

Endophytic bacteria associated with halophyte *Seidlitzia rosmarinus* Ehrenb. ex Boiss. from saline soil of Uzbekistan and their plant beneficial traits

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Abstract: Endophytic bacteria of halophytic plants play essential roles in salt stress tolerance. Therefore, an understanding of the true nature of plant–microbe interactions under extreme conditions is essential. The current study aimed to identify cultivable endophytic bacteria associated with the roots and shoots of *Seidlitzia rosmarinus* Ehrenb. ex Boiss. grown in the salt-affected soil in Uzbekistan and to evaluate their plant beneficial traits related to plant growth stimulation and stress tolerance. Bacteria were isolated from the roots and the shoots of *S. rosmarinus* using culture-dependent techniques and identified by the 16S rRNA gene. RFLP (Restriction Fragment Length Polymorphism) analysis was conducted to eliminate similar isolates. Results showed that the isolates from the roots of *S. rosmarinus* belonged to the genera *Rothia*, *Kocuria*, *Pseudomonas*, *Staphylococcus*, *Paenibacillus* and *Brevibacterium*. The bacterial isolates from the shoots of *S. rosmarinus* belonged to the genera *Staphylococcus*, *Rothia*, *Stenotrophomonas*, *Brevibacterium*, *Halomonas*, *Planococcus*, *Planomicrobium* and *Pseudomonas*, which differed from those of the roots. Notably, *Staphylococcus*, *Rothia* and *Brevibacterium* were detected in both roots and shoots, indicating possible migration of some species from roots to shoots. The root-associated bacteria showed higher levels of IAA (indole-3-acetic acid) synthesis compared with those isolated from the shoots, as well as the higher production of ACC (1-aminocyclopropane-1-carboxylate) deaminase. Our findings suggest that halophytic plants are valuable sources for the selection of microbes with a potential to improve plant fitness under saline soils.

Keywords: endophytic bacteria; phylogenetic analysis; halophyte; auxin; plant beneficial traits

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1 Introduction

Salinity is considered to be a major threat to the environment and for food security, as it can affect biodiversity and reduce agricultural production and yield (Announ et al., 2004; Egamberdieva et al.,

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2017a, b). Under saline conditions, only a few highly tolerant plants, i.e., halophytes withstand high salinity levels. Thus, revegetation of saline soils with halophytes is considered as a potential phytoremediation approach (El Shaer, 2010; Egamberdieva et al., 2015; Mishra and Tanna, 2017). Moreover, the introduction of perennial and annual halophytes in salt-affected soils helps to improve soil structure and to increase soil organic biomass, soil biodiversity and activity, as well as crop productivity (Toderich et al., 2008; Hasanuzzaman et al., 2014; Flowers and Colmer, 2015). Halophytes have developed different morphological and physiological strategies to thrive under salinity stress, including the regulation of stress-responsive genes (Grigore et al., 2014; Muchate et al., 2016), the generation of reactive oxygen species and active osmoregulation (Hashem et al., 2016). There is also evidence that salt stress tolerance of halophytes is modulated by endophytic bacteria that colonize internal tissues (Etesami and Beattie, 2017).

The roles of endophytes in plant stress tolerance and growth stimulation under abiotic stress conditions have been reported in several studies (Ludwig-Mueller, 2015; Hashem et al., 2016; Egamberdieva et al., 2017; Etesami and Beattie, 2018). There is now clear evidence for the mechanisms used by endophytic bacteria to induce stress tolerance and to stimulate plant growth, such as the synthesis of plant growth hormones, the modulation of plant metabolites and the inhibition of ethylene synthesis in roots through 1-aminocyclopropane-1-carboxylate (ACC) deaminase (Kaplan et al., 2013; Cho et al., 2015; Egamberdieva et al., 2017; Etesami and Beattie, 2018). Mora-Ruiz et al. (2016) found a clear difference in the diversity of endophytes in the aerial and below-ground parts of *Arthrocnemum macrostachyum*, a flowering plant in the Amaranthaceae family. The colonization of plant tissue by endophytes originated in the rhizosphere, and followed by migration to the aerial parts of the plant.

Halophytic plants harbor diverse endophytic bacteria, e.g., *Chromohalobacter canadensis*, *Rudaea cellulositytica*, *Psychrobacter* sp., *Bradyrhizobium* sp. and *Halomonas* sp. were found in *A. macrostachyum* (Mora-Ruiz et al., 2016). *Bacillus* sp., *Serratia* sp., *Rhodococcus* sp., *Thalassospira* sp., *Rhizobium* sp., *Salinicola* sp., *Hafnia* sp., *Streptomyces* sp., *Microbacterium* sp. were isolated from the halophyte *Salicornia europaea* L. (Szymańska et al., 2016), belonging to the family Chenopodiaceae. Zhao et al. (2016) isolated the endophytes *Bacillus endophyticus*, *B. tequilensis*, *Planococcus rifietoensis*, *Variovorax paradoxus* and *Arthrobacter agilis* from tissues of *S. europaea*. The endophytic isolates associated with *Salicornia brachiata* L. were identified as *Brachybacterium saurashtrense* sp. nov., *Zhihengliuella* sp., *Brevibacterium casei*, *Haererehalobacter* sp., *Halomonas* sp., *Vibrio* sp., *Cronobacter sakazakii*, *Pseudomonas* sp., *Rhizobium radiobacter* and *Mesorhizobium* sp. (Jha et al., 2012).

To date, there have been no reports of endophytes associated with halophytes from salt-affected arid lands of Central Asia. Thus, here, we report on the endophytic bacteria associated with *Seidlitzia rosmarinus* Ehrenb. ex Boiss., a perennial woody shrub belonging to the Amaranthaceae family found in deserts and the arid and salt-affected regions in Jordan, Iraq, Iran, Saudi Arabia, and Central Asia. The plant is highly salt-tolerant and can grow on substrates up to 500 mM NaCl and is extremely well adapted even to hypersaline conditions (Kurkova et al., 2002). The shrub is used as a fodder crop for camels and is also considered for phytoremediation of salt-affected soils (Hadi, 2009), thus providing commercial value and ecological importance. To improve plant fitness to abiotic and biotic stresses, an enhanced understanding of plant and microbial interactions under extreme conditions is essential. The aims of our study were: (1) to isolate and identify cultivable endophytic bacteria associated with the roots and shoots of *S. rosmarinus* grown in salt-affected soil by using 16S rRNA gene analysis; and (2) to evaluate plant beneficial traits of endophytic bacteria related to plant growth stimulation and stress tolerance.

2 Materials and methods

2.1 Plant sample collection

Seidlitzia rosmarinus Ehrenb. ex Boiss was collected from the foothills of a mountain not far from Tuzkon salt cave in the Surkhandarya Province, Uzbekistan in August 2017. The soil has an EC (electrical conductivity) value of 6.5 mS/cm. Three individual plants over a distance of 10–12 m

were collected as a whole, stored in zip-lock plastic bags using sterile gloves and transported to the laboratory. The roots were separated from the stems with a sterile scalpel and were rinsed in water to remove the soil attached to the roots. Approximately 10 g of the plant roots and shoots were prepared for the isolation of endophytic bacteria.

2.2 Isolation of endophytic bacteria

The roots and shoots (stems with leaves together) were separated with a sterile scalpel, sterilized with 99.9% ethanol for 2 min and subsequently with 10% NaClO and were rinsed five times in sterile distilled water. The sterile shoots and roots (10 g fresh weight) were cut into 3–4 cm pieces and macerated using a sterile mortar and pestle (Mora-Ruiz et al., 2015). The macerated tissue (1 g) was transferred into plastic tubes with 9 mL of sterile phosphate-buffered saline (PBS) (20 mM sodium phosphate, 150 mM NaCl, pH 7.4) and shaken for 1 min using a Biosan B-1 Vortex (Microspin FV-2400; BIOSAN, Riga, Latvia). About 100 μ L aliquot from dilutions (10^1 – 10^5) was spread on 30% Tryptic Soy Agar plates (TSA) (BD, Difco Laboratories, Detroit, USA) supplemented with nystatin 50 μ g/mL, and the plates were incubated for 4 d at 28°C. The content of 30% TSA (tryptone soya agar) was as follows: 4.5 g/L pancreatic digest of casein, 1.5 g/L enzymatic digest of soya bean, 5.0 g/L NaCl and 15.0 g/L agar. After 4 d, colonies with different shape, color and density were picked and carefully transferred by streaking on nutrient agar plates, and incubated for the next 72 h to check the purity of the isolates. Visually homological colonies in sizes, shapes and colors were checked under a microscope for purity and used for DNA isolation. In order to test the sterility of the outer surfaces of the plant parts after sterilization with ethanol, we put two uncut pieces of roots and shoots onto TSA media, and the absence of any colonies after 72 h confirmed that sterilization was successful.

2.3 DNA isolation

For DNA extraction, the heat treatment method (Dashti et al., 2009) was used. The small parts of the colonies were transferred into 2-mL Eppendorf tubes with 1.5 mL of sterile MQ-water and were mixed with a Biosan B-1 Vortex for 10 s. The tubes were incubated at 90°C for 20 min in a Dry Block Heater (IKA Works, Inc., Wilmington, USA) and centrifuged at 12,000 rpm for 5 min. The DNA-containing supernatant was taken and stored at –20°C. The presence of DNA was checked by horizontal gel electrophoresis (0.8% agarose) and was quantified with a NanoDrop™ One (Thermo Fisher Scientific Inc., Waltham, USA).

2.4 Polymerase chain reaction (PCR)

Extracted DNA was used as a template for 16S rRNA gene analysis. The 16S rRNA genes were amplified via PCR using the following primers: 27F (5'-GAGTTTGATCCTGGCTCAG-3') and 1492R (5'-GAAAGGAGGTGATCCAGCC-3') (Sigma-Aldrich, St. Louis, Missouri, USA) (Lane, 1991). Each 25 μ L of reaction mixture contained 1 μ L (15–40 ng) DNA, 5 μ L 5×OneTaq standard reaction buffer (BioLabs, New England), 0.5 μ L 10 mM dNTP mix (Thermo Scientific), 0.5 μ L 10 mM primer 16SF (Merck), 0.5 μ L 10 mM primer 16SR (Merck) (25 μ mol/mL), 1 μ L 0.1% bovine serum albumin (TaKaRa Bio Inc., USA), 0.125 μ L One Taq polymerase (BioLabs, New England), and 16.375 μ L Milli Q water. The PCR was performed using a PTC-200 thermocycler (Bio-Rad Laboratories, USA). The PCR program was as follows: a primary heating step for 30 s at 94°C, followed by 30 cycles of denaturation for 15 s at 94°C, annealing for 30 s at 55°C and extension for 1.5 min at 68°C, followed by the final step for 20 min at 68°C. The PCR-amplified products were examined by electrophoresis in a 0.8% agarose gel containing GelRed.

2.5 Restriction fragment length polymorphism (RFLP) analysis

To determine the difference of the isolates similar in their color, shape and size, we conducted RFLP analysis of PCR amplicons of 16S rRNA gene as described by Jinneman et al. (1996). The fragments of digested PCR amplicons were checked via gel electrophoresis (1% agarose gel). After the electrophoresis, the gel was visualized using a digital gel imaging system (Gel-Doc XR TM+,

Bio-Rad Laboratories, USA) to identify identical isolates and reduce the number of strains to be sequenced.

2.6 Sequencing and phylogenetic analysis

Before being sequenced, we purified the PCR products with the USB® ExoSAP-IT® PCR Product Cleanup Kit (Affymetrix, USB® Products, USA) according to the manufacturer's protocol. Sequencing was performed using an ABI PRISM BigDye 3.1 Terminator Cycle Sequencing Ready Reaction Kit (Applied Biosystems, USA) by the manufacturer's protocol. Received data were analyzed and corrected using Chromas software v2.6.5. Corrected sequences were manually merged using EMBOSS Explorer (<http://emboss.bioinformatics.nl/>). The sequences were compared with those registered in GenBank from the National Center for Biotechnology Information (NCBI) (<http://www.ncbi.nlm.nih.gov/>) using basic local alignment search tool (BLAST).

All sequences were multiply aligned using ClustalX software v2.1, and a FASTA format file was used to construct the phylogenetic tree. The evolutionary history was inferred using the Neighbor-Joining method (Saitou and Nei, 1987). The optimal tree with the sum of branch length of 0.96928586 is shown. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (500 replicates) is shown next to the branches (Felsenstein, 1985). The tree is drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. The evolutionary distances were computed using the Maximum Composite Likelihood method (Tamura et al., 2004) and were in the units of the number of base substitutions per site. The analysis involved 35 nucleotide sequences. All positions containing gaps and missing data were eliminated. There were a total of 1278 positions in the final dataset. Evolutionary analyses were conducted in Molecular Evolutionary Genetics Analysis v6.0 (Tamura et al., 2013).

2.7 Accession numbers

The 16S rRNA gene sequences of the endophytic bacteria of *S. rosmarinus* were deposited into GenBank under the accession numbers: MH311985–MH311991 for root endophytes and MH311992–MH312003 for shoot endophytes.

2.8 Plant beneficial traits

IAA (indole-3-acetic acid) production was studied following the description of Bano and Musarrat (2003). The calibration curve of pure IAA was used as a standard to calculate IAA concentrations in culture supernatants. The utilization of ACC deaminase by endophytic bacteria as the sole N source was also determined. The bacterial isolates were grown in basal medium supplemented with 3.0 mM of ACC (Sigma Chemical Co., St. Louis, Missouri, USA) to test ACC utilization, or of $(\text{NH}_4)_2\text{SO}_4$ (positive control) as the sole N source or without added N source (negative control) (Egamberdieva et al., 2011).

2.9 Plant growth stimulation assay

Analysis of the plant growth stimulation capacity of endophytic bacteria was performed by using cress-lettuce (*Lepidium sativum* L.) seedlings. The bacterial isolates were grown for 3 d in nutrient broth at 28°C. Seeds of cress-lettuce were sterilized with 1% sodium hypochlorite solution followed by 95% ethanol for 3 min and were rinsed five times with sterile distilled water. The sterilized seeds were soaked for 10 min in bacterial suspension at a cell density of 1×10^7 cells/mL and were germinated on 1/2 agar plate supplemented with 100 mM NaCl. The untreated seeds were soaked with corresponding medium only. Each plate received 15 seeds and was kept in a plant growth chamber for 5 d. The germination rate, lengths of the roots and stems of seedlings were measured. All experiments were conducted in three replications.

2.10 Statistical analysis

Data were tested for statistical significance using the analysis of variance package included in Microsoft Excel 2010. Comparisons were performed using Student's *t*-test. Mean comparisons were conducted using the least significant difference (LSD) test ($P=0.05$).

3 Results

3.1 Isolation and identification of cultivable endophytic bacteria

A total of 45 bacterial isolates were isolated from root and shoot tissues of the halophytic plant *S. rosmarinus* (Fig. 1).



Fig. 1 *Seidlitzia rosmarinus* growing on saline soil in the Surkhandarya Province, Uzbekistan

Only 19 isolates were obtained after RFLP analysis, in which 7 isolates were obtained from the roots (Table 1) and 12 from the shoots (Table 2). The 16S rRNA gene sequence similarities of endophytic bacteria isolated from the roots of *S. rosmarinus* with sequences from GenBank are shown in Table 1.

Table 1 Sequence similarities of endophytic bacteria isolated from the roots of *Seidlitzia rosmarinus* with sequences registered in GenBank

Isolated strain sequence			Closest match among bacteria (16S rRNA gene)		
Strain	Length (bp)	Accession number	Species	Accession number	Identity (%)
JRT1	1452	MH311985	<i>Rothia terrae</i>	NR_043968	99.1
JRT2	1450	MH311986	<i>Kocuria palustris</i>	NR_026451	98.9
JRT3	1452	MH311987	<i>Pseudomonas baetica</i>	NR_116899	98.9
JRT4	1450	MH311988	<i>Staphylococcus warneri</i>	NR_025922	99.1
JRT5	1471	MH311989	<i>Staphylococcus epidermidis</i>	NR_113957	99.1
JRT6	1460	MH311990	<i>Paenibacillus amylolyticus</i>	NR_025882	98.7
JRT7	1466	MH311991	<i>Brevibacterium frigoritolerans</i>	NR_115064	99.6

All strains provided 98.4%–99.6% identities to those registered in GenBank. Isolated strains belonged to three phyla: Actinobacteria (JRT1, JRT2), Proteobacteria (JRT3) and Firmicutes (JRT4, JRT5, JRT6 and JRT7). The isolates were identified as *Rothia terrae* JRT1, *Kocuria palustris* JRT2, *Pseudomonas baetica* JRT3, *Staphylococcus warneri* JRT4, *Staphylococcus epidermidis* JRT5, *Paenibacillus amylolyticus* JRT6 and *Brevibacterium frigoritolerans* JRT7 (Table 1; Fig. 2). The diversity of isolates from the shoots of *S. rosmarinus* is shown in Table 2.

In total, 12 different isolates from the shoots of *S. rosmarinus* were selected after RFLP analysis. Isolated strains belonged to three phyla: Actinobacteria (JST2), Proteobacteria (JST3, JST7 and JST12) and Firmicutes (JST1, JST4, JST5, JST6, JST8, JST9, JST10 and JST11). The isolates JRT1, JRT2 and JST2 represent the same family Micrococcaceae, and it should be noted that the strains representing *R. terrae* inhabit both the roots and the shoots of *S. rosmarinus*. The same applies to *S. warneri*, *S. epidermidis* and *B. frigoritolerans*.

All isolated strains of the phylum Proteobacteria were predominantly related to the class Gammaproteobacteria, but from different orders, i.e., Pseudomonadales (JRT3 and JST12), Xanthomonadales (JST3) and Oceanospirillales (JST7). Representatives of the phylum Firmicutes were related to the order Bacillales and four families: Bacillaceae (JRT7 and JST5), Paenibacillaceae

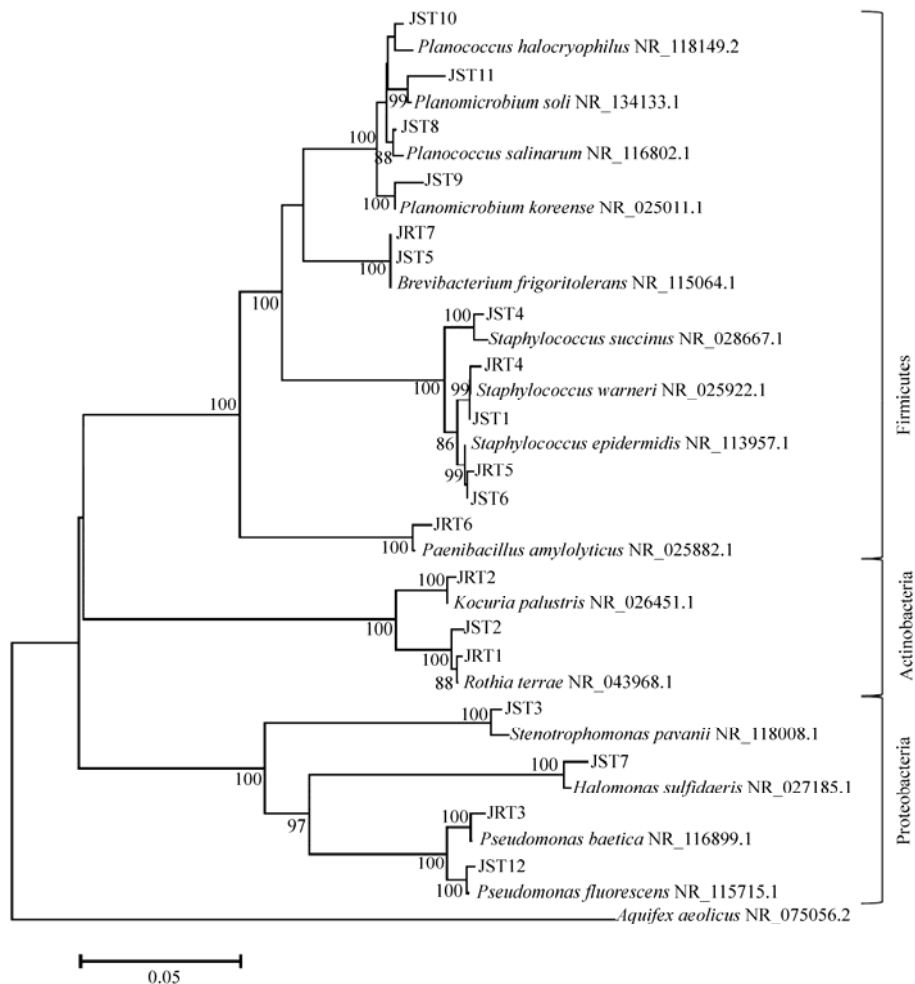


Fig. 2 Neighbor-joining phylogenetic tree based on 16S rRNA gene sequences isolated from endophytic bacteria of *Seidlitzia rosmarinus*, showing the relationship of isolated strains to their closest relatives in GenBank. All presented strains were divided into three groups: Firmicutes, Actinobacteria and Proteobacteria.

Table 2 Sequence similarities of endophytic bacteria isolated from the shoots of *Seidlitzia rosmarinus* with sequences registered in GenBank

Isolated strain sequence			Closest match among bacteria (16S rRNA gene)		
Strain	Length (bp)	Accession number	Species	Accession number	Identity (%)
JST1	1474	MH311992	<i>Staphylococcus warneri</i>	NR_025922	99.6
JST2	1445	MH311993	<i>Rothia terrae</i>	NR_043968	98.7
JST3	1467	MH311994	<i>Stenotrophomonas pavanii</i>	NR_118008	98.4
JST4	1474	MH311995	<i>Staphylococcus succinus</i>	NR_028667	98.6
JST5	1471	MH311996	<i>Brevibacterium frigiditolerans</i>	NR_115064	99.4
JST6	1458	MH311997	<i>Staphylococcus epidermidis</i>	NR_113957	99.4
JST7	1442	MH311998	<i>Halomonas sulfidaeris</i>	NR_027185	98.8
JST8	1478	MH311999	<i>Planococcus salinarum</i>	NR_116802	98.7
JST9	1471	MH312000	<i>Planomicrobium koreense</i>	NR_025011	98.5
JST10	1476	MH312001	<i>Planococcus halocryophilus</i>	NR_118149	98.9
JST11	1454	MH312002	<i>Planomicrobium soli</i>	NR_134133	98.5
JST12	1464	MH312003	<i>Pseudomonas fluorescens</i>	NR_115715	98.6

(JRT6), Planococcaceae (JST8, JST9, JST10 and JST11) and Staphylococcaceae (JRT4, JRT5, JST1, JST4 and JST6).

According to species diversity, the representatives of Firmicutes phyla (12 species) were more numerous as compared with Actinomycetes (3 isolates) and Proteobacteria (4 isolates) in both the roots and shoots of *S. rosmarinus*. A phylogenetic tree constructed showed the closest relatives of the isolates to known species (Fig. 2).

3.2 Plant beneficial traits

We characterized several plant growth traits of endophytes isolated from *S. rosmarinus*. The results showed that 6 out of 7 root-associated bacteria produced IAA, whereas 7 out of 12 shoot-associated bacteria produced IAA (Table 3).

Among the studied isolates from the roots, the following isolates showed the IAA production potential: *R. terrae* JRT1, *K. palustris* JRT2, *P. baetica* JRT3, *S. warneri* JRT4, *S. epidermidis* JRT5, *P. amylolyticus* JRT6 and *B. frigoritolerans* JRT7. The highest IAA synthesis was observed in the root-associated bacteria *P. baetica* JRT3 (14.9 µg/mL) and *P. amylolyticus* JRT6 (15.8 µg/mL). The

Table 3 Production of IAA (indole-3-acetic acid) and ACC (1-aminocyclopropane-1-carboxylate) deaminase activities in endophytic isolates of *Seidlitzia rosmarinus* and plant growth promoting abilities

Isolated strain	IAA production (µg/mL)		ACC deaminase	Plant growth stimulation (cm)		Seed germination percentage (%)
	Tr-	Tr+		Roots	Shoots	
<i>R. terrae</i> JRT1	8.4±0.7	11.7±1.0	+	5.2±0.5*	5.4±0.5	89±4
<i>K. palustris</i> JRT2	8.9±0.7	12.5±0.8	+	5.4±0.6*	5.5±0.6	90±6
<i>P. baetica</i> JRT3	10.1±0.8	14.9±1.0	+	5.5±0.5*	5.5±0.6	92±5
<i>S. warneri</i> JRT4	3.8±0.4	4.0±0.4	-	4.7±0.5	4.8±0.5	82±5
<i>S. epidermidis</i> JRT5	4.8±0.5	8.9±0.5	-	4.9±0.5	5.1±0.5	85±5
<i>P. amylolyticus</i> JRT6	11.4±0.9	15.8±1.1	+	5.4±0.6*	5.5±0.5	91±6
<i>B. frigoritolerans</i> JRT7	0.0	0.0	+	4.8±0.5	5.1±0.5	85±6
<i>S. warneri</i> JST1	0.0	0.8±0.4	-	4.6±0.4	5.0±0.5	85±5
<i>R. terrae</i> JST2	8.4±0.7	11.7±1.0	+	5.2±0.5*	5.4±0.5	89±5
<i>S. pavanii</i> JST3	9.5±0.7	20.5±0.9	+	5.5±0.6*	5.6±0.6*	93±4
<i>S. succinus</i> JST4	7.1±0.6	10.9±0.9	-	5.1±0.5	5.3±0.5	87±6
<i>B. frigoritolerans</i> JST5	0.0	0.0	+	4.9±0.5	5.2±0.4	85±5
<i>S. epidermidis</i> JST6	3.3±0.5	6.5±0.5	-	4.7±0.4	5.1±0.5	85±4
<i>H. sulfidaeris</i> JST7	0.0	1.7±0.8	+	4.9±0.5	5.1±0.4	85±4
<i>P. salinarum</i> JST8	0.0	0.0	-	4.6±0.5	4.9±0.4	83±5
<i>P. koreense</i> JST9	7.8±0.7	9.9±0.9	+	5.1±0.6	5.3±0.6	88±6
<i>P. halocryophilus</i> JST10	0.0	1.0±0.4	-	4.7±0.4	5.0±0.5	85±5
<i>P. soli</i> JST11	9.3±0.7	12.3±0.8	+	5.4±0.5*	5.4±0.5	90±6
<i>P. fluorescens</i> JST12	7.2±1.3	11.6±1.5	+	5.6±0.6*	5.7±0.6*	94±4
Control				4.8±0.5	5.1±0.5	85±5

Note: Tr-, without tryptophan; Tr+, with tryptophan; +, ACC deaminase production; -, non-ACC deaminase production. * means significant difference between isolated strain and control at $P<0.05$ level. Mean±SE.

shoot-associated bacteria that produced IAA belonged to *R. terrae* JST2, *S. pavanii* JST3, *S. succinus* JST4, *S. epidermidis* JST6, *P. koreense* JST9, *P. soli* JST11 and *P. fluorescens* JST12. ACC deaminase production was observed in 5 out of 7 bacterial isolates from the root system and 7 out of 12 isolates from the shoots (Table 3).

Table 3 illustrates plant growth stimulation capabilities of endophytic isolates on lettuce. The results showed that *K. palustris* JRT2, *P. baetica* JRT3 and *P. amylolyticus* JRT6 isolated from roots, stimulated roots and shoots of lettuce seedlings by 12%, 15% and 13%, respectively, as compared

with the control seedlings. However, only *S. warneri* JRT4 reduced the growth of lettuce seedlings. Similarly, the isolates from the shoots also showed significant plant growth stimulatory capabilities, although four isolates, i.e., *S. warneri* JST1, *S. epidermidis* JST6, *P. salinarum* JST8 and *P. halocryophilus* JST10, showed inhibitory activity. The highest stimulatory percentage was obtained with *P. fluorescens* JST12 (17%), followed by *S. pavanii* JST3 (14%).

4 Discussion

To our knowledge, this is the first report in which endophytic bacteria associated with the halophyte *S. rosmarinus* growing on salt-affected soils were analyzed. In total, 19 isolates were selected, and we found that the majority of the isolates (i.e., 12 isolates) were from the Firmicutes phylum, divided into four clusters: Bacillaceae, Paenibacillaceae, Planococcaceae and Staphylococcaceae. The Actinobacteria phylum contained three isolates, belonging to *Kocuria* and *Rothia* genera. The Proteobacteria phylum contained four isolates, belonging to *Pseudomonas*, *Stenotrophomonas* and *Halomonas* genera. In our study, the endophytic bacteria *P. halocryophilus* and *P. salinarum* were associated with the roots of *S. rosmarinus*. Zhao et al. (2016) also identified *P. rifietoensis* associated with *Salicornia europaea* growing on a highly saline soil. *P. rifietoensis* was also found in permafrost soil from the Canadian Arctic region (Mykytczuk et al., 2012). Yoon et al. (2010) observed a strain of *P. salinarum* in a marine solar saltern in Korea. The strains *R. terrae* JRT1 and *R. terrae* JST2 were isolated from root and shoot tissues of *S. rosmarinus* in the present study. The occurrence of this bacterium was also observed in a soil from Taiwan, China (Chou et al., 2008). In our study, one of the shoot-associated bacterial species was identified as *P. koreense*. Yoon et al. (2001) observed a strain similar to *P. koreense* JST9 from fermented seafood in Korea. The species *P. soli* was first isolated from soil of Alxa National Geological Park in Inner Mongolia Autonomous Region, China (Luo et al., 2014). The identification of the isolate *H. sulfidaeris* in our study is the first report of the occurrence of halotolerant *H. sulfidaeris* in plant tissue. You et al. (2015) also observed several *Halomonas* species, including *H. sulfidaeris* from rhizosphere soils of coastal plants from the Dokdo Islands of South Korea. Previously, this species was found in Pacific hydrothermal vents, similar to many other halotolerant microorganisms (Kaye and Baross, 2000).

We have observed five isolates from the roots and the shoots of *S. rosmarinus* belonging to *Staphylococcus* species, such as *S. warneri* JRT4, *S. epidermidis* JRT5, *S. warneri* JST1, *S. succinus* JST4 and *S. epidermidis* JST6. The representatives of the genus *Staphylococcus* are commonly known as potential human or animal pathogens (Kloos and Schleifer, 1975; Nováková et al., 2006). For example, *S. warneri* causes bovine abortion (Barigye et al., 2007) or multifocal discitis (Announ et al., 2004). There are also several reports of the occurrence of salt-tolerant *Staphylococcus* species in diverse environments, including saline soils (Roohi et al., 2012; Nanjani and Soni, 2014). For example, *S. saprophyticus* was isolated from the rhizosphere of wheat grown in saline soil (Egamberdieva et al., 2008), from carrot (Surette et al., 2003), or from chestnut phyllosphere (Valverde et al., 2005).

Ramos et al. (2011) isolated and identified *Stenotrophomonas pavanii* JST3 from the stems of Brazilian sugar cane, which possesses nitrogen-fixing capacity. Notably, we have observed *S. warneri*, *S. epidermidis* and *B. frigoritolerans* both in the roots and the shoots of the plant. It has been reported that bacteria in plant tissues are capable of migrating from soil to aerial parts of the plant through chemotaxis (Chi et al., 2005; Bulgarelli et al., 2013).

Bacteria use different mechanisms to improve plant health and fitness under stressed environmental conditions, such as salinity or drought. There are numerous reports in which endophytic bacteria stimulated plant growth and improved the resistance of a host plant to abiotic and biotic stresses, including drought and salt stress (Rashid et al., 2012; Egamberdieva et al., 2017c). The production of IAA by endophytic bacteria is considered to be an essential trait that modulates plant growth and physiology (Egamberdieva et al., 2017c). In our study, root-associated bacteria showed higher IAA synthesis activities compared with shoot-associated bacteria. The phytohormones produced by bacteria stimulate the growth and development of the root system architecture, which then facilitates the nutrient acquisition of plants under saline conditions (Piccoli et al., 2011; Egamberdieva et al., 2017a, c). Sorty et al. (2016) observed IAA production by several bacterial species isolated from a halotolerant weed (*Psoralea corylifolia* L.), including

Acinetobacter, *Enterobacter*, *Pseudomonas* and *Bacillus*. The salt-tolerant strain *P. fluorescens* SPB2145 produced IAA, through which the root and shoot growth, nutrient uptake and plant stress tolerance of cucumber significantly increased (Egamberdieva et al., 2011). In our study, we observed that eight bacterial isolates produced IAA and stimulated root length. Interestingly, all of them were also able to produce ACC deaminase, which could cleave the plant ethylene precursor ACC, and thereby lowered the level of ethylene in a stressed plant (Glick, 2014). Our observations are in line with the results of Sgroj et al. (2009), showing that the endophytic bacteria isolated from the halophyte *Prosopis strombulifera* were positive for ACC deaminase activity. Gupta and Pandey (2019) reported that ACC deaminase producing bacteria *Paenibacillus* sp. alleviated the negative effects of salinity stress and increased root and shoot growth of French bean seedlings subjected to salinity stress. However, in our study two isolates of *B. frigiditolerans*, which produced ACC deaminase, but not IAA, or *Staphylococcus* strains that produce IAA but not ACC deaminase did not show any stimulation on plant growth. This finding indicates that multiple plant growth promoting activities vary with the trait and the isolate.

5 Conclusions

For the first time, we revealed the diversity of endophytic bacteria isolated from the roots and the shoots of the halophyte *S. rosmarinus*, grown on saline soil of Uzbekistan. The isolates belong to the genera *Rothia*, *Kocuria*, *Pseudomonas*, *Staphylococcus*, *Paenibacillus*, *Brevibacterium*, *Stenotrophomonas*, *Halomonas*, *Planococcus* and *Planomicrobium*. The most frequently isolated genus in the plant roots and shoots was *Staphylococcus*, which is known for its capacity of high salt tolerance. The root-associated bacteria showed higher levels of IAA synthesis compared with bacteria isolated from the plant shoots, as well as the production of ACC deaminase. Our findings suggest that halophytic plants could be a source for the selection of microbes that could improve plant fitness under saline soils. However, these findings also show that further research is necessary in pot as well as field experiments to resolve the impact of endophytic bacteria with selected plant growth promoting traits on plant growth and stress tolerance.

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