

Isolation and characterization of endophytic bacteria from some halophytes in saline desert regions of Uzbekistan

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Abstract. Alikulov B, Gulboev D, Maxammadieva D, Tillaeva Z, Olimjonova S, Ismailov Z. 2023. Isolation and characterization of endophytic bacteria from some halophytes in saline desert regions of Uzbekistan. *Biodiversitas* 24: 1279-1288. The present study described the experimental results of isolating, characterizing, and selecting promising endophytic bacteria strains from halophytic plants, such as *Haloxylon aphyllum*, *Halocnemum strobilaceum*, and *Halostachys belangeriana*. These common bacteria in the southwestern regions of Uzbekistan are recommended for practical applications. In the present experiments, the effect of treatment with 65 isolates of endophytic bacteria isolated from these plants on the germination of seeds of agricultural crops such as wheat, cotton, and cucumber was evaluated. The result shows 15 promising strains of endophytic-bacteria from halophytic plants were selected and identified using molecular genetic methods. Furthermore, for the first time, we researched the isolation of endophytic bacteria from *H. aphyllum*, *H. strobilaceum*, and *H. belangeriana* plants and studied their properties. The results are useful for efficiently cultivating agricultural crops in areas affected by stress factors.

Keywords: Cotton, cucumber, endophyte, *Halocnemum strobilaceum*, Halophyte, *Halostachys belangeriana*, *Haloxylon aphyllum*, seed, wheat

INTRODUCTION

Today, less than one billion hectares of the world's territory are under primary salinity, more than 77 million hectares are under secondary salinity, and 58% of the secondary salinity areas are irrigated areas (<https://wad.jrc.ec.europa.eu/> 2022). The expansion of areas affected by salinity leads to a decrease in the yield of cultivated crops, as a result of which the population's demand for food products is insufficiently met. This situation requires the development and application of effective biotechnologies based on innovative trends in increasing the resistance of agricultural crops to stress factors, including growth in salinity conditions. One urgent problem that needs to be determined is the extremophilic endophyte bacteria properties of halophyte plants, widespread in saline areas. Those bacteria properties stimulate the development of cultivated plants and evaluate their biotechnological potential.

Research is being conducted worldwide on microbial preparations based on the activity of plant microorganisms in agricultural regions affected by various stress factors. Therefore, developing competitively promising biotechnologies for practical application is important in researching endophytic bacteria of halophytic plants. Furthermore, those bacteria are widespread in saline areas but are not often used in economic sectors.

The specific communities of endophytic bacteria are found in the different tissues of plants. In addition to stimulating the growth of plants, it has been reported that

they are important in protecting against phytopathogenic microorganisms (Hassan 2017). Endophytic bacteria have several advantages over rhizosphere bacteria. When they enter the tissues, they are in direct contact with the plant, resulting in easy communication between cells, directly benefiting the host plant (Jayakumar et al. 2020).

Foreign and local scientists have investigated the endophytic bacteria of various plants and their properties that promote plant development. However, information about the endophytic bacteria of some halophytic plants widely distributed in the desert regions of Uzbekistan is rarely found in scientific sources. Among such plants, *Haloxylon aphyllum* (Minkw.) Iljin, *Halostachys belangeriana* (Moq) Botsch and *Halocnemum strobilaceum* (Pall) Bieb can be included (Alikulov et al. 2022; Shurigin et al. 2022).

Haloxylon aphyllum is one of the common plants in the deserts of Uzbekistan, Kazakhstan, and Turkmenistan. It creates biomass in the ecosystem, stabilizes the movement of sand, maintenance of soil layer and specific microclimate, and growth and development of related plants. It also performs important tasks such as creating animal living conditions and supporting permanent microbial communities in the root system and endophytic microorganisms living in plant tissues. In addition, *H. aphyllum* forests are a source of food for farm animals and valuable fuel for the local population (Shuyskaya et al. 2009).

Halostachys belangeriana is widespread in the saline areas of the deserts of China and Central Asia (Alikulov et

al. 2022). In desert regions, it is used for livestock feed with good nutritional properties. In addition, the antimicrobial and antioxidant properties of the plant have been noted by experts. Yang et al. (2019) evaluated the antimicrobial activity of the ethyl acetate fraction of the ethanol extract from the stem of *H. belangeriana* (Moq) Botsch.

The plant is a halophyte distributed in the deserts of northern Africa and western Asia (Jurakulov et al. 2023). Acheuk et al. (2018) reported the potential of the crude extract of this plant as an insecticide against harmful insects, the plant composition being rich in saponins, tannins, flavonoids, anthocyanins, and alkaloids. Similarly, Handoussa et al. (2019) proved that natural antioxidants from *H. strobilaceum* possessed anticancer activity. It is also noted that *H. strobilaceum* may be a promising phytoremediation species due to its high adaptability to live in contaminated soil, which could stabilize and accumulate metals in its tissues (Bobtana et al. 2019).

Based on these observations, an association of endophytic bacteria with *H. aphyllum*, *H. belangeriana*, and *H. strobilaceum* plants should be explored to evaluate their great scientific and practical importance.

MATERIALS AND METHODS

Plant collection

The collection of halophytic plant samples was carried out in the springs of 2019 and 2020 based on segments isolated from the roots and stems of *H. aphyllum*, *H. belangeriana*, and *H. strobilaceum* plants (Figure 2). Moreover, these plants are common in the saline bogs of the southwestern regions of Uzbekistan, including Kyzylkum and Karnobchol (Figure 1). First, samples were taken from plants growing at a distance of not less than 10 m, for this study. Next, the roots and stem were cleaned in sterile water to remove soil particles.

The endophytic bacterial isolation

A total of 15 g stem and roots were sterilized in beakers filled with 99.9% ethanol for 2 minutes and 10% sodium hypochlorite for 1 minute. Afterward, they were placed in sterile water cups for 2 minutes (Coombs et al. 2003). Pieces of stem and 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 Tryptic Soy Agar (TSA) nutrient medium (Kuklinsky-Sobral et al. 2004 at 30°C. After four days, the colonies that changed in color and shape were transferred to petri dishes with TSA for purification.

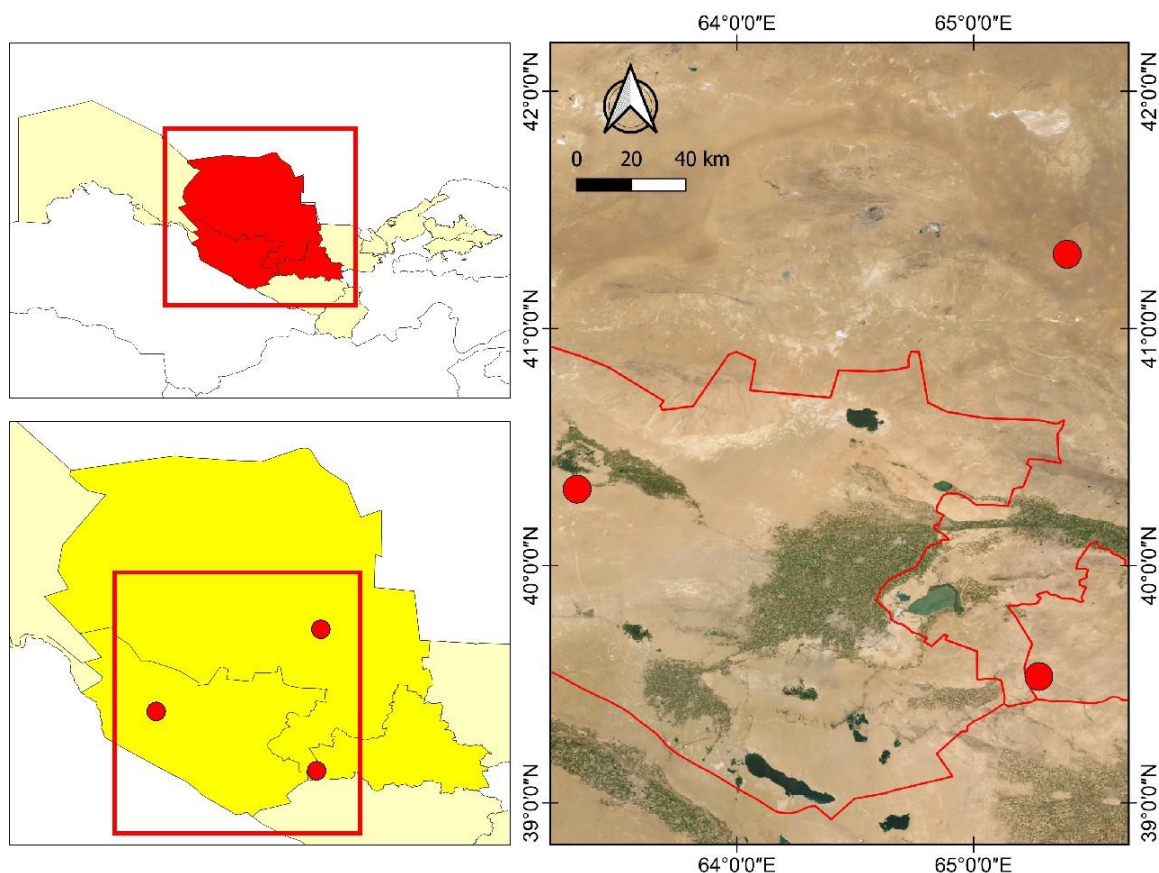


Figure 1. Locations where samples were taken for research in Uzbekistan, including Kyzylkum and Karnobchol

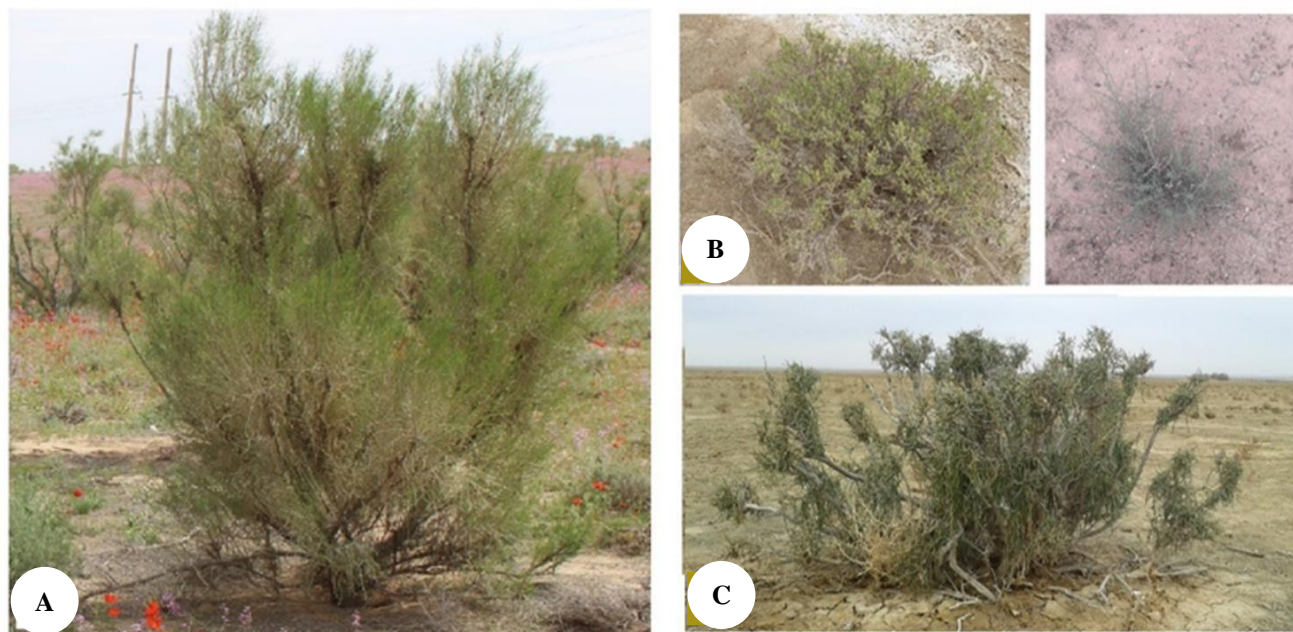


Figure 2. Research objects. A. *Haloxylon aphyllum* (Minkw.) Iljin, B. *Halocnemum strobilaceum* (Pall) Bieb, C. *Halostachys belangeriana* (Moq) Botsch)

Selection of promising strains of halophytic endophytic bacteria

The effect of treatment with bacterial isolates was evaluated on wheat, cotton and cucumber seed germination to isolate promising strains among the isolated isolates. The seeds were collected between 5-35°C in 5°C intervals to determine the effect of temperature on seed germination of selected crops. The seeds' germination level was checked on the 4th and 7th days.

The isolated endophytic bacterial strains were grown individually in nutrient broth at 30°C for 96 hours, and the cell concentration was brought up to 10⁸ CFU/ml. Plant (cotton, wheat and cucumber) seeds were surface sterilized by soaking in sodium hypochlorite (2%) for 3 minutes and then washing with sterile water (Egamberdieva et al. 2017). Sterile seeds were inoculated with bacteria (10 seeds of cotton, 20 seeds of wheat, and 20 seeds of cucumber for each isolate) by soaking in bacterial suspension and transferring to sterile petri dishes with moist filter paper for germination. Furthermore, to determine the time and concentration dependence of seed germination on bacterial suspension treatment, seeds were treated with bacterial suspensions at concentrations ranging from 0 to 30% for 6, 12, 18, and 24 hours before being placed in a thermostat set to 25°C. Petri dishes were kept in a refrigerator at 25°C. The seeds' germination level was observed on the 4th and 7th days.

Bacteria identification

The genomic DNA used for molecular identification was extracted as per the method given by Dashti et al. (2009). 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) (<http://www.ncbi.nlm.nih.gov/>) (Alikulov et al. 2022).

Antifungal activity of bacteria

The pathogenic fungi, namely, *Fusarium solani*, *Fusarium oxysporum*, and *Rhizoctonia solani*, used in antifungal studies were collected 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 passed to test plates in the same medium as the fungi. The plates were incubated at 28°C for seven 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.

RESULTS AND DISCUSSION

More than 700 segments of stem and roots of common halophytes were studied in the present study. A total of 65 isolates of endophytic bacteria were found growing on the surface of the nutrient medium, of which 20 isolates (HAPH1- HAPH20) were from *H. aphyllum*, 25 isolates (SSU1- SSU25) from *H. belangeriana* and 20 isolates (HAST1-HAST20) were isolated from *H. strobilaceum* (Table 1).

The effect of treatment with bacterial isolates was evaluated on wheat, cotton, and cucumber seed germination to isolate promising strains among the isolated isolates. Initially, the optimal temperature for germination of the seeds of selected crops was determined on the seeds collected at 5-35°C in variants of the 5°C interval. The results showed that the temperature had a certain effect on the germination of wheat, cotton, and cucumber seeds, i.e., the germination level of seeds increased up to 25-30°C (Figure 3). The level of germination at 5°C is equal to 55% in wheat, 30% in cotton and 25% in cucumber. In contrast, at 25°C, it is 90%, 80%, and 95%, respectively. The seeds germination rate at 30°C is reduced by 5-10% compared to 25°C; this temperature (25°C) was chosen as the optimal temperature for the seeds germination of this research subject.

During our research, experiments were conducted to determine the optimal concentration and processing time of bacterial isolates. The seeds were collected in a thermostat at a temperature of 25°C after being treated with 0-30% bacterial suspensions for 6, 12, 18, and 24 hours (Figure 4). Fertilization is high in seeds treated for 12 hrs. at all concentrations of the bacterial suspension. Fertilization of seeds was equal to 80% in wheat, 70% in cotton, and 90% in cucumber after 12 hrs. of treatment with a 5% bacterial suspension. These indicators did not differ from the control (untreated). When treated for 12 hours with a 20% concentration of bacterial suspension, seeds germination was 95% in wheat, 90% in cotton, and 100% in cucumber. The parameters were improved by 15-20% in comparison to the control. When treated with bacterial suspension at 25% concentration, a decrease in fertility was noted in all variants as compared to variants treated with bacterial suspension at 20% concentration. Therefore, in our research, 12 hours of treatment with a 20% concentration suspension of bacterial isolates was chosen as the optimal condition, and further experiments were conducted under these conditions.

The effect of 12 hrs. treatment with 20% concentration suspensions of 65 isolates of endophytic bacteria isolated from halophytic plants on the seed germination of agricultural crops, namely wheat, cotton, and cucumber, was determined (in Figures. 5-6). It was observed that 6 of the 20 isolated isolates in wheat plants (HAPH2, HAPH8, HAPH12, HAPH13, HAPH15, HAPH16) and 6 in cotton (HAPH2, HAPH8, HAPH12, HAPH14, HAPH15,

HAPH16) in 80% (16) of bacterial isolates, the germination of wheat, cotton, and cucumber seeds was found to be higher than 90%. Treatment with *H. aphyllum* endophytic bacterial isolates HAPH2, HAPH8, HAPH12, HAPH15, and HAPH16 ensured 100% seed germination across all research sites.

During determining the effect of treatment with isolates of *H. strobilaceum* endophytic bacteria on the germination of seeds of agricultural crops (Figure 4.B), 7 out of 20 isolates were isolated in wheat plants (HAST2, HAST6, HAST7, HAST9, HAST10, HAST13, HAST17), 8 in cotton (HAST1, HAST2, HAST7, HAST9, HAST10, HAST11, HAST14, HAST17) and nine cucumbers (HAST2, HAST5, HAST7, HAST9, HAST10, HAST13, HAST16, HAST17, HAST18) showed 100% seed germination. In 85% (17) of the bacterial isolates, the germination of wheat, cotton, and cucumber seeds was found to be higher than 90%. Treatment with the endophytic bacteria *H. strobilaceum* HAST2, HAST7, HAST9, HAST10, and HAST17 was proven to ensure 100% seed germination in all research sites. Treatment of wheat, cotton, and cucumber seeds with bacterial isolates improved germination in all variants compared to the control variant.

As a result of the effect of treatment with isolates of *H. belangeriana* endophytic bacteria on the germination of seeds of agricultural crops (Figure 4.C), 7 out of 25 isolates were isolated from wheat plants (SSU2, SSU4, SSU7, SSU11, SSU16, SSU18, SSU21), six from cotton. (SSU4, SSU7, SSU11, SSU16, SSU18, SSU21) and seven cucumbers (SSU1, SSU4, SSU7, SSU12, SSU16, SSU18, SSU21) had 100% seed germination. In 72% (18) of the bacterial isolates, the germination of wheat, cotton, and cucumber seeds was found to be higher than 90%. In experiments, when treated with isolates of *H. belangeriana* endophyte bacteria SSU4, SSU7, SSU16, SSU18, and SSU21, seed germination was equal to 100% in all cases.

Moreover, it was also observed that endophytic bacteria of some halophytic plants stimulate the germination and development of agricultural crop seeds. Therefore, it is also necessary to identify HAPH2, HAPH8, HAPH12, HAPH15, and HAPH16 isolates of *H. aphyllum* endophyte bacteria, HAST2, HAST7, HAST9, HAST10, HAST17 isolates of *H. strobilaceum* endophyte bacteria and SSU4, SSU7, SSU16, SSU18, SSU21 isolates of *H. belangeriana* endophyte bacteria as the promising strains.

The 16S rRNA nucleotide sequences of 15 promising strains of endophytic bacteria isolated from halophytes were deposited in the National Center for Biotechnology Information (NCBI) database: MZ443975.1, MZ443981.1, MZ443985.1, MZ443988.1, MZ443989.1 (*H. aphyllum* (Minkw.) Iljin), OK594050.1, OK594051.1, OK594052.1, OK594053.1, OK594054.1 (*H. strobilaceum* (Pall) Bieb) and OK559720.1, OK559721.1, OK559722.1, OK559723.1, OK559724.1 (*H. belangeriana* (Moq) Botsch) listed with (<http://www.ncbi.nlm.nih.gov/>).

Based on molecular characterization, it was found that there are 13 types of endophytic bacteria belonging to three classes, four families, and five genera (Table 2). When

analyzing the taxonomic units of the studied endophytic bacterial species, 62% of the detected species (eight types) belonged to the *Bacillus* class. These species showed that this class's representatives dominate the endophytes of halophytic plants. Also, 31% of the identified species (four types) belonged to the Proteobacteria class and 7% (one type) to the Actinobacteria class. *Bacillales* (five types) and *Pseudomonadales* (four types) were discovered to be the dominant groups in the endophytic bacteria of the investigated halophytic plants. On the other hand, in the taxonomic unit of the family, 54% of the identified species (seven types) belonged to the *Bacillaceae* family. That shows representatives of this family dominate among the endophytes of halophytic plants. Endophytic bacteria identified in the studied halophyte plants belong to five genera: *Bacillus* (five types), *Pseudomonas* (four types), and *Priestia* (two types) dominate among the genera (Figure 7).

The antifungal activity of selected promising strains of halophytic endophytic bacteria was determined. *Bacillus amyloliquefaciens* HAPH2, *Bacillus pumilus* SSU-4, and *Pseudomonas chlororaphis* HAST-17 were observed

among the selected prospective strains to have higher antifungal activity against *Fusarium solani*, *Fusarium oxysporum*, and *Rhizoctonia solani* than other strains (Table 3).

Plant growth and production are substantially affected by numerous environmental factors. Salinity and drought are two major abiotic stresses encountered by plants. The application of plant endophytic bacteria to increase plant growth and abiotic stress tolerance has become a less time-consuming approach than traditional breeding and genetic engineering of abiotic stress-tolerant crop varieties (Hwang et al. 2022). Furthermore, isolation, identification, and the study of endophytes from halophytic plants could be essential for developing bioinoculants to facilitate salt soil phytoremediation. That would be beneficial for mitigating the stress on the plants growing in such salt-affected habitats (Chebotar et al. 2022). On the other hand, endophytic bacteria of halophytic plants common in Ethiopian rift valley lakes (Enquahone et al. 2022), Saudi Arabia (Bibi et al. 2017), and the coastal shoal of Nantong (Bian et al. 2011) resulted in an exhibit of high biodiversity of endophytic bacteria.

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

Plant	Geographical coordinates of sample collection areas	Root		Shoot		Total	
		A	B	A	B	A	B
<i>Haloxylon aphyllum</i> (Minkw.) Iljin	40°-82'50.3"N, 64°-81'82.7"E 41°-04'14.4"N, 64°-32'29.0"E 40°-20'14.0"N, 63°-21'06.7"E	178	13	144	7	322	20
<i>Halocnemum strobilaceum</i> (Pall) Bieb	39°-31'45.5"N, 65°-16'41.6"E 41°-04'14.4"N, 64°-32'29.0"E	115	11	101	9	216	20
<i>Halostachys belangeriana</i> (Moq) Botsch	41°-04'14.4"N, 64°-32'29.0"E 41°-03'45.9"N 64°-54'03.3"E	102	14	80	11	182	25
Total		395	38	325	27	720	65

Note: *A- Segment, piece; B-Isolation, piece.

Table 2. The effective plant growth-promoting endophytes isolated from halophytic 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
<i>Haloxylon aphyllum</i> (Minkw.) Iljin					
HAPH2	1465	MZ443975	<i>Bacillus amyloliquefaciens</i>	LN864483.1	99.79
HAPH8	1411	MZ443981	<i>Bacillus toyonensis</i>	MK424259.1	99.79
HAPH12	1456	MZ443985	<i>IsotERICOLA halotolerans</i>	AB489222.1	99.66
HAPH15	1454	MZ443988	<i>Planomicrobium soli</i>	NR_134133.1	99.72
HAPH16	1466	MZ443989	<i>Pseudomonas kilonensis</i>	LN995719.1	99.59
<i>Halocnemum strobilaceum</i> (Pall) Bieb					
SSU-4	1500	OK559720	<i>Bacillus pumilus</i>	MN750426.1	99.73
SSU-7	1478	OK559721	<i>Bacillus endophyticus</i>	KR085883.1	99.73
SSU-16	1543	OK559722	<i>Bacillus subtilis</i>	MT491101.1	99.74
SSU-18	1469	OK559723	<i>IsotERICOLA halotolerans</i>	AB489222.1	99.66
SSU-21	1480	OK559724	<i>Pseudomonas kilonensis</i>	LN995719.1	99.8
<i>Halostachys belangeriana</i> (Moq) Botsch					
HAST-2	1467	OK594050	<i>Priestia megaterium</i>	KY660610.1	99.93
HAST-7	1485	OK594051	<i>Priestia aryabhatai</i>	KU179345.1	99.87
HAST-9	1427	OK594052	<i>Pseudomonas plecoglossicida</i>	MH165359.1	99.79
HAST-10	1488	OK594053	<i>Pseudomonas putida</i>	MK680517.1	99.87
HAST-17	1478	OK594054	<i>Pseudomonas chlororaphis</i>	GU947817.1	99.73

Table 3. Antifungal properties of isolated endophytic bacteria

Bacterial strains	Zone of fungal growth inhibition (mm)		
	<i>F.solani</i>	<i>F.oxysporum</i>	<i>R.solani</i>
<i>Haloxylon aphyllum</i> (Minkw.) Iljin			
<i>Bacillus amyloliquefaciens</i> HAPH2	9±1	8±1	11±1
<i>Bacillus toyonensis</i> HAPH8	4±1	5±1	4±1
<i>Isoptericola halotolerans</i> HAPH12	0	0	0
<i>Planomicrobium soli</i> HAPH15	0	0	0
<i>Pseudomonas kilonensis</i> HAPH16	12±1	12±1	10±1
<i>Halostachys belangeriana</i> (Moq) Botsch			
<i>Bacillus pumilus</i> SSU-4	10±1	11±1	9±1
<i>Bacillus endophyticus</i> SSU-7	0	0	0
<i>Bacillus subtilis</i> SSU-16	4±1	5±1	0
<i>Isoptericola halotolerans</i> SSU-18	8±1	10±1	8±1
<i>Pseudomonas kilonensis</i> SSU-21	6±1	6±1	4±1
<i>Halocnemum strobilaceum</i> (Pall) Bieb			
<i>Priestia megaterium</i> HAST-2	3±1	4±1	4±1
<i>Priestia aryabhatai</i> HAST-7	11±1	9±1	7±1
<i>Pseudomonas plecoglossicida</i> HAST-9	0	0	0
<i>Pseudomonas putida</i> HAST-10	4±1	5±1	4±1
<i>Pseudomonas chlororaphis</i> HAST-17	9±1	8±1	12±1

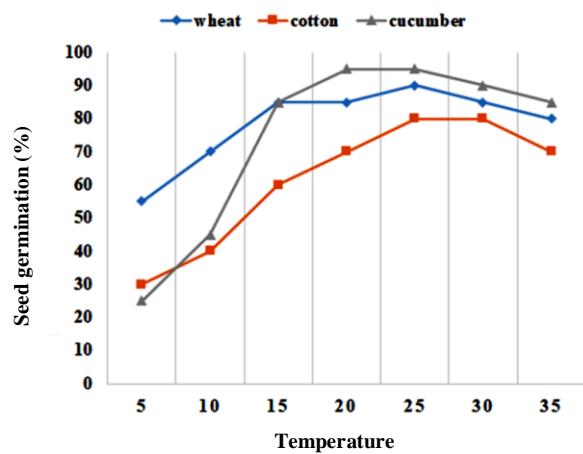


Figure 3. Effect of temperature on seed germination of some agricultural crops

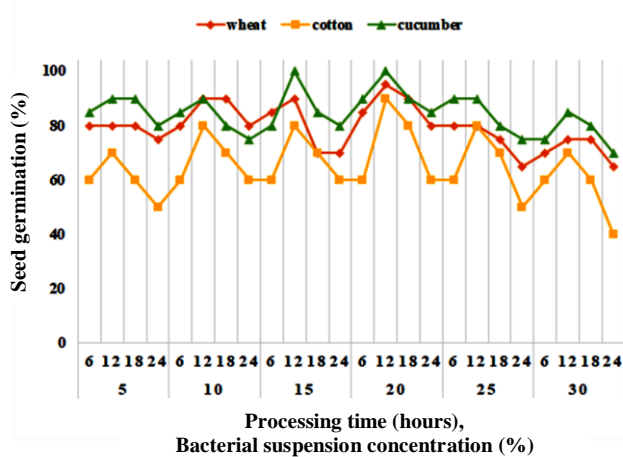


Figure 4. Dependence of the germination of seeds of some agricultural crops on the time and concentration of treatment with bacterial suspensions

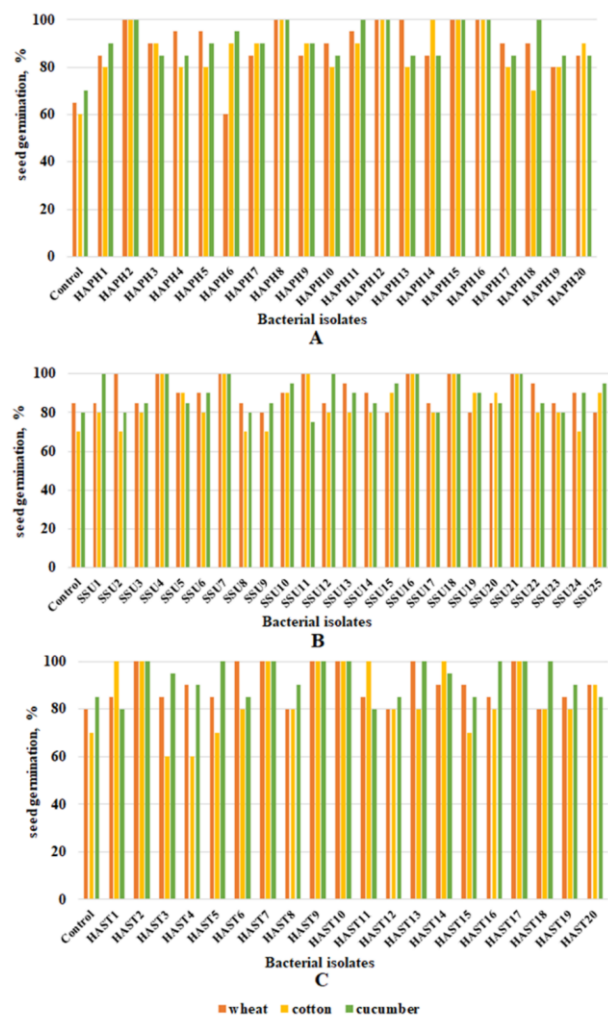


Figure 5. Effects of treatment with endophytic bacterial isolates isolated from *Haloxylon aphyllum* (Minkw.) Iljin (A), *Halostachys belangeriana* (Moq) Botsch (B) and *Halocnemum strobilaceum* (Pall) Bieb (C) on seed germination of some agricultural crops

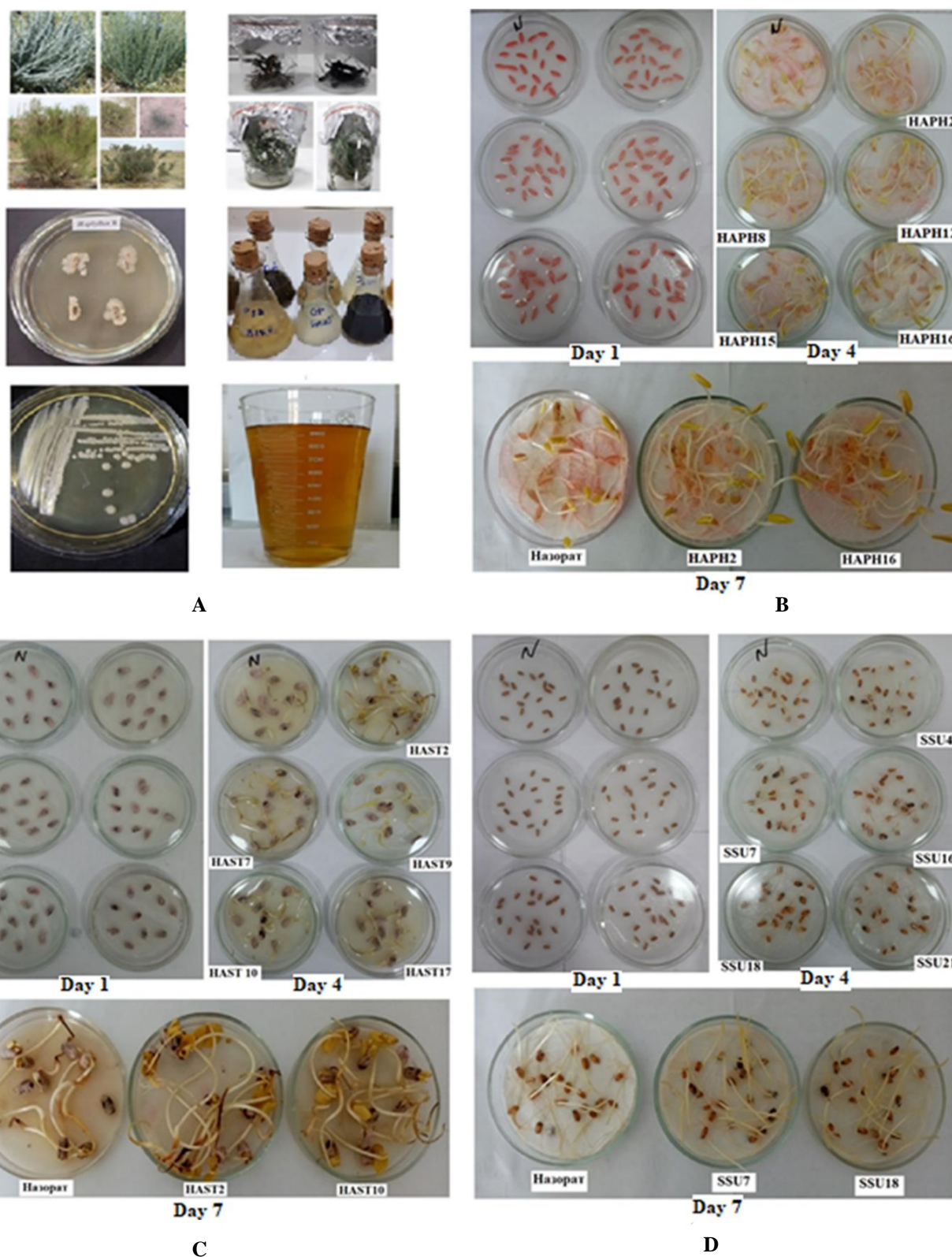


Figure 6. A. Seeds of some agricultural crops treated with endophytic bacterial isolates isolated from halophytic plants. B. Treatment of cucumber seeds with endophytic bacteria isolates of *Haloxylon aphyllum* (Minkw.) Iljin; C. Treatment of cotton seed with endophytic bacterial isolates of *Halocnemum strobilaceum* (Pall) Bieb; D. Wheat seeds with endophyte of *Halostachys belangeriana* (Moq) Botsch treatment with bacterial isolates) effect on fertility

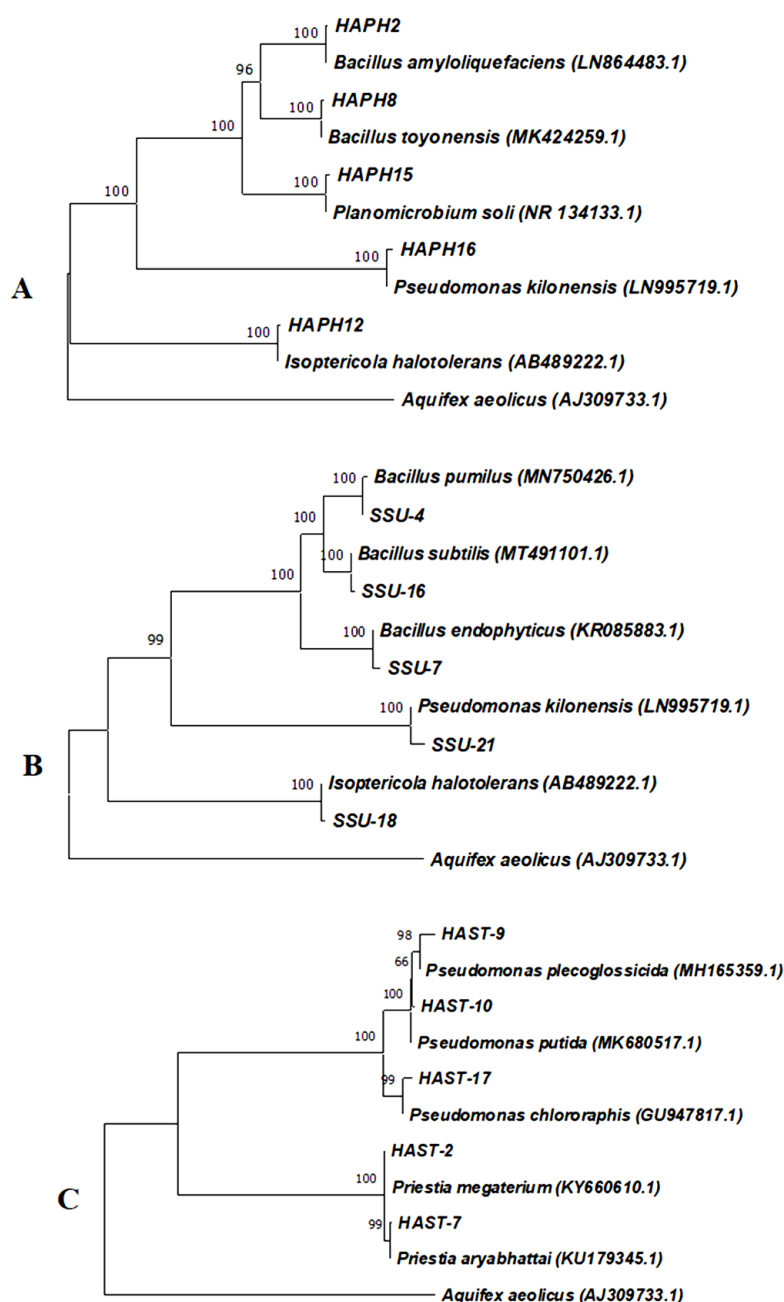


Figure 7. Phylogenetic tree of endophytic bacteria from *Haloxylon aphyllum* (Minkw.) Iljin (A), *Halostachys belangeriana* (Moq) Botsch (B), and *Halocnemum strobilaceum* (Pall) Bieb (C) with the closest relatives registered in GenBank

Numerous studies have shown that halotolerant endophytic bacteria and PGPR effectively improve the growth of various crops under saline conditions (Goswami et al. 2014; Ji et al. 2014; Kim et al. 2014; Kaushal et al. 2016). Inoculating crops with halotolerant PGPR and endophytic bacteria isolated from halophytes successfully improved crop growth and tolerance under salt stress conditions. Halotolerant PGPR and endophytic bacteria can benefit plants, helping halophytes and glycophytes overcome salt stress. For example, salt-tolerant PGPRs isolated from the rhizosphere soil of the halophytes

Haloxylon salicornicum, *Lespedeza bicolor*, *Atriplex leucoclada*, *Suaeda fruticosa*, and *Salicornia virginica* are also enhanced the growth of saline-exposed maize (Ullah et al. 2015). These plants showed accumulation of osmolytes (e.g., sugar and proline) and increased activity of antioxidant enzymes (e.g., superoxide dismutase, peroxidase, catalase, and ascorbate peroxidase) compared to uninoculated plants. Similarly, studies by Siddikee et al. (2010) showed a significant increase in growth after inoculation of canola seedlings with halotolerant bacterial isolates isolated from halophyte plants under salt stress on

gnotobiotic conditions. The resulting study was evidenced by a 35-43% increase in dry weight and an increase in root length by 29-47%.

In several previous studies, endophytic fungi were isolated from common medicinal plants in Uzbekistan, and their secondary metabolites were characterized (Gulyamova et al. 2022). In addition, endophytic bacteria were isolated from *Iris pseudacorus* L. (Shurigin et al. 2022) and *Ajuga turkestanica* (Rgl.) Brig (Mamarasulov et al. 2022) and their biochemical-physiological, plant-stimulating properties were evaluated. The present study confirmed that some halophytes distributed in Uzbekistan saline bogs are a new endophytic bacteria source.

In conclusion, halophytes distributed in the saline desert areas of Uzbekistan (*H. aphyllum*, *H. belangeriana*, and *H. strobilaceum*) are a promising new source of endophytic bacteria. Molecular-genetic analyses showed that 13 species of endophytic bacteria belonging to three classes, four families, and five genera were found associated with these plants. *Bacillus amyloliquefaciens* HAPH2, *Bacillus pumilus* SSU-4, and *Pseudomonas chlororaphis* HAST-17 strains isolated halophytic plants could be used as stimulants for growing wheat, cotton, and cucumber in saline areas.

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