaf senescence [5, 6]. Understanding the toxic effects of salinity on plants and the mechanisms by which plants can achieve tolerance to these stresses can assist in developing salt-tolerant crops.

The generation of salt-tolerant crops using traditional crop breeding techniques and genetic engineering are expensive, challenging and time-consuming. Recently, microbes, such as bacteria or fungi, were suggested as a simple and cost-efficient alternative for enhancing salinity stress tolerance of crops [7, 8]. Indeed, plant growth promoting bacteria (PGPB) may serve as bio-fertilizers for promoting the growth of many plant species. Several direct and indirect mechanisms of their mode of action are well documented [9-12]. Bacteria possess the ability to produce, or modulate the levels of different plant hormones such as auxin (e.g. indole or indole-3-acetic acid/IAA), ethylene (e.g. lowering the level of its precursor 1-aminocyclopropane-1-carboxylate/ACC by the enzyme ACC deaminase), cytokinins, gibberellins and abscisic acid [13-17]. Many PGPB also have the ability to secrete acids, exopolysaccharides, enzymes and nutrient chelators for (1) increasing the bioavailability or acquisition of mineral nutrients (e.g. phosphate solubilization and siderophore production), (2) supporting the colonization of plant roots or (3) inducing abiotic stress tolerance [18-22]. It has also been demonstrated that some bacteria are able to induce abiotic stress tolerance via the emission of volatile organic compounds (VOCs) [23, 24]. However, the molecular mechanisms by which these bacteria induce salinity stress tolerance remains largely unknown.

The work presented in this study is part of a larger project, DARWIN21 (http://www.darwin21.org/), which aims to improve sustainable agriculture on arid and semi-arid lands by using beneficial bacteria isolated from plants living in one of the harshest terrestrial ecosystems on Earth, the desert. We previously isolated an endophytic bacterium,

Enterobacter sp. SA187, from the endosphere of the desert plant *Indigofera argentea* from Jizan, Saudi Arabia, and demonstrated its plant growth promoting properties and induction of salinity stress tolerance in *Arabidopsis thaliana* and *Medicago sativa* [25, 26]. In order to gain new insights into the molecular mechanisms underlying salinity stress tolerance promotion by bacteria, we isolated and identified taxonomically diverse bacteria from four deserts plants native to the Jizan region of Saudi Arabia. The isolates exhibiting enhancement of the salinity stress tolerance in the model plant *Arabidopsis thaliana* were further characterized for different plant growth promoting properties. Our results revealed that despite their highly diverse phylogenetic affiliation and plant growth promoting properties, the isolated PGPB promoted common ionic and transcriptional responses in Arabidopsis under salinity stress.

2. Materials and methods

2.1. Study site description and sample collection

Plant materials were collected from the hot, arid desert region of Jizan, Saudi Arabia, located in the in the southern Red Sea coast (16.8776N 42.6162E; 16.9412N, 42.6115E). The criteria of plant species collected was based on the plants being native/indigenous species to the region and adapted to its climate, perennials that persist for many growing seasons and woody shrubs and sub-shrubs with multiple stems arising at or near the base for easy access to the whole root system. The root systems of the annual halophytes *Tribulus terrestris* and *Zygophyllum simplex* (both Zygophyllaceae), a C_4 salt-tolerant perennial tussock-grass *Panicum turgidum* (Poaceae) and a prostrate, annual *Euphorbia granulata* (Euphorbiaceae) were collected in Zip-plastic bags and kept at ambient temperature.

2.2. Bacterial isolation

Plant roots were washed with ddH_2O and vortexed for 3 min to dislodge attached soil particles, then washed for 10 sec in 70% ethanol then 20 sec in 2% sodium hypochlorite to remove attached microbes from root surface and, subsequently, washed twice with sterilized ddH_2O, cut into small sections (0.5 mm in length) and macerated with 0.8% saline solution [27]. The liquid homogenate was diluted in 0.8% saline solution and 10^{-4} and 10^{-5} dilutions were used as inoculum for bacterial isolation. Four main culture media were used for the purification of bacterial isolates, namely Luria-Bertani (LB) agar (Sigma Aldrich, Germany), BD™ Difco™ R2A (R2A) agar (BD Diagnostics, Sparks, MD, USA) with and without 1.5 or 3.0% of added sodium chloride (NaCl) and Tryptone Soya Agar (TSA) agar (g/L: Bacto tryptone-15; Bacto soytone-5; NaCl-5; agar-15). A total volume of 100 µL of diluted root extract was spread on agar plates with different media and incubated at 28°C for 3-4 days. Isolated colonies were purified by re-streaking until pure cultures were achieved. Purified isolates were stored in 20% glycerol at -80°C.

2.3. Identification and taxonomic assignment of culturable bacteria

The amplification of the 16S rRNA gene fragment was carried out using Taq DNA polymerase PCR Master Mix (Promega, Madison, WI, USA) with universal primer sets 27F and 1492R (27F: 5'-AGAGTTTGATCCTGGCTCAG-3' and 1492R: 5'-TACGGYTACCTTGTTACGACTT-3') [28]. The PCR amplification of 16S rRNA genes was carried out using the following PCR conditions: an initial denaturation at 95°C for 1 min, followed by 30 cycles with steps of 95°C for 30 sec, 53°C for 45 sec and 72°C for 90 sec and a final extension step of 5 min at 72°C. Amplification

was confirmed by analyzing PCR products on a 1% agarose gel. PCR products were purified from incorporated primers and extra dNTPs using ExoSAP-IT (Affymetrix, Santa Clara, CA, USA) and sequenced using ABI 3730xl DNA Analyzer (Applied Biosystems, Foster City, CA, USA). The 16S rRNA gene sequences of the bacterial isolates were compared to known sequences listed in NCBI's GenBank using BLAST [29]. Proposed taxonomic assignments of bacteria were based on BLAST annotation using sequence identity and query cover as main criteria. The 16S rRNA gene sequences of the bacterial isolates in this study have been deposited in the GenBank database and are accessible under accession numbers KY194216, KY194225, KY194228, KY194238, KY194243 and KY194246. Multiple alignment of the nucleotide sequences was performed using MUSCLE [30]. The phylogenetic tree was constructed by the Neighbor-Joining method [31], based on the Kimura 2-parameter model [32], with bootstrap analysis (1,000 replications) using the software MEGA (version 7, https://www.megasoftware.net/) [33].

2.4. Plant assays

Arabidopsis thaliana Col-0 (wild-type) seeds were surface sterilized by shaking for 10 min in 70% ethanol + 0.05% sodium dodecyl sulfate (SDS), then washed twice with 99% ethanol and once with sterilized ddH_2O. The seeds were then sown on square Petri dishes (12x12 cm) containing half-strength Murashige and Skoog Basal Salt Mixture pH 5.8, 0.9% agar (½MS) [34] (M5524, Sigma Aldrich, Germany). The plates were stored in the dark for 2 days at 4°C for seed stratification and then incubated vertically (~75° angle) in growth chambers (Percival Scientific Inc., USA) at 22°C with a photoperiod of 16/8 h (light/dark) for germination. Five-day old seedlings (~1.0-1.5 cm in root length) were then gently transferred to fresh ½MS agar plates supplemented with 100 mM NaCl as a salt stress (5 seedlings/plate). Bacterial isolates were spread on LB agar plates as "lawn" and incubated at 28°C for 24 hours prior to transfer of seedlings. From these plates, square-shaped (3 mm^2, approximately $5x10^4$ CFU/plug) plugs were cut out and laid beside the root of each seedling, bacteria-free LB agar plugs were used as a control. For assessment of the effect of inoculation with cultured bacteria, phenotypic and molecular characterization was performed on plants 16 days after inoculation (DAI) and compared to control (bacteria free LB).

The number of lateral roots (NLR) was counted under a light microscope and primary root lengths (PRL) were measured using image analysis software ImageJ (https://imagej.nih.gov/ij/). Lateral root density (LRD) was calculated by dividing the number of lateral roots by the primary root length. Fresh weight (FW) measurements were taken 16 DAI. For dry weight (DW) measurements, plant material was dried for 2 days at 65°C.

2.5. Quantification of ion content

Dry plant material was digested in 2 mL of 1% nitric acid (Sigma Aldrich, Germany) for 2 days at 65°C and allowed to cool. The concentrations of Na^+ and K^+ ions of samples were measured relative to standard solutions using a model 425 flame photometer (Sherwood Scientific Ltd., UK).

2.6. RNA extraction, cDNA synthesis, and quantitative RT-PCR analysis

For RNA extraction, 3-4 leaves or 3-4 root systems from 4-6 different plants per biological replicate were collected at 16 DAI in liquid nitrogen. Samples were ground for 30 sec using Tissue Lyser II (Qiagen) and glass beads until samples were completely ground. Total RNA was obtained using the NucleoSpin® RNA Plant Kit (Macherey-Nagel) following the manufacturer's instructions. RNA concentration and purity was determined by Qubit™ RNA BR assay (Thermo Fisher Scientific) and NanoDrop 2000 spectrophotometer. For cDNA synthesis, 800 ng of total RNA were reverse transcribed with oligo dT primers using SuperScript® III First-Strand Synthesis SuperMix (Invitrogen), according to the manufacturer's protocol. Quantitative Real-time PCR was performed using SsoAdvanced™ Universal SYBR® Green Supermix Kit (Bio-Rad) and the CFX384 Real-Time System (Bio-Rad). The PCR mixture (10 µL) contained 300 nM of each primer and 2 µL of template cDNA (diluted 1:8). Amplification was performed under the following conditions: 50°C for 2 min, 95°C, 8 min; followed by 40 cycles of 98°C, 10 sec; 60°C, 45 sec, followed by a melting curve from 65°C-95°C. Primer efficiencies, expression stability of the housekeeping (HK) genes and relative gene expression calculations of gene of interest were performed using the Bio-Rad CFX Manager software (version 3.1). Considering the housekeeping genes, transcripts of *Actin* (ACTIN) were slightly more stable than transcripts of *Ubiquitin* (UBQ) in all treatments and, thus, was used as the reference gene. The gene sequences of all primers can be found in the supporting information (Table S1).

2.7. Phosphate solubilization and siderophore production

Calcium phosphate solubilization ability of bacteria was determined based on formation of clear halo on Pikovskaya's (PVK) (g/L: yeast extract-0.5; dextrose-10; calcium phosphate-5; ammonium sulphate-0.5; potassium chloride-0.2; magnesium sulphate-0.1; manganese sulphate-0.0001; ferrous sulphate-0.0001; agar-15) agar plates (M520, Himedia, France) [35]. Siderophore production was determined by formation of orange halo as described by [36]. The casamino acids were extracted with 3% (w/v) 8-quinolinol hemisulphate salt in chloroform by mixing, allowing the phases to separate overnight at 4°C and then recovering the upper phase until no colored 8-quinolinol-iron complex is formed. The extracted casamino acids were then used in the media preparation of agar plates. The phosphate solubilization and siderophore production assays were performed in triplicates by spot inoculating 10 µL of 10^7 CFU/mL of bacterial culture on the plates and incubated at 28°C for 3-5 days until appearance of orange zones around colonies was observed.

2.8. Tolerance to salt, drought and heat stress

Tolerance to salt, drought and heat stress assays were performed by inoculating bacteria into 48-well plates containing LB broth (Lennox L Broth Base, Invitrogen) or TSA at OD_{600} of 0.01 for 2 days at 28°C (or 42°C for heat stress) and shaking at 210 rpm. For drought and salinity stress, media was supplemented with 10% or 20% Polyethylene-glycol (PEG) 8,000 (Fisher Scientific, Belgium) and 0.5 or 1.0 M NaCl, respectively.

2.9. Biofilm formation, motility assay and exoprotease production

Biofilm formation was qualitatively determined based on crystal violet staining [37]. Motility assay was performed by spot inoculation of 10 µL of 10^7 CFU/mL culture on LB or TSA plates containing 0.3% (swimming) and 0.6%

(swarming) agar, and the plates were incubated at 28°C for 3 days. Exoprotease production, demonstrated by casein degradation, was assessed by spot inoculating 10 µL of 10^7 CFU/mL of bacterial culture on milk agar (% w/v: skim milk powder-2; YPD broth-2; NaCl-0.5, agar-1.5) and monitoring clearing zone after incubation at 28°C for 3-5 days [38].

2.10. Bacterial colonization of *A. thaliana* roots

For microscopic observation of root colonization, 5 day-old seedlings of *A. thaliana* (Col-0) grown on vertical ½MS agar were transferred to ½MS plates with or without 100 mM NaCl. The root tips were inoculated with a 100 µL of LB-grown bacterial suspension (OD_{600} = 0.1), and examined for colonization after 3 days. Seedlings were gently washed in sterile water before mounting on microscopic slides in drop water as mounting medium. Axio Imager.Z2 (Zeiss) equipped with an EC Plan-Neofluar 40X/0.75 objective, differential interference contrast (DIC) optics and AxioCam 512c (Zeiss) was used to capture images at exposure times around ~30 msec. The upper face of each root (i.e. without any direct contact with agar) was imaged at the beginning of the root-hair zone. Epidermal cells in 1-2 files off the central file provided the best contract to visualize bacterial cells on the root surface.

2.11. ACC deaminase, indole-3-acetic acid and indole production, and emission of hydrogen sulfide and indole volatiles

ACC deaminase activity was semi-quantitatively assessed in 96-well plates as described by Penrose and Glick [39]. Strain *Sinorhizobium meliloti* was used as a positive control for ACC deaminase production. The amount of α-ketobutyrate (indicative of ACC deaminase activity) produced was determined based on a standard curve of pure α-ketobutyrate (Sigma Aldrich, Germany) and spectrophotometric measurement at 540 nm. Indole-3-acetic acid (IAA) production was qualitatively determined according to Bric, Bostock and Silverstone [40], however, either liquid LB or TSA media modified with 5 mM L-Tryptophan (Sigma Aldrich, Germany) were used instead of agar plates. The media was inoculated with an OD_{600} of 0.01 of culture and incubated for 2 days at 28°C and shaking at 210 rpm in 48-well plates. Cells were centrifuged and the supernatant was used for determination of IAA production. Salkowski reagent (2% of 0.5 M $FeCl_3$ in 35% $HClO_4$ solution) was added to supernatant in a 2:1 ratio, and formation of red-pink color indicated positive production. For production of indole, the same experimental conditions were performed as for IAA. However, Kovac's reagent (Sigma Aldrich, Germany) was used by adding to supernatant in a 1:1 ratio, and formation of purple-pink color indicated positive production. Production of volatiles compounds was determined by using test strips impregnated with lead(II) acetate (for hydrogen sulfide, H_2S) and Kovac's reagent (for indole) (Sigma Aldrich, Germany). Color change of strips from white to black (H_2S) or yellow to pink (indole) indicated positive production.

2.12. Statistical analysis

The data were subjected to non-parametric one-way ANOVA, or Kruskal-Wallis H test [41]. Data were expressed as the mean ± standard error of the mean (SEM). The differences among the various treatment means were compared and the values with a P value of ≤ 0.05 were considered statistically significant. Statistical analysis was done using

DEVELVE statistical software (https://www.develve.net/). Statistical analysis of qPCR data was performed by the Bio-Rad CFX Manager software (version 3.1).

3. Results

3.1. Endophytic bacteria isolated from the root endosphere of Jizan desert plants are taxonomically diverse

Following the isolation from four different plants, *T. terrestris*, *Z. simplex*, *P. turgidum* and *E. granulata*, on different media (LB, TSA and R2A), a bacterial collection of 116 isolates was established. The choice of media was in order to select for highly versatile bacteria that can be easily cultivated should they ever be used in mass production for agricultural purposes. Our preliminary screening using the plug-based plant assay found many of them having the salinity stress tolerance promoting (SSTP) abilities. By visual comparison of the shoot size of Arabidopsis plants inoculated by the tested strains with mock plants, five bacterial isolates (JZ2, JZ12, JZ29, JZ34 and JZ37) exhibited reproducible and significant enhancement under salinity stress conditions. Thus, these isolates were used for further characterization along with JZ18, which did not show any SSTP effect, as a control. The six strains were different in terms of their isolation source (host plant species) and taxonomic classification (Figure 1).

3.2. SSTP bacteria promote growth of *A. thaliana* plants under salinity stress

Quantitative measurements of plant growth parameters such as shoot fresh weight (FW) and dry weight (DW) were recorded (Figure 2; Table S2). Growth of mock plants under salinity stress (MS agar with 100 mM NaCl, hereafter referred to as MS100) was severely retarded compared to normal conditions (MS agar with no NaCl, hereafter referred to as MS0) as observed by the significant reduction of shoot and root system (Figure 2, a). However, the inoculation of bacterial isolates JZ2, JZ12, JZ29, JZ34 and JZ37 alleviated the toxic effects of salinity, with promotion of both shoot and root growth. Isolates JZ2 and JZ29 exhibited the highest increase in shoot DW by 187% and 235%, respectively, followed by JZ34 (174%), JZ37 (149%) and JZ12 (127%); measurements of fresh weight provided similar values (Figure 2, b). Plants inoculated with isolate JZ18 did not exhibit any growth promotion and had a negative effect on plant growth. The FW and DW of mock plants grown on MS100 were 88% and 84%, respectively, lower compared to mock plants on MS0 (Figure 2, b).

3.3. SSTP bacteria variably alter the root architecture of *A. thaliana* under salinity stress

Changes were also observed in the root system of plants inoculated with the SSTP bacterial isolates (Figure 2, a). The root system was characterized for biomass (FW and DW) and architecture (Figure 3; Table S3). Mock plants on MS100 exhibited 93% and 85% lower root FW and DW, respectively, compared to plants on MS0 (Figure 3, a). Inoculation of SSTP bacteria led to a significant increase in FW and DW of roots with isolate JZ29 exhibiting the highest increase in root DW (166%) and isolate JZ12 exhibiting the lowest increase (94%).

In terms of root architecture, several phenotypic parameters influence/modulate the weight of the root system; primary root length (PRL), number of lateral roots (NLR), lateral root length and, to a lesser extent, thickness of the primary and lateral roots. In this study, NLR (Table S2) and PRL (Figure 3, b) were determined at 12 DAI, from which the

overall lateral root density (LRD; Figure 3, b) was calculated. Three of the five SSTP bacteria (JZ2, JZ12 and JZ29) displayed a common root phenotype, with a significant increase in NLR (37%, 48% and 62%, respectively) and PRL (31%, 39% and 39%, respectively) compared with mock plants on MS100. However, isolate JZ29 resulted in an overall higher LRD (17%) due to the higher increase in NLR. In contrast, isolate JZ34 did not affect NLR but significantly increased PRL (24%). On the other hand, isolate JZ37 resulted in higher NLR (27%) and no effect on PRL. In addition, isolate JZ34 was the only strain causing a significantly lower LRD (29%) than the mock, while JZ37 had a significantly higher LRD (33%). The control isolate JZ18 did not alter the LRD but did significantly lower the NLR (19%) and PRL (18%).

3.4. SSTP bacteria induce similar changes in the distribution of Na^+ and K^+ in *A. thaliana* shoots and roots

The content of Na^+ and K^+ was measured in both dry shoots and roots of inoculated and mock Arabidopsis plants grown on MS100 and MS0. In shoots, the measurements revealed a common effect of decreased Na^+ and increased K^+ content (i.e. lower Na^+/K^+ ratio) as a result of SSTP bacterial inoculation (Figure 4; Table S2). Mock-treated control plants grown on MS100 accumulated 22-fold higher Na^+ content (2.82 mmol/g DW) compared to mock-treated control plants grown on MS0 (0.127 mmol/g DW), but accumulated less K^+ content (MS0, 1.29 ; MS100, 0.343 mmol/g DW) (Figure 4, a). Inoculation of SSTP bacteria resulted in decreased Na^+ content ranging between 1.18 mmol/g DW (JZ2, 58% decrease) and 1.58 mmol/g DW (JZ37, 44% decrease) and in increased K^+ content ranging between 0.82 mmol/g DW (JZ34, 140% increase) and 1.02 mmol/g DW (JZ29, 198% increase). Roots of control plants under salinity stress (MS100) accumulated much more Na^+ (more than 8-fold) compared to under non-stress conditions (MS0), but accumulated less K^+. The inoculation of SSTP bacteria resulted in an overall higher accumulation of Na^+. Roots of MS100 plants contained 0.84 mmol/g DW Na^+ while the roots of inoculated plants contained between 0.96 mmol/g DW (JZ29, 15% increase) and 1.05 mmol/g DW (JZ34, 25% increase). The K^+ content in roots of inoculated plants was not significantly different from that of mock plants under salinity stress.

In terms of Na^+/K^+ ratios in shoots and roots, all SSTP bacteria induced similar effects – they decreased the Na^+/K^+ ratio in the shoots and increased the Na^+/K^+ ratio in the roots (Figure 4, c). Inoculation of isolates JZ2 and JZ29 resulted in the lowest Na^+/K^+ shoot ratio of 1.22 (86% decrease) compared with MS100 plants (ratio of 8.75), while isolate JZ12 led to the least reduction of 79 % in Na^+/K^+ shoot ratio of 1.88 among the SSTP bacteria . In contrast, the negative control (JZ18) induced an increase in the Na^+/K^+ ratios in both shoots and roots.

3.5. SSTP bacteria reveal similar transcriptional changes of genes responsible for Na^+ and K^+ transport in *A. thaliana* shoots and roots

In order to understand the molecular mechanisms contributing to the differences in Na^+ and K^+ levels and salinity stress tolerance, transcript levels of genes involved in sodium and potassium transport under salinity stress and normal conditions were examined. The SSTP bacteria exhibited common transcriptional responses in a number of genes (Figure 5). In shoots, the transcript levels of *AKT1*, *GORK*, *KUP6*, *SOS1* and *SOS3* were significantly higher in mock-

treated control plants on MS100 than on MS0 (Figure 5, a). However, the inoculation of SSTP bacteria led to significantly lower transcript levels of these genes (except JZ12 for *SOS1*, -1.37-fold change (FC)). Furthermore, while the shoot expression of *HKT1* in mock-treated plants was slightly lower under salinity conditions (FC = -1.33) compared to non-stress conditions, upon inoculation of SSTP bacteria, the expression of *HKT1* was significantly increased (except for JZ12, FC = 1.44).

In roots, salinity stress resulted in significantly lower transcript levels of *HAK5*, *SKOR* and *HKT1*, while it led to higher levels of *SOS3* (Figure 5, b). SSTP bacteria-inoculated plants exhibited an opposite expression pattern; higher levels of *HAK5*, *SKOR* and *HKT1* and lower levels of *SOS3*. For most genes, the expression of plants inoculated by the negative control JZ18 exhibited similar expression patterns to mock plants on MS100, one exception being *HAK5* in the roots, where SSTP bacteria-inoculated plants exhibited patterns similar to mock plants on MS0. For a few genes, the expression was regulated differently between the isolates (e.g. shoot and root *AKT2/3;* shoot *HAK5* and *NHX2*) (Figure S1, a). In other cases, genes were not differentially expressed by salinity (e.g. shoot and root *NHX1;* root *KUP6*) or SSTP bacterial inoculation (e.g. root *NHX2*, *AKT1* and *GORK*) (Figure S1, b).

3.6. Bacterial endophytes possess variable plant growth promoting traits, tolerance to abiotic stresses and colonization abilities

The six isolates were characterized for their biochemical abilities and their potential as plant growth promoters (Table 1). In terms of nutrient acquisition, isolates JZ2 and JZ29 were the only positive isolates able to solubilize phosphate and produce siderophores. The ability of all isolates to grow under abiotic stress conditions (salinity up to 1 M NaCl, osmotic stress generated by 20% PEG 8000 and heat stress at 42°C) was demonstrated for all bacterial isolates except for isolate JZ12 which did not grow in 1 M NaCl or at 42°C.

The ability to colonize the *A. thaliana* root surface under salinity stress was examined by microscopically observation of the roots 3 DAI (Figure S2). As a result, isolates JZ2, JZ18, JZ29, JZ34 and JZ37 but not JZ12 colonized the root surface. The swimming/swarming assay for bacterial motility showed that the most motile isolates were JZ34 and JZ37 as they were able to swarm the 0.6% agar plates with colonies. Isolates JZ2 and JZ29 displayed only swimming ability (0.3% agar), while isolates JZ12 and JZ18 did not display any motile ability. The ability to produce biofilms was exhibited by all isolates, with JZ34 and JZ37 being the highest biofilm-producing isolates. Finally, the exoprotease production assay revealed that isolates JZ12 and JZ18 exhibit high production, others moderate production and JZ37 no production.

The SSTP bacterial isolates were also tested for the production of some plant hormones (e.g. IAA) or enzymes that modulate their levels (e.g. ACC deaminase), signaling molecules (e.g. indole) and volatile compounds (e.g. H_2S). The only isolate that could significantly produce ACC deaminase was JZ34 with 47% higher amounts (219.57 µM) of α-ketobutyrate produced compared to the positive control *S. meliloti* (149.36 µM). The production of IAA was exhibited by every isolate except for JZ12 and JZ18, and the production of indole in liquid and as a volatile was only evident

by isolates JZ2 and JZ29. The production of sulfur-based volatile, H_2S, was detected by all SSTP bacterial isolates but JZ18 (not promoting plant growth).

4. Discussion

It is understood that plants can interact with soil microorganisms, altering the microbiome of the rhizosphere and selecting beneficial bacteria that promote plant growth or health [42, 43]. The 16S bacterial community analysis of the soil, rhizosphere and endosphere of the four desert plant species presented in this study revealed similar finding showing an overlap between the endosphere, rhizosphere and soil compartments and the endosphere containing less diversity and species richness (Eida et al. 2018, *in press*, https://doi.org/10.1371/journal.pone.0208223). Subsequently, a plant screening assay was developed to test the effects of the bacterial endophytes (from the four desert plants) on the growth of the model plant *A. thaliana* under salinity stress conditions. The inoculation strategy of the SSTP assay we used offers a quick method of screening for both direct (e.g. requiring physical interaction) and indirect (e.g. mediated by emission of volatile compounds) effects by which bacterial isolates induce salinity stress tolerance in *A. thaliana*. Indeed we found highly diverse endophytic bacteria that were able to promote growth of the *A. thaliana* plants under salinity stress conditions.

It is well established that spatial rearrangement of the root system is an important mechanism by which plants optimize their water and nutrient uptake and, thereby, increase their abiotic stress tolerance or adapt to nutrient deficiency [44, 45]. Salinity stress has inhibitory effects on the growth of the primary and lateral roots [46, 47]. Under high salt concentrations, the growth of the primary root and the development of lateral roots are inhibited as a consequence of suppressing cell division and elongation [48, 49]. A similar response was also observed in our assays, where salinity-stressed plants were significantly inhibited in NLR and PRL. Our SSTP bacterial isolates, however, had significant variable effects on the root system architecture. A similar pattern of increased shoot and root biomass and higher LRD was observed upon inoculation and under salinity stress as in the case of the endophytic bacterium *Enterobacter* sp. SA187 also isolated from Jizan desert plants in another study [25]. Nevertheless, presented results suggest that neither primary root length nor lateral root number alone is a determinant of salinity stress promotion by bacteria, instead the overall increase in the root system biomass is correlated with growth promotion.

Plants differ in their responses and tolerance to salinity, possessing different mechanisms to cope with the stress of toxic ions. For example, plants can counter the harmful effects of Na^+ by retaining K^+ and actively excluding Na^+ from the roots and/or the shoots and/or detoxification of Na^+ by sequestering and compartmentalizing it into specialized tissues and cell organelles (e.g. vacuoles) [5, 50-52].

The high-affinity plasma membrane Na^+/K^+ symporter HKT1, which we found important in our study, is involved in retrieval of Na^+ from the root xylem by unloading it from xylem vessels into the xylem parenchyma [53, 54]. Root stele-specific overexpression of *HKT1* was shown to reduce Na^+ accumulation in the leaves by increased retrieval from xylem vessels, leading to higher salinity tolerance [55, 56]. In shoots, HKT1 is thought to play a role in unloading

Na^+ from the phloem sap, suggesting recirculation of Na^+ from the shoot to the root [54, 57, 58]. In our study, all SSTP isolates (except for JZ12 in shoots) significantly upregulated *HKT1* in both shoots and roots. These results suggest that the transcriptional regulation of *HKT1* by SSTP may have given rise to the decreased Na^+ content in shoots and their accumulation in roots, possibly by both unloading Na^+ from xylem vessels and recirculation from shoots back to the roots. *HKT1* is known to be transcriptionally upregulated in *A. thaliana* under salinity stress by volatiles emitted from *Bacillus subtilis* GB03 [59], *Bacillus amyloliquefaciens* strains FZB42 and SQR9 [60] and by the endophytic fungus *Piriformospora indica* [61]. Therefore, the upregulation of *HKT1* by our SSTP bacteria further confirms a possible common mechanism for salinity stress tolerance.

Plants can also shield the toxic effects of salinity by the compartmentalization of Na^+ into vacuoles by tonoplast Na^+/H^+ antiporters (e.g. NHXs). It is suggested that efficient sequestration or pumping of Na^+ into the vacuoles increases Na^+ tolerance by detoxifying the cytoplasm [62-64]. Except for JZ12 in the shoots, expression of *NHX1* and *NHX2* did not change upon inoculation by SSTP bacterial isolates under salinity stress. However, the expression of *NHX2* was significantly higher in plants on MS100 compared to MS0. This suggests that higher Na^+ levels in mock plants compared with bacteria-inoculated plants under salinity stress may result in increased expression of *NHX2*.

The accumulation of Na^+ in the roots but an overall lower total plant content observed by SSTP inoculation indicated that Na^+ ions must have been either taken up from the soil into the roots but extruded back into the soil at a later stage or their overall uptake into the roots from the soil was decreased, in addition to being prevented from accumulating in the shoots. The Ser/Thr CBL-interacting protein kinase SOS2/CIPK24 interacts with a calcium-binding protein, calcineurin B-like protein SOS3/CBL4 forming the SOS3/SOS2 complex [65]. This complex activates a putative Na^+/H^+ antiporter, SOS1, which plays a role in removal of Na^+ from the cytosol into the surrounding medium in epidermal cells (root-soil boundary) and from surrounding parenchyma cells into the xylem vessels where it can be transported to the shoots [66]. However, the exact function of SOS1 likely depends on the salinity stress treatment, where low to moderate salinity induces xylem loading while high salinity induces removal from xylem [66]. The expression of *SOS1* and *SOS3* in the roots of plants grown on MS100 in this study was significantly higher than plants on MS0. Inoculation of SSTP bacterial isolates under salinity stress did not affect the *SOS1* expression in the roots, but did significantly decrease *SOS3* expression. One possible explanation is that plants are not sensing severe salinity stress and, thus, downregulation of *SOS3* would prevent loading of Na^+ into xylem vessels by decreasing SOS1 activity.

Quantification of K^+ revealed another common effect of SSTP inoculation whereby the total amount of K^+ in plants was significantly higher (due to significant accumulation in shoots) when inoculated with SSTP bacterial isolates under salinity stress. The high-affinity K^+ transporter, HAK5, has been shown to mediate K^+ uptake in the roots upon K^+ starvation (i.e. external $[K^+] < 10$ µM), allowing plants to survive and thrive under such conditions [67-70]. Our bacterial isolates, including the non-SSTP isolate JZ18, upregulated *HAK5* in the roots under salinity stress conditions. Another transporter involved in K^+ uptake from the roots is the inward rectifying, plasma membrane transporter AKT1

[71, 72]. Although plants on MS100 plants displayed significant upregulation of *AKT1* in the roots compared to those on MS0, its regulation was unaltered when inoculated with SSTP bacterial isolates. There are approximately 35 genes encoding for K^+ transporters and channels in Arabidopsis [73], and other transporters may explain the increased uptake of K^+ into the shoot.

Several potassium transporters have been implicated in stomatal closure/aperture via ABA regulation. The Arabidopsis *akt1* mutants displayed reduced water loss (via reduced transpiration) under osmotic stress and improved stomatal closure in response to ABA [74]. The outward rectifying channel, GORK, is strongly expressed in guard cells and plays a crucial role in K^+ efflux and subsequent stomatal closure [75, 76]. The double knockout mutant *kup6 gork* exhibits enhancement of lateral root formation in response to salinity stress (100 mM NaCl), but has lower survival rates under water deficit stress [77]. These authors proposed that the K^+ uptake transporter, KUP6, is regulated by ABA and, thus, both KUP6 and GORK are responsible for K^+ efflux from guard cells and stomatal closure via ABA. The inoculation of plants with SSTP bacteria resulted in the downregulation of *AKT1*, *KUP6* and *GORK* in shoot under salinity stress. These results suggest that stomata may also play a role, possibly via ABA, in controlling SSTP-induced sodium and potassium distribution and salinity stress tolerance. However, this needs to be further investigated in the future. Furthermore, GORK has been suggested to be activated by salinity-induced membrane depolarization, resulting in an increased K^+ loss from the roots [78, 79]. However, inoculation of SSTP bacteria did not alter the gene expression of *GORK* in the roots.

Finally, we investigated the regulation of another outward rectifying channel, SKOR, which is exclusively localized and/or expressed in the pericycle and xylem parenchyma of roots, where it is responsible for loading K^+ into the xylem sap for long-distance transport to shoots [80]. The Arabidopsis *skor* mutants display a significant decrease in shoot K^+ levels and K^+/Na^+ ratios under salinity stress (80 mM NaCl) [81]. The decreased K^+ content in the shoots of plants growing under salinity stress and its increased content upon SSTP bacterial inoculation in our study may be explained by the downregulation of *SKOR* in the roots of plants grown on MS100 compared to MS0 and its upregulation upon bacterial inoculation, respectively.

Quantification of the Na^+ and K^+ content revealed that mock-treated plants grown on MS0 contained significantly lower Na^+ and higher K^+ total plant content compared to plants grown on MS100. It also revealed that SSTP bacteria shared a common effect of increasing and decreasing the total amount of K^+ and Na^+ in the plants, respectively, similar to MS0 mock plants. The shoots had an overall increased K^+ content and decreased Na^+ content while the roots contained higher Na^+ content. The volatiles emitted from *B. subtilis* GB03 resulted in similar total plant (shoot and root combined) Na^+ and K^+ contents in Arabidopsis under 100 mM NaCl, however, K^+ content was lower in the shoots and higher in the roots and the Na^+ content was higher in the roots [59]. The overall increase in total plant K^+ content was also observed by *Enterobacter* sp. SA187 under similar conditions, however, the Na^+ contents were not changed in both shoots and roots and the K^+ content was higher in the roots [25]. Furthermore, colonization by *P. indica* led to lower Na^+/K^+ shoot, but also root, ratios in Arabidopsis under salinity stress [61]. In terms of Na^+/K^+ ratios, our five

SSTP bacterial isolates resulted in a decreased ratio in the shoots but an increased ratio in the roots. The effect of decreased Na^+/K^+ ratio was also exhibited by strain SA187 inoculation but not strain GB03. However, it is important to point out that the quantification of these ions was performed at different time points for SA187 (12 DAI) than our isolates (16 DAI). Furthermore, inoculations were performed differently in our assay compared with others (e.g. in-plate inoculation for SA187 and contactless for GB03).

The gene expression pattern of the ion transporters in the shoots and roots revealed that plants inoculated with SSTP bacterial isolates under salinity stress resembled plants grown on MS0, suggesting that plants either do not perceive the salinity stress or they adapt faster to the stress. Perhaps an investigation of the expression of these transporters at earlier time points (several hours or days post inoculation) would reveal significant differences in the responses by the different bacterial isolates. Indeed, the expression of some of these transporters, induced by *Burkholderia phytofirmans* PsJN inoculation, was significantly different at early and late time points under salinity stress [82]. For example, *SOS1* was significantly upregulated in the shoots after 2 and 24 hours of exposure to 150 mM NaCl but not after 72 hours [82]. Yet again, the method of inoculation, age of plants when transferred (11 days after sowing), number of bacteria (10^8 CFU/mL) and salt concentration (150 mM) were different and, thus, could cause the differences in expression data.

Despite having relatively similar effects on Arabidopsis, the SSTP possessed different PGP traits and biochemical abilities. The only strains with the ability to solubilize phosphate and sequester iron via siderophore production were the *P. stewartii* isolates JZ2 and JZ29. Indeed, isolates identified as *P. stewartii* have been shown to produce siderophores [83]. Several bacterial isolates belonging to the *Pantoea* genus, such as *P. dispersa* and *P. agglomerans*, have also been shown to promote salinity stress tolerance in chickpea [84], sweet pepper [85] and tropical corn [86], and to possess PGP traits, such as phosphate solubilizing and siderophore producing abilities [84, 87]. In terms of abiotic stresses which occur in arid and semi-arid soils, most of the isolated bacterial strains were able to adapt to and grow under salinity, osmotic stress and high temperatures. The ability of the SSTP bacterial isolates to tolerate a range of different abiotic stresses could provide them with a fitness advantage to be used as bio-fertilizers in soils where extreme, unfavorable conditions are present.

The motile nature of bacteria and ability to produce biofilms are thought to not only contribute to successful colonization of the roots, but also to plant growth promotion [88-90]. Although different isolates exhibited different abilities, there was no clear correlation between their motile ability and their colonization of root surfaces, despite all being able to produce biofilms. For example, isolates JZ12 and JZ18 were not able to swarm or swim but isolate JZ18 was able to colonize the root surface and JZ12 was not. The SSTP effect of isolate JZ12, although not colonizing the root surface, may be explained by its ability to produce volatile compounds inducing the plant growth, but this needs to be investigated. Another possibility could be that the time point at which the root colonization was examined may have been too early for JZ12.

Many studies demonstrated the ability of bacteria (e.g. Pseudomonas or Bacillus strains) possessing ACC deaminase genes or activity to ameliorate the effects of salinity stress in crops such as canola [91], rice [92], tomato [93] and wheat [94, 95]. Among the SSTP bacterial isolates, the only one that could significantly produce ACC deaminase was JZ34. Exogenous application of indole-3-acetic acid (IAA) has been shown to promote growth and elongation of primary roots of wheat and cotton seedlings under salinity stress [96, 97]. The ability of some species of the genus Pseudomonas, Bacillus and Microbacterium to produce IAA has been linked to alleviate salinity stress of crop plants such as wheat, pepper and cotton [96-99]. The production of IAA was exhibited by all the bacterial isolates except JZ12 and JZ18. There was no correlation between the ability to produce IAA and changes in the roots system, but this does not exclude its role in promoting salinity stress tolerance or changes in PRL or LRD. Indole is produced by a large variety of bacteria to act as an intercellular signaling molecule (e.g. quorum sensing signal), and it can influence biological functions such as motility, biofilm formation, virulence and antibiotic resistance [100]. Indole also plays a role as an interspecies and inter-kingdom signaling molecule in animals, plants and bacteria [101]. Indole volatiles have also been shown to be potent plant growth modulators by interfering with the auxin-signaling machinery and positively affecting the shoot biomass of *A. thaliana* and its root system (e.g. primary root length and secondary roots) when present at specific concentrations [102]. This is believed to be partly due to indole acting as a precursor for IAA synthesis, whereby indole is converted into tryptophan by the tryptophan synthase-β subunit (TSB1 and TSB2) [103, 104]. In this study, the production of indole in liquid medium and as a volatile was only evident in isolates JZ2 and JZ29. However, the exact role and extent by which bacteria-produced indole might play a role in promoting salinity stress tolerance is unknown and further investigations are needed.

There is accumulating evidence of the involvement and effect of H_2S in inducing and regulating salinity stress tolerance in plants, e.g. in Arabidopsis [105, 106], alfalfa [107], barley [108], rice [109] or strawberry [110]. However, the H_2S production ability of bacteria to promote salinity stress tolerance has not been shown to our knowledge. Indeed, the production of H_2S by the SSTP bacterial isolates but not JZ18 could be a mechanism by which bacteria promote salinity stress tolerance.

In conclusion, five phylogenetically diverse bacteria isolated from the endosphere of three different desert plants induced salinity stress tolerance in *A. thaliana* through similar tissue-specific Na^+ and K^+ distribution patterns and transcriptional regulation of ion transporters. It is important to point out that the changes in transcript levels of the genes investigated may not only be due to regulation of gene expression but also due to mRNA stability by post-transcriptional modifications especially under abiotic stress conditions [111]. However, other mechanisms of salinity stress tolerance exist, such as detoxification via glyoxalase pathway [112], adjustment of the osmotic potential via accumulation of compatible solutes (e.g. glycine betaine or proline) [113] or ROS scavenging by enzymes (e.g. ascorbate peroxidases, catalase, superoxide dismutases or glutathione-s-transferase) [114-116]. Therefore, a complex array of responses occurring at different time points is highly probable in achieving salinity stress tolerance in plants. A comparative transcriptomic analysis (RNA-seq) between the different isolated SSTP bacteria at different time points is necessary in order to get a global picture of the transcriptional regulation mechanism. In addition, the use of mutants

impaired in the expression of ion transporters are required to confirm their involvement in salinity stress tolerance. Furthermore, the transfer of biological inoculants from controlled laboratory conditions to variable field conditions is highly challenging [117, 118], but successful in many cases [25, 119, 120]. Thus, we are currently investigating the ability of some of the isolates in this study to promote plant growth of wheat and barley under field conditions using non-saline and saline irrigation in Saudi Arabia and the United Arab Emirates.

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Author Contributions

H.H. collected the desert plant samples. F.L. performed the bacterial isolation and 16S rRNA PCR amplification. A.Z. established the plant screening assay. A.E. performed the biochemical assays and plant screening. A.E. and H.A. determined the ion contents. A.E. and L.S. analyzed root colonization. M.S. and W.S. performed ACC deaminase assay. M.S. performed biofilm formation assay. A.E. and A.Z. extracted RNA. A.E. performed the qPCR. A.E constructed the figures and performed the data analysis. A.E and M.S. wrote the manuscript. M.S. and H.H. conceived the overall study and coordinated the project.

CRediT Author Statement

Abdul Aziz Eida: Conceptualization, Formal analysis, Investigation, Methodology, Validation, Vizualization, Writing – original draft, review & editing.

Hanin S. alzubaidy: Formal analysis, Investigation.

Axel de Zélicourt: Conceptualization, Formal analysis, Methodology, Writing – review & editing.

Lukáš Synek: Formal analysis, Investigation, Visualization, Writing – review & editing.

Wiam Alsharif: Formal analysis, Investigation.

Feras F. Lafi: Investigation, Methodology.

Heribert Hirt: Conceptualization, Funding acquisition, Project administration, Supervision, Writing – original draft, Writing – review & editing.

Maged M. Saad: Conceptualization, Formal analysis, Investigation, Methodology, Project administration, Supervision, Writing – original draft, Writing – review & editing.

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Tables

Table 1 Qualitative assessment of plant growth promoting potential of endophytic bacterial isolates.

	JZ2	JZ12	JZ18	JZ29	JZ34	JZ37
Phosphate solubilization	++	-	-	++	-	-
Siderophore production	++	-	-	++	-	-
Salt stress (1 M / 0.5 M NaCl)	+++/+++	-/+++	++/+++	+++/+++	++/++	++/+++
Drought stress (20% / 10% PEG 8000)	+/++	+/++	+/++	+/++	+/++	+/++
Heat stress (42°C)	++	-	++	++	+++	+++
Motility (Swimming / Swarming)	+/-	-/-	-/-	+/-	+++/+++	++/++
Biofilm production	++	++	++	++	+++	+++
Root surface colonization	+	-	+	+	+	+
Exoprotease production	+	+++	++	+	+	-
ACC deaminase production (μM) [*S. meliloti*: 149.36 μM]	3.08	53.79	20.64	53.79	219.57	14.78
IAA production (liquid)	++	-	-	++	+	++
Indole production (liquid)	++	-	-	++	-	-
Indole production (volatile)	+++	-	-	+++	-	-
H_2S production (volatile)	+++	++	-	+++	+	++

(+): weak ability; (++): moderate ability; (+++): strong ability; (-): no positive ability was observed.

Figure Legends

Figure 1 Phylogenetic tree of the five salinity stress promoting bacteria, and a negative control strain, based on 16S rRNA gene sequence comparison. Evolutionary relationships of the six selected bacterial isolates (JZ2, JZ12, JZ18, JZ29, JZ34, and JZ37) inferred using the Neighbor-Joining method. The evolutionary distances were computed using the Kimura 2-parameter method. GenBank accession numbers of isolates are presented in parentheses and type strains are indicated by a ^{T} after the parentheses. The percentages of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) are shown next to the branches. Plant species from which the strains were isolated from: *T. terrestris* (4 point star), *Z. simplex* (7 point star), *P. turgidum* (6 point star) and *E. granulata* (5 point star).

Figure 2 Effects of bacterial inoculation on *A. thaliana* growth under salinity stress. Representative images of *A. thaliana* plants inoculated with bacteria (JZ) or mock on ½MS + 100 mM NaCl at 16 DAI (days after inoculation) and non-stressed control grown without 100 mM NaCl **(a)**. Quantitative measurements of fresh weight **(FW, black)** and dry weight **(DW, grey)** of *A. thaliana* plant shoots **(b)**. Data are means of 3 biological replicates of 8 plants per treatment. Error bars represent standard error of the mean (SEM). Asterisks indicate significant differences between mock (100 mM NaCl) and bacteria-inoculated (100 mM NaCl) or non-stressed mock (0 mM NaCl) plants (Kruskal–Wallis test, $p \leq 0.05$). White bars in photographs correspond to 1 cm.

Figure 3 Phenotypic characterization of *A. thaliana* root system under salinity stress upon bacterial inoculation. Relative fresh weight **(FW, black)** and dry weight **(DW, grey)** of *A. thaliana* plant roots inoculated with bacteria (JZ) or mock on ½MS + 100 mM NaCl at 16 DAI (days after inoculation); and non-stressed control plants grown on 0 mM NaCl **(a)**. Primary root length **(PRL/black)** and lateral root density **(LRD/grey)** measurements collected 12 DAI **(b)**. Data are means of 3 biological replicates of 4 plants (100 mM NaCl control and JZ18), 8 plants (100 mM NaCl all strains except JZ18; 0 mM NaCl control) and 15 plants (root parameters) per treatment. Error bars represent standard error of the mean (SEM). Asterisks indicate significant differences between mock (100 mM NaCl) and batceria-inoculated (100 mM NaCl) or non-stressed mock (0 mM NaCl) plants (Kruskal–Wallis test, $p \leq 0.05$).

Figure 4 Sodium (Na^+) and potassium (K^+) ion content of *A. thaliana* shoots and roots upon bacterial inoculation. Na^+ **(black)** and K^+ **(grey)** contents in the shoots **(a)** and roots **(b)** of *A. thaliana* plants inoculated with (JZ) or mock on ½MS + 100 mM NaCl at 16 DAI (days after inoculation); and non-stressed control plants grown on 0 mM NaCl and the Na^+/K^+ ratios in the shoots **(black)** and roots **(grey)** were calculated **(c)**. Data are means of 3 biological replicates of 4 (roots of control and JZ18) and 8 (roots of all strains except JZ18; shoots) plants per treatment. Error bars represent standard error of the mean (SEM). Asterisks indicate significant differences between mock (100 mM NaCl) and bacteria-inoculated (100 mM NaCl) plants (Kruskal–Wallis test, $p \leq 0.05$).

Figure 5 Effects of bacterial inoculation on the expression of genes involved in sodium and potassium transport in *A. thaliana* shoot and roots under salinity stress. Normalized qPCR expression levels of genes involved in potassium **(left)** and sodium **(right)** transport in the shoots **(a)** and roots **(b)**; *AKT1* (*K^+ Transporter 1)*, *GORK* (*Gated Outwardly-Rectifying K^+ Channel*), *KUP6* (*K^+ Uptake Permease 6*), *HKT1* (*High-Affinity K^+ Transporter 1*), *SOS1* (*Salt Overly Sensitive 1*), *SOS3* (*Salt Overly Sensitive 3*), *HAK5* (*High Affinity K^+ Transporter 5*) and *SKOR* (*Stellar K^+ Outward Rectifier*). RNA was extracted from the shoots or roots of *A. thaliana* 16 DAI with or without bacteria on ½MS + 100 mM NaCl and 0 mM NaCl as non-stress control. Data are means of 3 biological replicates collected from 4-6 different plants per replicate and are normalized to non-inoculated control plants (100 mM NaCl). Error bars represent standard error of the mean (SEM). Asterisks indicate significant differences between mock (100 mM NaCl) and bacterua-inoculated (100 mM NaCl) or non-stressed mock (0 mM NaCl) plants (regulation threshold ≥ 1.5, $p \leq 0.05$).

Figures

Figure 1

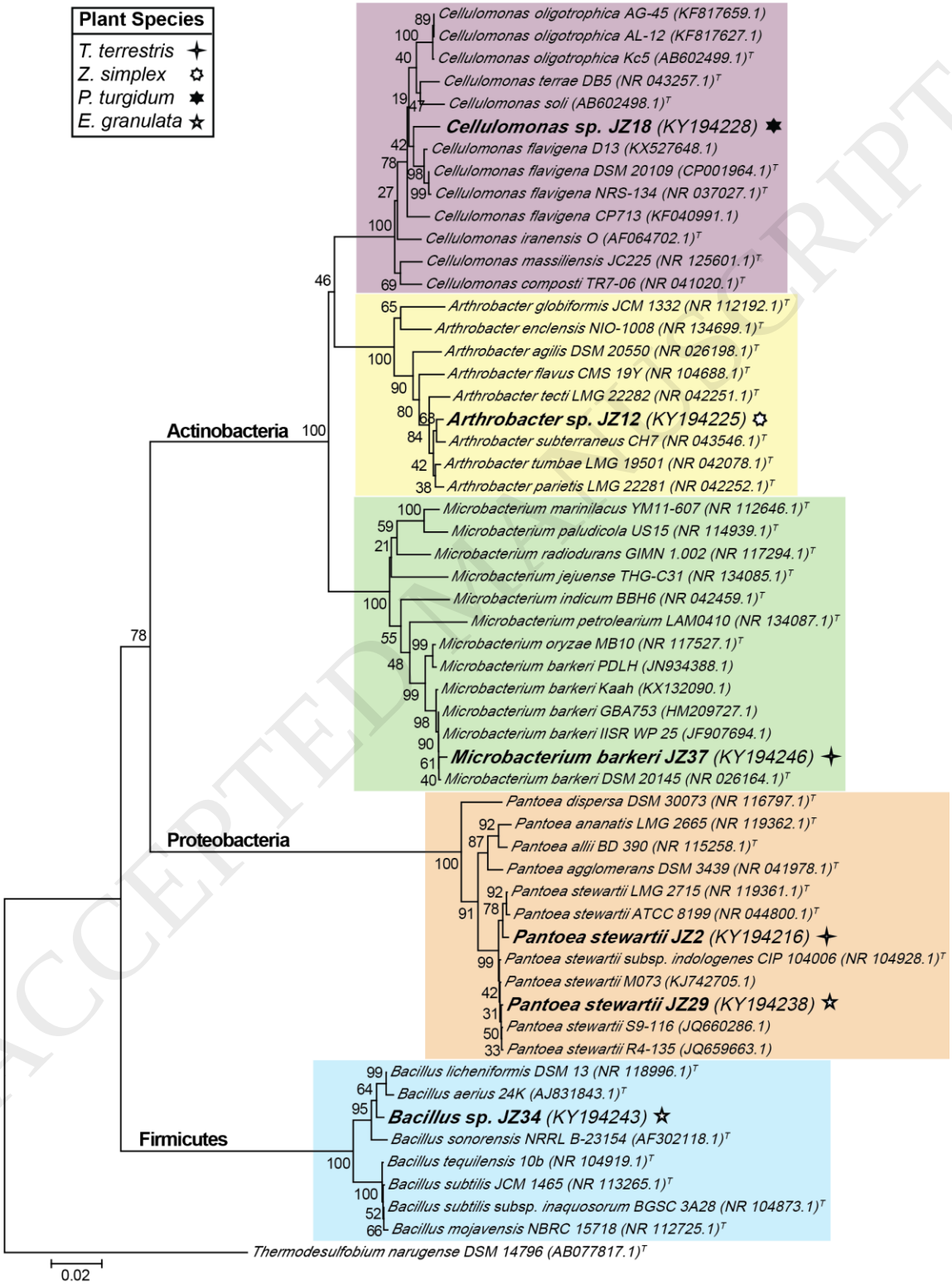

Figure 2

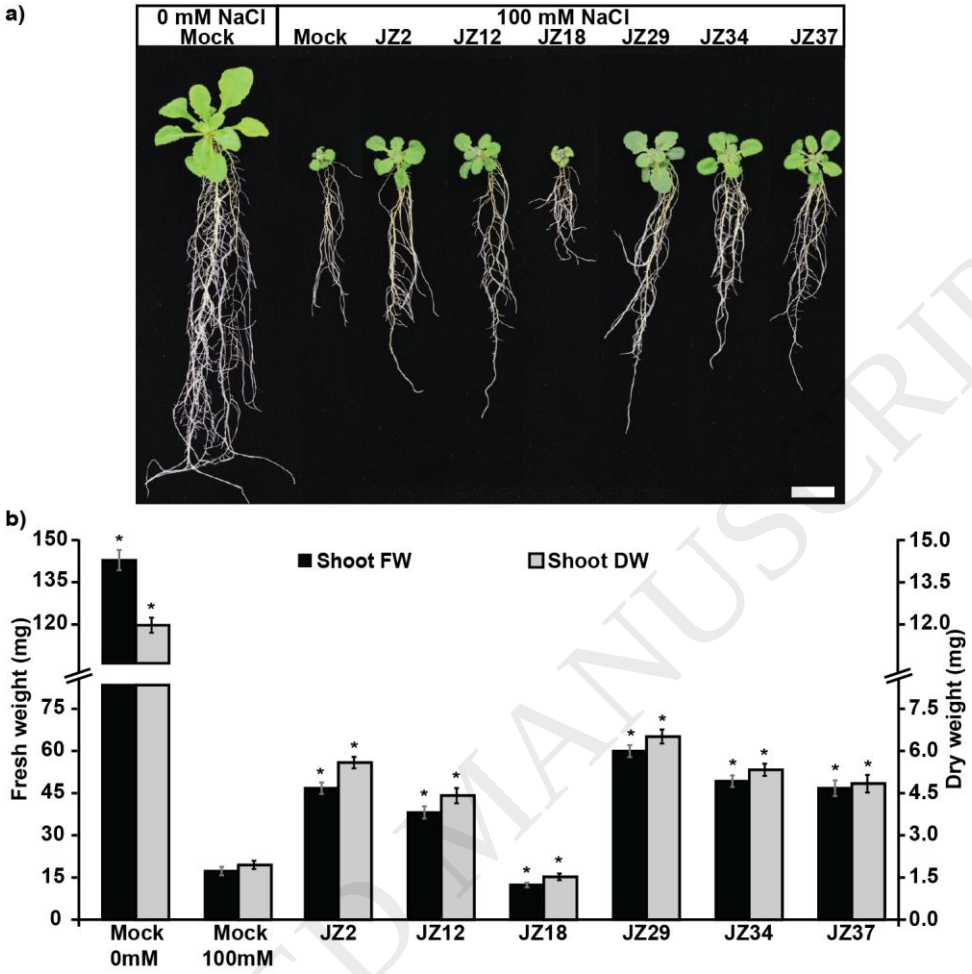

Figure 3

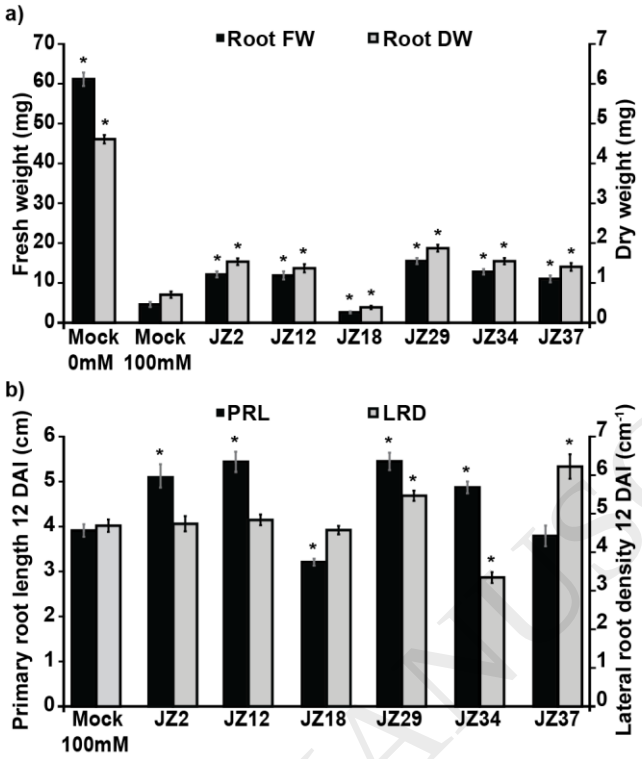

Figure 4

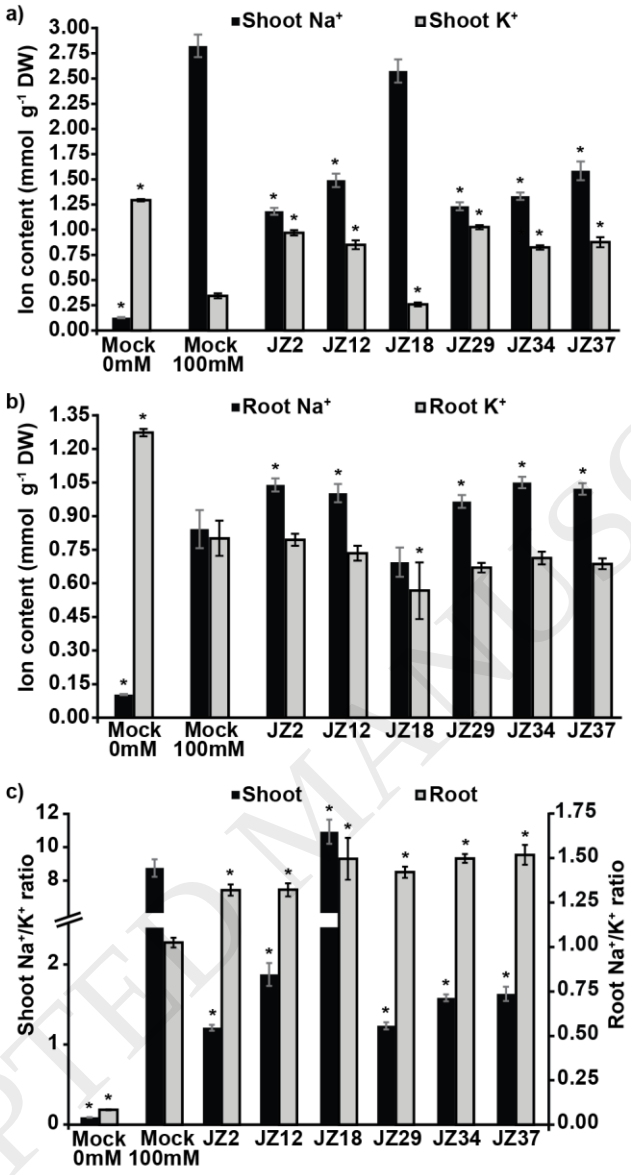

Figure 5