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RESEARCH ARTICLE

Plant growth-promoting properties of endophytic bacteria isolated from some xerophytic plants distributed in arid regions (Uzbekistan)

Ixtiyor Akramov[1], Shahzod Axanbayev[1], Begali Alikulov[1]*, Sitora Mukhtorova[2], Azamat Ergashev[3] & Zafar Ismailov[1]

[1]Department of Genetics and Biotechnology, Samarkand State University named after Sharof Rashidov, University Boulevard, 15, Samarkand, 140100, Uzbekistan
[2]Department of Agrobiotechnology,, Tashkent branch of Samarkand State Veterinary Medicine, Animal Husbandry and Biotechnology University, Chilonzor district 20, 35A, Tashkent, 100117, Uzbekistan
[3]Department of Biology, ,Denau institute of entrepreneurship and pedagogy, Guli Rano street, 1A, Denau district, Surkhandarya region, 190507, Uzbekistan

*Email: balikulov87@gmail.com

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Abstract

Recent advancements in the development of endophytic microorganisms-based stimulants have shown promising potential in various fields. Research on the identification of these endophytic microorganisms has been well reported, however, there are limited studies of these endophytes isolated from plants in arid regions. Thus, isolation and identification of promising microbial endophytes from xerophytic plants is essential in technology development for sustainable agriculture in arid regions. This study aims to identify the endophytic bacteria isolated from *Kochia prostrata* (L.) Schrad. and *Ceratoides eversmanniana* (Stschegl. ex I.G.Borshch.) Botsch. & Ikonn. in vertical zones of arid regions of Uzbekistan, and examine their potential plant growth-promoting properties. Using Luria-Bertani (LB) medium, 70 distinct bacterial colony were isolated from the different segments of *K. prostrata* and *C. eversmanniana*. These isotypes were screened using NaCl-supplemented LB medium in which nine promising bacterial isolates showed tolerance to 15% NaCl. The nine promising halophytes were subjected to molecular identification using specific primers. The isolates from *K. prostrata* are identified as *Bacillus amyloliquefaciens, Bacillus pumilus, Priestia aryabhattai, Pseudomonas putida*, and *Priestia endophytica*. On the other hand, *Priestia megaterium, Pseudomonas putida, Bacillus subtilis* and *Brevibacillus parabrevis* were isolated from *C. eversmanniana*. The identified isolates also showed significant plant growth-promoting properties (N_2-fixation, IAA production, phosphates solubilization, ACC deaminase production, siderophores production) and shows ability to inhibit pathogenic fungal growth. Based on the result, the identified bacterial endophytes can be processed as growth-stimulants and biological control of fungal pathogens in crops in arid regions.

Keywords

Antifungal property; arid region; *Ceratoides eversmanniana*; endophytic bacteria; *Kochia prostrata* ; plant growth-promoting properties

Introduction

Drought, salinity, and desertification have become global problem. It was reported by UNCCD (1) that the severity of drought affected area increase by 29% since 2000, affecting 55 million people across the globe. Drought poses

a major challenge in agriculture, resulting in a substantial reduction in agricultural crop production. Considering this threat, it is important to prioritize the development and implementation of innovative technologies based on biotechnological approaches. This is essential for creating sustainable farming opportunities in drought conditions (2). One solution being explored is the use of endophytic bacteria that are naturally occurring and adapted to drought conditions, such as those in xerophytic plants. Understanding the beneficial effects of endophytic bacteria in drought-adapted plants can stimulate the development of crops in arid areas.

Endophytic bacteria are microorganisms that live inside the vegetative organs of plants, and they contribute to the growth and development of the host plant organism to a certain extent (3). Endophytic bacteria can form a mutualistic relationship with the host plant by fixing nitrogen, breaking down insoluble phosphates, producing phytohormones and siderophores, and controlling phytopathogens, in exchange for shelter and conducive environment (4).

Endophytic bacteria isolated from different plants have been reported by several studies. Hwang et al. (5) isolated *Priestia megaterium* Strain BP-R2 from *Bolboschoenus planiculmis* in which result revealed that these specific endophytes produce a significant amount of specific plant growth-promoting hormone. *Bacillus halotolerans* was also been isolated from *R. soongorica* which was reported to have beneficial effect base on the result (6). Egamberdieva et al. (7) found that the endophytic bacterium *Pseudomonas kilonensis* isolated from *Tetragonia tetragonioides* has high antifungal activity against the parasitic fungus *Fusarium solani*. The endophytic bacterium *Oceanobacillus manasiensis* isolated from *Salicornia europaea* L. has been reported to be effective in producing IAA and siderophore (8). As a result of a series of studies, endophytic bacteria were isolated from plants *Haloxylon aphyllum* (9), *Helianthus annuus* (10), *Halocnemum strobilaceum* (11, 12), *Seidlitzia rosmarinus* (13) and *Halostachys belangeriana* (14), their potential was evaluated and identified using molecular genetic methods. The analysis showed that *Bacillus*, *Pseudomonas* and *Priestia* representatives dominate among plant endophytic bacteria. However, there were limited studies on endophytic bacteria from xerophytes, specifically in *K. prostrata* and *C. Eversmanniana*, in arid regions.

Kochia prostrata is a 10-50 cm tall perennial plant belonging to the *Amaranthaceae* family. This plant is a xerophyte-halophyte with high nutritional value for livestock (15). According to electronic databases (16), *K. prostrata* is distributed in Central Asia, Southwest Asia, most of Europe and North Africa (Fig.1A).

Ceratoides eversmanniana is a perennial plant up to 1 meter tall, belonging to the *Amaranthaceae* family. This plant has a robust root system that penetrates to a depth of 1-3 m in the first year and 6 m at the age of 10. It flowers in yellow in July and August, and the long hairy seeds ripen in September and October. *Ceratoides eversmanniana* is distributed in the foothills and semi-desert region and is a high forage source for livestock (15). According to electronic databases (17), *C. eversmanniana* is distributed throughout most of Eurasia and northern Africa (Fig.1B).

Both *K. prostrata* and *C. eversmanniana* are adopted in the drought conditions of Uzbekistan. Symbiotic relationship between plants and microorganisms are common in nature. However, endophytic bacteria associated with *K. prostrata* and *C. eversmanniana* have not yet been explored. Hence, isolation and identification of these endophytic bacteria is important to enhance the performance *K. prostrata* and *C. Eversmanniana* and other crops.

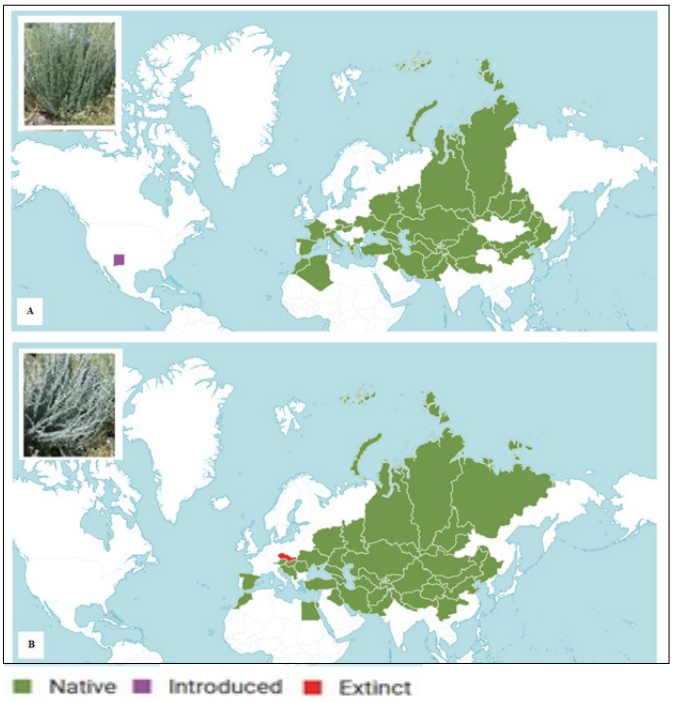

Fig. 1. Distribution area of *Kochia prostrata* (L.) Schrad (**A**) and *Ceratoides eversmanniana* (Stschegl. ex I.G.Borshch.) Botsch. & Ikonn (**B**) (*https://*

Materials and Methods

Plant collection

The collection of xerophytic plant samples was carried out during the springs of 2022 in the arid lands of the southwestern regions of Uzbekistan, including Nurata, Gallaorol and Qamashi (Fig. 2). Segments from roots, leaves and stems of *K. prostrata* and *C. eversmanniana* were used as source of inoculum. . First, samples were taken from plants growing at a distance of not less than 10 m, for this study. Next, the roots, leaves and stem were cleaned in sterile water to remove soil particles.

The endophytic bacterial isolation

A total of 15 g stem, leaves and roots were sterilized in beakers filled with 99.9% ethanol for 2 min. and 10% sodium hypochlorite for 1 minute. Afterward, they were placed in sterile water cups for 2 min. (18). Pieces of stem and

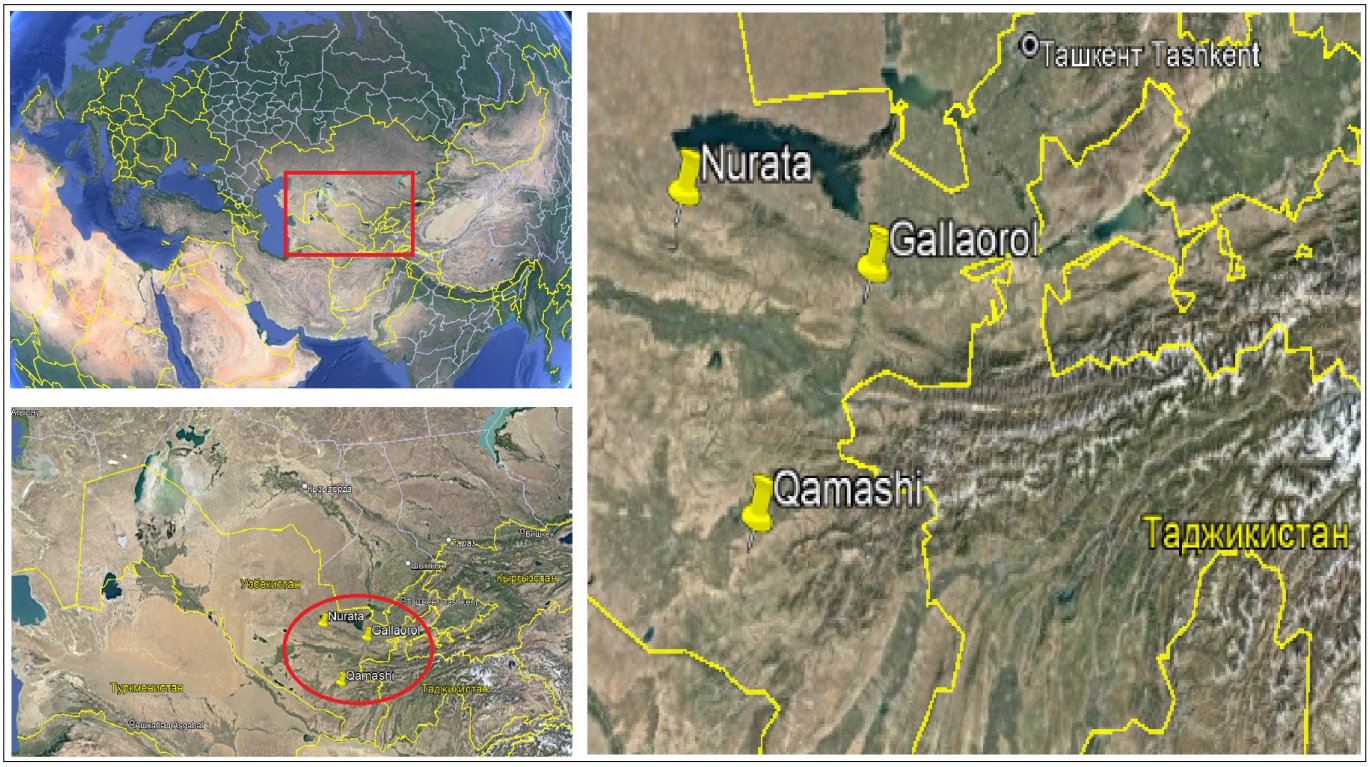

Fig. 2. Locations where samples were taken for research.

roots were cut lengthwise into thin slices. For serial dilutions, 5 g of each sample was taken and transferred to test tubes containing 9 ml of sterile water (10^1–10^5). After each dilution, 100 ml of the suspension was taken and inoculated onto Luria-Bertani (LB) nutrient medium at 30°C (19). After four days, the colonies that changed in color and shape were transferred to petri dishes with LB for purification.

Selection of promising strains of endophytic bacteria

Colonization capabilities of the isolates at different levels of salinity were considered when isolating promising strains of endophytic bacteria of xerophytes. The working medium was prepared by mixing LB medium and NaCl solutions with different concentrations (0%, 5.0%, 7.5%, 10.0%, 12.5% and 15%) 1:1. Isolated isolates were grown in this nutrient medium at 30°C for 7 days. Isolates colonized in nutrient medium with 15% NaCl solution were selected as promising strains.

Bacteria identification

The genomic DNA used for molecular identification was extracted as per the method given by Dashti et al. (20). The 16S rRNA gene of the extracted DNA was amplified using PCR with the following primers: 27F 5′-GAGTTTGATCCTGGCTCAG-3′ (Sigma-Aldrich, St. Louis, Missouri, USA) and 1492R 5′-GAAAGGAGGTGATCCAGCC-3′ (Sigma-Aldrich, St. Louis, Missouri, USA). The PCR program was used as follows: a primary heating step for 30 sec. at 94°C, followed by 30 cycles of denaturation for 15 sec. at 94°C, annealing for 30 sec. at 55°C and extension for 1.5 min at 68°C, then followed by the final step for 20 min at 68°C. The PCR products were checked by electrophoresis using GelRed.

The ABI PRISM BigDye 3.1 Terminator Cycle Sequencing Ready Reaction Kit (Applied Biosystems, USA) was used for the sequencing. The obtained sequences were compared with the sequences of the closest relatives from GeneBank at the National Center for Biotechnology Information (NCBI) (21).

Antifungal activity of bacteria

The pathogenic fungi, namely, *Rhizoctonia solani, Fusarium oxysporum* and *Alternaria alternata,* used in antifungal studies were obtained *from* the Department of Microbiology and Biotechnology of the National University Uzbekistan. The bacterial endophyte strains were checked *in vitro* for the presence of antagonistic activity against the fungi mentioned earlier by using the plate method. Within 5-7 days, the fungi were grown on Czapek Dox Agar medium at 28°C. Agar discs containing grown fungi cultures were cut into small squares (7-8 mm on each side) and placed in the center of Petri plates (9 cm in diameter). Bacteria were grown in a TSA medium and then inoculated to test plates in the same medium as the fungi. The plates were incubated at 28°C for 7 days until the fungi covered the control plates without bacteria. Antifungal activity was measured as the width of the growth inhibition zone between fungi and test bacteria.

Tests for plant growth-promoting traits

The production of indole-3-acetic acid (IAA) was tested according to the method of Sarwar and Kremer (22). Bacterial suspension was adjusted to 1 x 10^8 CFU/mL and added into flasks with 10%LB supplemented with 5 mmol/L^{-1}of L-tryptophan and cultivated at 30°C for 24 h in the dark. The grown bacteria were centrifuged at 8000×g for 15 min and supernatant was poured into fresh tubes. The Salkowski reagent (mixture of $FeCl_3$ - 0.5 mol/L and H_2SO_4 - 7.9 mol/L) was added in a 1:1 ratio (v/v) to supernatant and incubated at roomtemperature for 30 min in the dark. The appearance of pink color indicated the production of

indole-3-acetic acid. For measurement of IAA, spectrophotometer at 530 nm was used. Different concentrations of IAA solutions were used to construct standard curve.

The ability of endophytes to solubilize inorganic phosphate was tested according to Mehta and Nautiyal (23). The bacteria were cultured on solid NBRIP medium (%): glucose - 1, $Ca_3(PO_4)_2$ - 0.5, $MgCl_2$ – 0.5, $(NH_4)_2SO_4$ - 0.01, $MgSO_4.7H_2O$ - 0.025, KCl - 0.02, agar - 1.5). Plates with bacteria were incubated at 28°C for 96 hours. The formation of colonies indicated on ability to use inorganic phosphate in the form of $Ca_3(PO_4)_2$ as a sole phosphate source.

To test the strains for nitrogen fixation assay the colonies of each endophyte were streaked onto solid nitrogen-deficient malate medium (g/L): $CaCl_2$ - 0.02, NaCl - 0.1, $FeCl_3$ - 0.01, KH_2PO_4 - 0.4, K_2HPO_4 - 0.5, $MgSO_4·7H_2O$ - 0.2, $Na_2MoO_4·2H_2O$ - 0.002, sodium malate - 5, agar - 15, pH 7.2–7.4) supplemented with 50 mg/L yeast extract. The plates were incubated at 30°C during 96 h and the appearance of growth indicated the ability to fix N_2. The new grown single colonies were streaked onto plates with same medium to confirm the ability of nitrogen fixation (24).

Siderophores production was determined by using chrome azurol S (CAS) agar. Isolates were streaked onto CAS agar, incubated at 30°C for 96 hours. The appearance of orange halo around the bacterial colony indicated on production of siderophores (25).

The 1-aminocyclopropane-1-carboxylate (ACC) deaminase production by bacteria was tested based on utilization of ACC as a sole N-source. The endophytes were cultivated on basal medium supplemented with 3.0 mM of ACC. $(NH_4)_2SO_4$ was used as a positive control and without added N source as a negative (26).

Statistical analysis

The statistical significance of data was tested by the analysis of variance of Microsoft Excel 2013 package. Mean comparisons were conducted using the least significant difference (LSD) test (P=0.05). The average values of plant growth parameters, IAA production and the standard deviation were counted based on several replications.

Results and discussion

More than 600 segments of stem, leaf and roots of *K. prostrata* and *C. eversmanniana* were studied in the present study. A total of 70 isolates of endophytic bacteria were found growing on the surface of the nutrient medium, of which 36 isolates (KoPr101- KoPr136) were from *K. prostrata* and 34 isolates (CREW1001- CREW1034) were isolated from *C. eversmanniana* (Table 1). Roots contains the most percentage of bacterial isolates with 45.7%, while leaves and roots have 35.7% and 18.6%, respectively, of the total isolates.

The colonization potential of the isolates isolated from xerophytic plantsin nutrient media with different concentrations of NaCl was determined. In the experiments, all 36 isolates of *K. prostrata* have tolerance to nutrient medium with 5% and 7.5% concentrations of NaCl. The same result was observed in all 34 isolates from *C. eversmanniana*. However, it was observed that 66.7% of isolates from *K. prostrata* and 58.8% isolates from *C. eversmanniana* were able to grow in nutrient medium with 10.0% NaCl concentration. Increasing NaCl concentration in the nutrient medium has been shown to reduce the growth of bacterial isolates isolated from xerophytic plants (10, 14). It was further observed that 47.2% of isolates from *K. prostrata* and 38.2% of isolates from *C. eversmanniana* were able to grow in nutrient medium with 12.5% concentration of NaCl. When cultured in medium with a concentration of 15.0% NaCl, which is the critical level for most organisms, 5 isolates from *K. prostrata* (KoPr101, KoPr113, KoPr118, KoPr129, KoPr131), 4 isolates from *C. eversmanniana* (CREW1004, CREW1015,

Table 1. Distribution of segments and isolates isolated from xerophytic plants by vegetative organs

Plant	Geographical coordinates of sample collection areas (Name of Location)	Root		Shoot		Leaf		Total	
		A	B	A	B	A	B	A	B
Kochia prostrata (L.) Schrad	40°31'39.2"N, 65°55'55.5"E (Nurata District, Navoi Region), h=368 m	46	6	78	8	36	3	**160**	**17**
	40°02'20.8" [illegible] (Gallaorol district, Jizzakh region), h=534 m	22	3	26	3	18	2	**66**	**8**
	38°46'35.2" [illegible] (Qamashi District, Kashkadarya Region), h=1304 m	34	4	54	5	28	2	**116**	**11**
Total per plant		102	13	158	16	82	7	**342**	**36**
Ceratoides eversmanniana (Stschegl. ex I.G.Borshch.) Botsch. & Ikonn.	40°31'39.2"N, 65°55'55.5"E (Nurata District, Navoi Region), h=368 m	56	7	84	9	42	3	**182**	**19**
	38°46'35.2" [illegible] Kashkadarya Region), h=1304 m	40	5	62	7	38	3	**140**	**15**
Total per plant		96	12	146	16	80	6	**322**	**34**
Total		**198**	**25**	**304**	**32**	**162**	**13**	**664**	**70**

Note: * A-number of segments; B- Number of isolates

CREW1018, CREW1021) show a high degree of resistant. These isolates were identified as promising strains and were subjected to molecular identification (Table 2).

The 16S pRNA nucleotide sequences of 15 promising strains of endophytic bacteria isolated from halophytes were deposited in the National Center for Biotechnology Information (NCBI) database: ON567219, ON567220, ON567221, ON567222, ON567223 (*K. prostrata*), ON567363, ON567362, ON567361, ON567360 (*C. eversmanniana*) (21).

The molecular analysis and DNA sequencing revealed the identity of the bacterial isolates, at species lev-

Table 2. Colonization of endophytic bacterial isolates of xerophytic plants under salinity conditions

Isolate name	NaCl concentration (in %)					
	0	5	7.5	10	12.5	15
***Kochia prostrata* (L.) Schrad**						
KoPr-101*	+++	++	++	++	+	+
KoPr-102	+++	++	+	-	-	-
KoPr-103	+++	++	++	+	+	-
KoPr-104	+++	++	+	-	-	-
KoPr-105	+++	++	++	+	+	-
KoPr-106	+++	++	++	+	+	-
KoPr-107	+++	++	++	+	+	-
KoPr-108	+++	++	+	-	-	-
KoPr-109	+++	++	+	-	-	-
KoPr-110	+++	++	++	+	+	-
KoPr-111	+++	++	++	+	+	-
KoPr-112	+++	++	++	+	-	-
KoPr-113*	+++	++	++	+	+	+
KoPr-114	+++	++	++	+	+	-
KoPr-115	+++	++	+	-	-	-
KoPr-116	+++	++	++	+	-	-
KoPr-117	+++	++	++	+	-	-
KoPr-118*	+++	++	++	++	+	+
KoPr-119	+++	++	++	+	+	-
KoPr-120	+++	++	++	+	+	-
KoPr-121	+++	++	+	-	-	-
KoPr-122	+++	++	++	+	-	-
KoPr-123	+++	++	++	+	-	-
KoPr-124	+++	++	+	-	-	-
KoPr-125	+++	++	+	-	-	-
KoPr-126	+++	++	++	+	+	-
KoPr-127	+++	++	+	-	-	-
KoPr-128	+++	++	++	+	-	-
KoPr-129*	+++	++	++	++	+	+
KoPr-130	+++	++	+	-	-	-
KoPr-131*	+++	++	++	++	+	+
KoPr-132	+++	++	+	-	-	-
KoPr-133	+++	++	++	+	-	-
KoPr-134	+++	++	+	+	+	-
KoPr-135	+++	++	+	-	-	-
KoPr-136	+++	++	+	+	+	-

***Ceratoides eversmanniana* (Stschegl. ex I.G.Borshch.) Botsch. & Ikonn.**						
CREW-1001	+++	++	+	-	-	-
CREW-1002	+++	++	++	+	+	-
CREW-1003	+++	++	+	-	-	-
CREW-1004*	+++	+++	++	++	+	+
CREW-1005	+++	++	++	+	+	-
CREW-1006	+++	++	+	-	-	-
CREW-1007	+++	++	++	+	+	-
CREW-1008	+++	++	+	+	-	-
CREW-1009	+++	++	++	+	+	-
CREW-1010	+++	++	+	-	-	-
CREW-1011	+++	++	++	+	+	-
CREW-1012	+++	++	+	-	-	-
CREW-1013	+++	++	++	+	+	-
CREW-1014	+++	++	++	+	+	-
CREW-1015*	+++	+++	++	++	+	+
CREW-1016	+++	++	++	+	-	-
CREW-1017	+++	++	+	-	-	-
CREW-1018*	+++	+++	++	++	+	+
CREW-1019	+++	++	+	-	-	-
CREW-1020	+++	++	++	+	+	-
CREW-1021*	+++	+++	++	++	+	+
CREW-1022	+++	++	+	-	-	-
CREW-1023	+++	++	+	-	-	-
CREW-1024	+++	++	+	+	-	-
CREW-1025	+++	++	+	-	-	-
CREW-1026	+++	++	++	+	+	-
CREW-1027	+++	++	+	+	-	-
CREW-1028	+++	++	+	-	-	-
CREW-1029	+++	++	++	+	+	-
CREW-1030	+++	++	+	-	-	-
CREW-1031	+++	++	+	+	-	-
CREW-1032	+++	++	+	-	-	-
CREW-1033	+++	++	+	-	-	-
CREW-1034	+++	++	++	+	+	-

el, after cross comparison with the kown sequence in the genebank. It was confirmed that five promising isolates from *K. prostrata* are *Bacillus amyloliquefaciens, Bacillus pumilus, Priestia aryabhattai, Pseudomonas putida,* and *Priestia endophytica*. On the other hand, the four isolates from *C. eversmanniana* are confirmed to be *Priestia megaterium, Pseudomonas putida, Bacillus subtilis* and *Brevibacillus parabrevis* (Table 3, Fig. 3).

The genus of the identified promising bacterial isolates was reported to have endophytic relationship with xerophytic and halophytic plants. In particular, Alikulov *et al*. (27) found that halophytic plants such as *Haloxylon aphyllum, Halocnemum strobilaceum* and *Halostachys belangeriana* formed an endophytic relationship with *Bacillus, Pseudomonas* and *Priestia*. In another study aimed at determining the species composition of endophytic bacteria of *Sporobolus specatus* and *Cyperus laevigatus* plants, it was reported that endophytic bacteria such as *Agrobacterium taibaishanense, Pseudomonas alcaliphila, Pseudomonas toyotomiensis, Halomonas johnsoniae* and *Halomonas alkaliantarctica* were found in these plants (28).

When the fungicidal properties of endophytic bacteria isolated from *K. prostrata* and *C. eversmanniana* plants against pathogenic fungi were studied, it was found that almost all of the selected promising strains were resistant to the effects of fungi to a certain extent (Table 4,

Table 3. The effective plant growth-promoting endophytes isolated from xerophytic plants and their closest relatives from GenBank

Isolated strains deposited to GenBank			Closest match (16S rRNA genes) (GenBank)		
Strain	**Length (bp)**	**Accession number**	**Reference strains**	**Acc. number**	**Percent identity (%)**
Kochia prostrata **(L.) Schrad**					
KoPr101	1448	ON567219	*Bacillus amyloliquefaciens*	LN864483.1	99.72
KoPr113	1479	ON567220	*Bacillus pumilus*	AB212862.2	99.66
KoPr118	1467	ON567221	*Priestia aryabhattai*	MG757377.1	99.73
KoPr129	1484	ON567222	*Pseudomonas putida*	MW522571.1	99.73
KoPr131	1479	ON567223	*Priestia endophytica*	OM960594.1	99.59
Ceratoides eversmanniana **(Stschegl. ex I.G.Borshch.) Botsch. & Ikonn**					
CREW1004	1432	ON567363	*Priestia megaterium*	MZ379521.1	99.58
CREW 1015	1475	ON567362	*Pseudomonas putida*	AB621834.1	99.66
CREW 1018	1504	ON567361	*Bacillus subtilis*	LC178546.1	99.73
CREW 1021	1455	ON567360	*Brevibacillus parabrevis*	MN204065.1	99.59

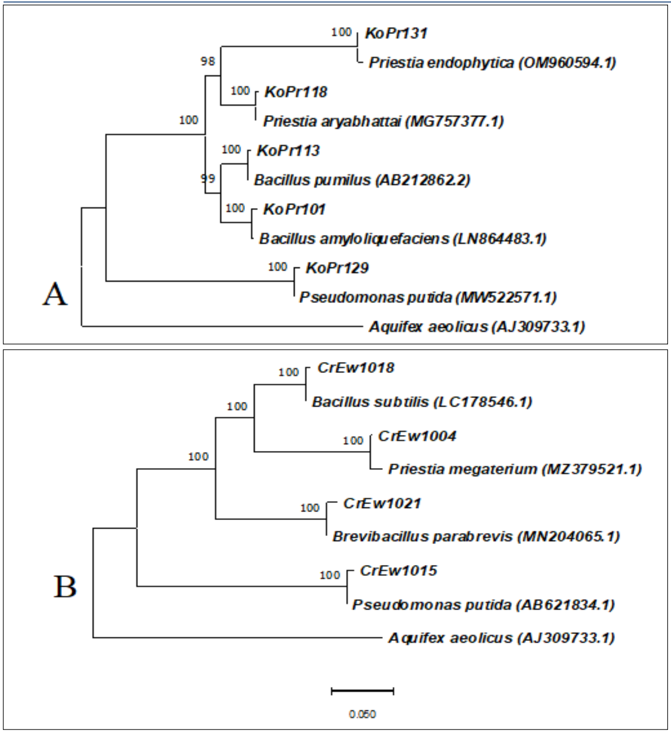

Fig. 3. Phylogenetic tree of endophytic bacteria from *Kochia prostrata* (L.) Schrad (**A**) and *Ceratoides eversmanniana* (Stschegl. ex I.G.Borshch.) Botsch.

Fig.4). Table 4 shows that *Bacillus amyloliquefaciens* (KoPr101) isolated from *K. prostrata* and *P. megaterium* (CREW1004) isolated from *C. eversmanniana* are the most resistant strains to pathogenic fungi. In the experiment, the zone of inhibition between *B. amyloliquefaciens* (KoPr101) strain against *R. solani* is 7±0.8 Ḇ Ḇ ṈBthe zone of inhibition against *F. oxysporum* and *A. alternata* is 9±0.5 Ḇ Ḇ B ÃÇẲB SUṂSFRB Ḇ Ḇ ṈB ĊẲĎĈẲẮḌĐẲĒÊFB ç̦B *P. megaterium* (CREW1004) strain isolated from *C. eversmanniana*, the inhibition zone was 9±0.7 mm against to *R. solani*, 15±1.0 Ḇ Ḇ Bagainst *F. oxysporum* and 16±1.0 mm against to *A. alternata*. Previous studies reported the endophytic bacteria from the genus *Bacillus* inhibit the growth of pathogenic fungi (29, 30). In addition, *Bacillus* species isolated *Prosopis strombulifera* showed protease activity and inhibited the fungus *Alternaria* sp., and reduce the damage caused by the phytopathogenic fungus up to 50% (31). In another study, a halotolerant strain *Pseudomonas* sp., isolated from *Suaeda salsa* halophyte, was found to inhibit the activity of phytopathogenic fungi *Fusarium oxysporum f. sp. cucumerinum* and *F. oxysporum f. sp. conglutinans* (32). The potential of halophilic bacteria in biological control may be related to their production of membrane-bound or extracellular hydrolytic

Table 4. Antifungal properties of isolated endophytic bacteria

Bacterial strains	Zone of fungal growth inhibition (mm)		
	R.solani	***F.oxysporum***	***A.alternata***
Kochia prostrata **(L.) Schrad**			
Bacillus amyloliquefaciens KoPr101	++	++	+++
Bacillus pumilus KoPr113	+	++	-
Priestia aryabhattai KoPr118	+	-	-
Pseudomonas putida KoPr129	+	++	+
Priestia endophytica KoPr131	-	+	-
Ceratoides eversmanniana (Stschegl. ex I.G.Borshch.) Botsch. & Ikonn.			
Priestia megaterium CREW1004	++	+++	+++
Pseudomonas putida CREW1015	+	+	-
Bacillus subtilis CREW1018	-	-	+
Isoptericola halotolerans CREW1021	+	-	-

Note: (-) – 0 mm; (+)-0-5mm; (++)-5-10mm; (+++)-10≤ mm

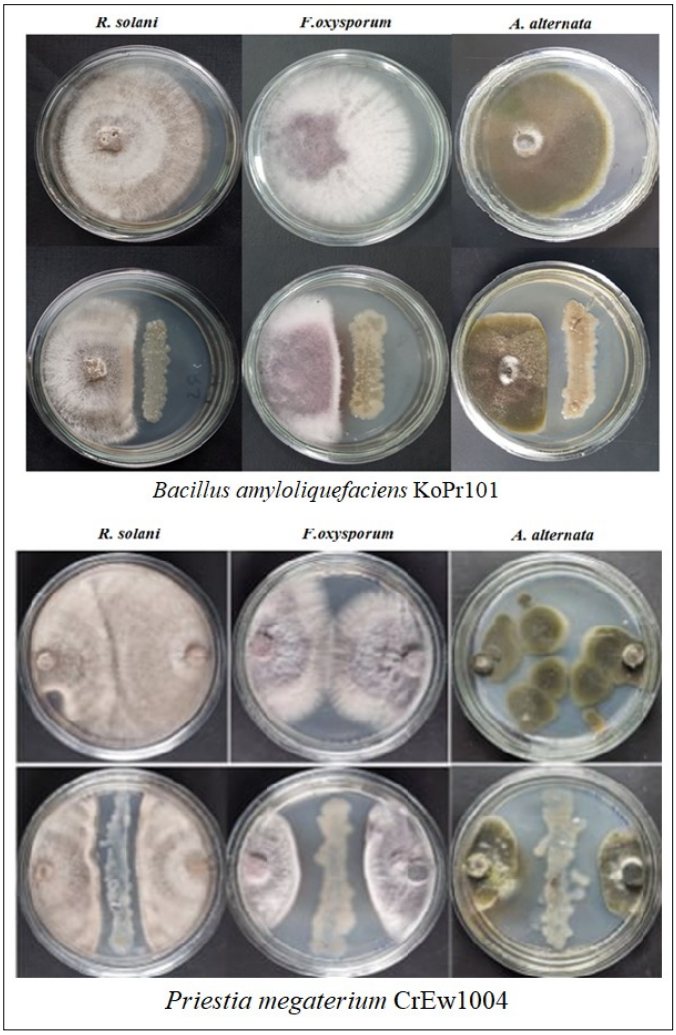

Fig. 4. Antifungal properties of active strains of endophytic bacteria isolated from xerophytic plants

Table 5. Plant growth-promoting properties of the isolated endophytes

Bacterial strains	N_2-fixation	IAA production	Phosphates solubilization	ACC deaminase production	Siderophores production
***Kochia prostrata* (L.) Schrad**					
Bacillus amyloliquefaciens KoPr101	+	+	+	+	+
Bacillus pumilus KoPr113	+	+	+	-	-
Priestia aryabhattai KoPr118	+	+	-	-	-
Pseudomonas putida KoPr129	+	+	-	+	+
Priestia endophytica KoPr131	-	+	-	+	-
Ceratoides eversmanniana (Stschegl. ex I.G.Borshch.) Botsch. & Ikonn.					
Priestia megaterium CrEw1004	+	+	+	+	+
Pseudomonas putida CrEw1015	+	+	+	-	-
Bacillus subtilis CrEw1018	+	-	-	+	+
Isoptericola halotolerans CrEw1021	+	-	-	-	-

enzymes. Although it is clear that antagonistic halotolerant endophytic bacteria can be an environmentally friendly alternative to fungicides, studies evaluating the antagonistic potential of halotolerant bacteria against plant pathogens and the effects of these pathogens on diseases under drought conditions do not lose their relevance (33).

In the current study, plant growth-promoting properties (N_2-fixation, IAA production, phosphates solubilization, ACC deaminase production, siderophores production) of promising strains of endophytic bacteria isolated from xerophytic plants *K. prostrata* and *C. eversmanniana* were determined (Table 5). Among the identified promising endophytic bacterial strains, *B. amyloliquefaciens* (KoPr101) and *P. megaterium* (CREW1004) show positive results to all the plant growth-promoting assays conducted. N_2 fixation by bacteria in the roots of xerophytic plants is an important process in providing nitrogen in saline soils. Data on the efficiency of biological nitrogen fixers for xerophytes increase interest in studying halotolerant N_2-fixing endophytic bacteria as potential sources of biofertilizers for saline soils (34). In previous studies, , halotolerant bacteria isolated from xerophytes also showed phosphate solubilizing activity. Screening of the rhizosphere of *Avicennia marina* identified 129 bacterial strains capable of solubilizing phosphate, of which *Oceanobacillus picturae* was shown to solubilize 97% of this mineral (35). Endophytic bacteria isolated from xerophytes and producing ACC deaminase were found to alleviate salinity stress and, to some extent, improve the growth of halophytes and salinity-tolerant crops. Such that, isolates of diazotrophic halotolerant bacteria such as *Brachybacterium saurashtrense, Brevibacterium casei, Cronobacter sakazakii, Haererehalobacter, Halomonas, Mesorhizobium, Pseudomonas, Rhizobium radiobacter, Vibrio* and *Zhihengliuella* isolated from the roots of *Salicornia brachiata* showed ACC deaminase activity. In addition, growth parameters were significantly increased after treatment with *Brachybacterium saurashtrense* and *Pseudomonas* in plants growing under drought conditions (36).

Conclusion

Based on the results, *K. prostrata* and *C. eversmannian* contains promising bacterial endophytes having resistance to high salinity. Molecular analysis confirmed that thse endophytic bacteria belong to genus *Bacillus*, *Priestia*, *Pseudomonas*, and *Brevibacillus*. Among the identified endophytes, *B. amyloliquefaciens* (KoPr101) and *P. megaterium* (CREW1004) have high inhibition capacity against plant pathogenic fungi, and shows positive plant growth-promoting properties. These endophytic bacteria can be

use as stimulants that can be use as growth-stimulants and biological control of fungal pathogens in crops in arid regions.

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Authors contributions

IA, SHA, BA and SM performed the experiments. BA and AE analyzed data. IA, BA and SHA statistically analyzed results. IA, BA and ZI wrote the draft of the manuscript. IT conducted the critical revision of the manuscript. ZI worked out the concept and design, supervised and funded the experiments. All authors read and approved the final manuscript.

Compliance with ethical standards

Conflict of interest: Authors do not have any conflict of interests to declare.

Ethical issues: None.

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