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Bacterial endophytes from halophyte black saxaul (*Haloxylon aphyllum* Minkw.) and their plant growth-promoting properties

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ABSTRACT

The purpose of the present research was to study the diversity of bacterial endophytes inhabiting the halophytic plant black saxaul (*Haloxylon aphyllum* Minkw.). A total of 20 bacterial isolates were isolated from tissues of black saxaul and identified based on their 16S rRNA genes analysis and comparison with the closest relatives registered in GenBank nucleotide data bank from the National Centre for Biotechnology Information. The endophytes were checked for plant growth-promoting activity toward cucumber plants and the strains *Bacillus amyloliquefaciens* HAPH2, *Priestia endophytica* HAPH5, *Bacillus subtilis* HAPH7, *Bacillus toyonensis* HAPH8, *Halomonas sulfidaeris* HAPH11, *Isoptericola halotolerans* HAPH12, *Planomicrobium soli* HAPH15, and *Pseudomonas kilonensis* HAPH16 demonstrated high plant growth-promoting activity of cucumber in four soil salinity levels (0, 25, 50, and 100 mM) after seeds' inoculation. These bacterial endophytes were able to fix nitrogen, solubilize phosphates, and produce indole-3-acetic acid, 1-aminocyclopropane-1-carboxylate deaminase, and siderophores which are considered as the main plant growth-promoting traits. After field experiments, the best plant growth-promoters can be used as bioinoculants for plants' growth improvement in salinity conditions.

1. INTRODUCTION

Black saxaul (*Haloxylon aphyllum* Minkw.) is one of the most well-known desert plants inhabiting the deserts of Uzbekistan, Kazakhstan, and Turkmenistan [1–3].

Saxaul forests perform the following functions in the ecosystem: biomass production, stabilization of sand movement, maintenance of soil layer and specific microclimate, ensuring the growth and development of associated plants (e.g., *Carex physodes* M. Bieb.), establishment of habitat conditions for animals, and support of permanent microbial communities in the root system and endophytic microorganisms living in plant tissues. Saxaul forests are a source of food for farm animals and a valuable fuel for the local population [4–7].

The tissues of various plants have their own unique communities of bacterial endophytes. There are many reports showing that endophytes play an important role in plant growth stimulation and their protection from phytopathogenic microorganisms [8–13]. Bacterial endophytes have advantages over the bacteria inhabiting the rhizosphere. Once inside the tissue, they have direct contact with the plant, and hence easy communication between cells can take place. Thus, they can have a direct beneficial effect on the host. In this process, compounds produced by bacteria directly affect the physiological activity of a host plant and can increase biomass production [14]. Bacteriogenic substances include indole-3-acetic acid (IAA) [15], siderophores [16], 1-aminocyclopropane-1-carboxylate (ACC) deaminase [17], and lytic enzymes, leading to plants beneficial processes such as phosphate solubilization [18], atmospheric nitrogen fixation [19], chelation of metal ions in an absorbable form [16], alleviation of stresses [14,20], and suppression of pathogenic microbiota [21].

Despite the fact that endophytic communities of various plants have been studied, there is still no information about bacterial

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endophytes living in black saxaul and their effect on it. So, this is the first report about bacterial endophytes of black saxaul.

The aim of this study was to isolate and identify bacterial endophytes from roots and stem of black saxaul, as well as check their plant beneficial traits.

2. MATERIALS AND METHODS

2.1. Black Saxaul Plants Collection

Five plants of black saxaul (*H. aphyllum* Minkw.) were carefully isolated from the soil of Kyzylkum Desert in Uzbekistan in springtime. The soil particles were eliminated from roots and shoots by washing them in sterile water.

2.2. Bacteria Isolation

The shoot and root were cut from each other and the pieces (15 g) were sterilized by putting them into glasses with 99.9% ethanol for 2 minutes and 10% sodium hypochlorite for 1 minute. After that, they were put into glasses with sterile water for 2 minutes [22]. The pieces of shoots and roots were longitudinally cut into thin slices. 5 g of each sample was transferred into tubes with 9 ml of sterile tap water for serial dilution (10^1–10^5). 100 µl of suspension from each dilution was transferred and spread on Tryptic Soy Agar. The plates were incubated in a thermostat at 30°C. In 4 days, the colonies changing in color and shape were transferred and streaked on plates with Tryptic Soy Agar for purification. The pure cultures were used for DNA isolation. We also checked the outer surface of the root and shoot pieces for sterility by putting them onto Tryptic Soy Agar (TSA) media and incubating for 4 days at 30°C. There were no colonies after incubation.

2.3. Bacteria Identification

The method of Dashti *et al.* [23] was used for bacterial DNA isolation. The bacterial colonies were transferred into Eppendorf tubes with 1 ml of Milli-Q water. The colonies were mixed with water by shaking in hand for 1 minute and incubated at 90°C for 20 minutes in a dry block heater. After that, they were centrifuged at 12,000 rpm for 5 minutes. The isolated DNA was visualized using gel electrophoresis.

The extracted DNA was exposed to 16S rRNA gene analysis by means of Polymerase chain reaction (PCR) using the following primers: 27F 5′-GAGTTTGATCCTGGCTCAG-3′ (Sigma-Aldrich, St. Louis, MO) and 1492R 5′-GAAAGGAGGTGATCCAGCC-3′ (Sigma-Aldrich, St. Louis, MO) [24]. The PCR program was as follows: a primary heating step for 30 seconds at 94°C, followed by 30 cycles of denaturation for 15 seconds at 94°C, annealing for 30 seconds at 55°C, and extension for 1.5 minutes at 68°C, then followed by the final step for 20 minutes 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 GenBank of the National Centre for Biotechnology Information (NCBI) (http://www.ncbi.nlm.nih.gov/).

The evolutionary history was inferred using the neighbor-joining method [25]. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (500 replicates) is shown above the branches [26]. 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 [27] and are in the units of the number of base substitutions per site. This analysis involved 41 nucleotide sequences. All ambiguous positions were removed for each sequence pair (pairwise deletion option). There were a total of 1,648 positions in the final dataset. Evolutionary analyses were conducted in MEGA X [28].

2.4. Test for Plant Growth Promotion by Bacterial Endophytes

Isolated bacterial endophytes were cultivated in a nutrient broth medium for 96 hours at 30°C and cells concentration was adjusted up to 10^8 CFU/ml. The seeds of a cucumber (genotype Orzu) were inoculated with bacteria by soaking in bacterial suspension and sown into 500 ml plastic pots containing 400 ml of soil by volume. We specially prepared four types of soil salinity with NaCl, 0, 25, 50, and 100 mM, by watering with water containing the appropriate NaCl concentration. All pots were set up randomly in five replications for each bacterial strain. Three seeds were sown into each pot. As a control, we used seeds without any inoculation. Plants were grown at 28°C–30°C during the day and 18°C–20°C at night, and after 14 days, the shoots and roots' dry weight was measured.

2.5. Tests for Plant-Growth-Promoting Properties

2.5.1. IAA production test

The production of IAA was tested according to the method of Sarwar and Kremer [29]. The bacterial suspension was adjusted to 1 × 10^8 CFU/ml and added to flasks with 10% TSA [30] supplemented with 5 mmol/l^{-1} of L-tryptophan and cultivated at 30°C for 24 hours in the dark. The grown bacteria were centrifuged at 8,000 × g for 15 minutes and the 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 the supernatant and left at room temperature for 30 minutes in the dark. The appearance of pink color indicated the production of IAA. For the measurement of IAA, a spectrophotometer at 530 nm was used. Different concentrations of IAA solutions were used to construct a standard curve.

2.5.2. Phosphate solubilization test

The ability of endophytes to solubilize inorganic phosphate was tested according to Mehta and Nautiyal [31]. The bacteria were cultured on solid National Botanical Research Institute's phosphate growth medium (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, and agar 1.5. Plates with bacteria were incubated at 28°C for 96 days. The formation of colonies indicated the ability to use inorganic phosphate in the form of $Ca_3(PO_4)_2$ as a sole phosphate source.

2.5.3. 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, $MgSO_4 \cdot 7H_2O$ 0.2, $Na_2MoO_4 \cdot 2H_2O$ 0.002, sodium malate 5, agar 15, and pH 7.2–7.4, supplemented with 50 mg/l yeast extract. The plates were incubated at 30°C for 96 hours and the appearance of growth indicated the ability to fix N_2. The newly grown single colonies were streaked onto plates with the same medium to confirm the ability of nitrogen fixation [32].

2.5.4. Siderophores production test

Siderophore production was determined by using chrome azurol S (CAS) agar. Isolates were streaked onto CAS agar and incubated at 30°C for 96 days. The appearance of an orange halo around the bacterial colony indicated the production of siderophores [33].

2.5.5. ACC deaminase production test

The ACC deaminase production by bacteria was tested based on the utilization of ACC as a sole N-source. The endophytes were cultivated on basal medium supplemented with 3.0 mM of ACC. We used $(NH_4)_2SO_4$ as a positive control without adding N-source as a negative [34].

2.6. Statistical Analysis

The statistical significance of data was tested by the analysis of variance of the Microsoft Excel 2010 package. Mean comparisons were conducted using the least significant difference test (p = 0.05). The average values of plant growth parameters, IAA production, and the standard deviation were counted based on several replications.

2.7. Accession Numbers

The 16S rRNA gene sequences of the endophytic bacteria of *H. aphyllum* Minkw. were deposited into GenBank under the following accession numbers MZ443974–MZ443993.

3. RESULTS

3.1. Bacterial Endophytes' Identification and Phylogenetic Analysis

A total of 20 bacterial isolates related to different species were isolated from tissues of roots and shoots of black saxaul. The isolates were identified based on a comparison of their 16S rRNA with the closest relatives registered in GenBank. The degrees of their 16S rRNA genes similarities are shown in Table 1.

The percent of identity of 16S rRNA gene from black saxaul isolates and the closest relatives from GenBank ranged from 99.38 to 99.79. The isolates represent three phyla: Firmicutes, Actinobacteria, and Proteobacteria. The most numerous is Firmicutes with 13 representatives: HAPH2, HAPH3, HAPH4, HAPH5, HAPH6, HAPH7, HAPH8, HAPH9, HAPH10, HAPH14, HAPH15, HAPH19, and HAPH20. Among 20 isolates, only 4 were related to Actinobacteria (HAPH1, HAPH12, HAPH13, and HAPH17) and 3

Table 1: Endophytes isolated from black saxaul (*H. aphyllum* Minkw.) and their closest relatives from GenBank.

Isolated strains deposited to GenBank			Closest match (16S rRNA genes) (GenBank)		
Strain	Length (bp)	Accession number	Reference strains	Accession number	Percent identity
HAPH1	1481	MZ443974	*A. agilis*	FR682668.1	99.66
HAPH2	1465	MZ443975	*B. amyloliquefaciens*	LN864483.1	99.79
HAPH3	1415	MZ443976	*B. aryabhattai*	MN889284.1	99.79
HAPH4	1456	MZ443977	*B. cereus*	MT538265.1	99.66
HAPH5	1447	MZ443978	*Bacillus endophyticus*	KR233758.1	99.38
HAPH6	1486	MZ443979	*B. pumilus*	MN750426.1	99.60
HAPH7	1463	MZ443980	*B. subtilis*	MT491101.1	99.52
HAPH8	1411	MZ443981	*B. toyonensis*	MK424259.1	99.79
HAPH9	1415	MZ443982	*B. frigoritolerans*	LN997924.1	99.65
HAPH10	1415	MZ443983	*E. faecalis*	LT745973.1	99.72
HAPH11	1452	MZ443984	*H. sulfidaeris*	MW282893.1	99.59
HAPH12	1456	MZ443985	*I. halotolerans*	AB489222.1	99.66
HAPH13	1429	MZ443986	*K. polaris*	MW872360.1	99.51
HAPH14	1450	MZ443987	*P. salinarum*	MH311999.1	99.59
HAPH15	1454	MZ443988	*P. soli*	NR_134133.1	99.72
HAPH16	1466	MZ443989	*P. kilonensis*	LN995719.1	99.59
HAPH17	1425	MZ443990	*R. terrae*	MH311993.1	99.51
HAPH18	1446	MZ443991	*S. tamaricis*	MT192575.1	99.65
HAPH19	1430	MZ443992	*S. epidermidis*	MH118521.1	99.65
HAPH20	1440	MZ443993	*S. warneri*	MT642942.1	99.58

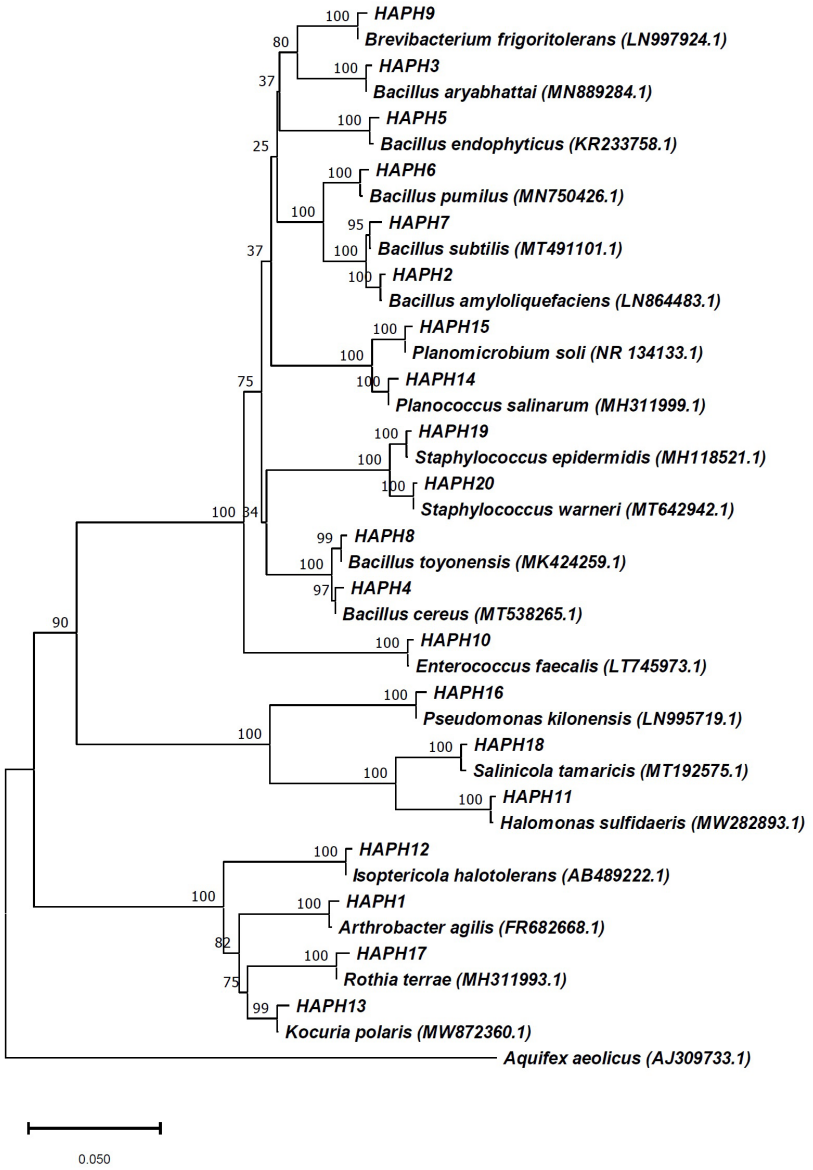

Figure 1: Phylogenetic tree of endophytic bacteria (HAPH1–HAPH20) of black saxaul with its closest relatives registered in GenBank of NCBI.

to Proteobacteria (HAPH11, HAPH16, and HAPH18). The isolates were divided into three classes: Bacilli (13 isolates), Actinobacteria (4 isolates), and Gammaproteobacteria (3 isolates). There were representatives of five orders: Bacillales (12), Micrococcales (4), Lactobacillales (1), Oceanospirillales (2), and Pseudomonadales (1). All isolates were related to 13 genera: *Bacillus* (HAPH9, HAPH4, HAPH6, HAPH7, HAPH8, and HAPH2), *Arthrobacter* (HAPH1), *Priestia* (HAPH3 and HAPH5), *Enterococcus* (HAPH10), *Halomonas* (HAPH11), *Isoptericola* (HAPH12), *Kocuria* (HAPH13), *Planococcus* (HAPH14), *Planomicrobium* (HAPH15), *Pseudomonas* (HAPH16), *Rothia* (HAPH17), *Salinicola* (HAPH18), and *Staphylococcus* (HAPH19 and HAPH20).

Based on 16S rRNA gene similarities using the neighbor-joining method, the phylogenetic tree was constructed (Fig. 1).

3.2. Plant Growth-Promotion Activity of Endophytic Bacteria

The strains of endophytic bacteria were tested for the ability to stimulate plant growth on the example of cucumber. The seeds of cucumber were inoculated with bacterial suspension and cultivated in pots. Although black saxaul is a halophyte, we decided to check whether its endophytes can benefit plants in salinity conditions. That is why for cucumber growing we used four types of soil salinization with NaCl: 0, 25, 50, and 100 mM. We measured shoot and root dry weight as the main growth parameter because the speed of a plant's dry biomass accumulation depends on the speed of cell division and its growth. The results are shown in Table 2.

It can be seen from Table 2 that the majority of endophytes stimulated plant growth of a cucumber less or more actively at different degrees of soil salinity. The strain *Bacillus amyloliquefaciens* HAPH2 appeared to be the most active plant

growth-promoting bacteria at 0 and 25 mM NaCl in soil. At 0 mM NaCl, the cucumber seeds' inoculation with this strain resulted in 17.9% and 18.8% increase in shoot and root dry weight as compared to the control, respectively. At 25 mM NaCl, the effect of this strain was even more and it increased shoot dry weight up to 22.3% and root dry weight up to 27.2% in comparison with the control. At 50 mM NaCl, the strain *B. amyloliquefaciens* HAPH2 increased cucumber plants shoot dry weight up to 38.1% and root dry weight up to 37.2% as compared to the control. At 100 mM NaCl, this strain raised the shoot dry weight up to 65.2% and root dry weight up to 216.7%. However, at 50 and 100 mM NaCl, the strain *B. amyloliquefaciens* HAPH2 was less effective than *Pseudomonas kilonensis* HAPH16. The seeds' inoculation with strain *P. kilonensis* HAPH16 resulted in 39.9% and 34.9% increase in shoot and root dry weight at 50 mM NaCl as compared to control, respectively. At 100 mM NaCl, *P. kilonensis* HAPH16 raised the shoot dry weight up to 68.3% and root dry weight up to 225%. It should be noted that strains *Arthrobacter agilis* HAPH1, *Bacillus cereus* HAPH4, *Bacillus pumilus* HAPH6, *Brevibacterium frigoritolerans* HAPH9, *Kocuria polaris* HAPH13, *Rothia terrae* HAPH17, *Staphylococcus epidermidis* HAPH19, and *Staphylococcus warneri* HAPH20 did not show or showed very low insignificant changes in shoot and root dry weight after seeds' inoculation.

3.3. Plant Growth-Promoting Traits of Endophytic Bacteria

The endophytic bacteria were analyzed for their plant growth-promoting characteristics: nitrogen fixation, phosphates solubilization, and production of IAA, ACC deaminase, and siderophores (Table 3).

It can be seen from Table 3 that, from 20 strains, only 5 possessed all checked plant growth-promoting characteristics: *B. amyloliquefaciens* HAPH2, *Priestia endophytica* HAPH5, *Bacillus subtilis* HAPH7, *Bacillus toyonensis* HAPH8, and *P. kilonensis* HAPH16. These strains showed N_2-fixation, phosphate solubilization, IAA, ACC, deaminase, and siderophores production. The highest amounts of produced IAA were observed in seven strains: *B. amyloliquefaciens* HAPH2 (172.12 µg/ml), *P. endophytica* HAPH5 (134.65 µg/ml), *B. subtilis* HAPH7 (163.86 µg/ml), *B. toyonensis* HAPH8 (119.03 µg/ml), *Isoptericola halotolerans* HAPH12 (123.35 µg/ml), *Planomicrobium soli*

Table 2: Effect of endophytic bacteria from black saxaul on the dry weight of cucumber (*Cucumis sativus*) (genotype Orzu) plants grown at different concentrations of NaCl after 14 days of pot experiment.

Bacterial strains	0 mM NaCl		25 mM NaCl		50 mM NaCl		100 mM NaCl	
	Shoot dry weight (g)	Root dry weight (g)	Shoot dry weight (g)	Root dry weight (g)	Shoot dry weight (g)	Root dry weight (g)	Shoot dry weight (g)	Root dry weight (g)
Control (without inoculation)	4.25 ± 0.31	1.17 ± 0.08	3.94 ± 0.30	1.03 ± 0.07	3.18 ± 0.21	0,86 ± 0.04	2.27 ± 0.18	0.36 ± 0.02
A. agilis HAPH1	4.26 ± 0.31	1.17 ± 0.08	3.95 ± 0.30	1.03 ± 0.07	3.18 ± 0.21	0.86 ± 0.04	2.27 ± 0.18	0.36 ± 0.02
B. amyloliquefaciens HAPH2	5.01 ± 0.42[a]	1.39 ± 0.1	4.82 ± 0.32	1.31 ± 0.09	4.39 ± 0.31	1.18 ± 0.08	3.75 ± 0.27	0.78 ± 0.04
P. aryabhattai HAPH3	4.37 ± 0.35	1.21 ± 0.09	4.26 ± 0.32	1.13 ± 0.08	3.82 ± 0.28	0.92 ± 0.05	2.58 ± 0.2	0.53 ± 0.03
B. cereus HAPH4	4.32 ± 0.34	1.19 ± 0.09	4.02 ± 0.31	1.08 ± 0.08	3.37 ± 0.25	0.89 ± 0.04	2.33 ± 0.18	0.37 ± 0.02
P. endophytica HAPH5	4.43 ± 0.36	1.28 ± 0.1	4.38 ± 0.31	1.17 ± 0.08	4.09 ± 0.3	0.96 ± 0.05	2.81 ± 0.19	0,58 ± 0.03
B. pumilus HAPH6	4.28 ± 0.31	1.17 ± 0.08	4.01 ± 0.32	1.09 ± 0.08	3.32 ± 0.24	0.87 ± 0.04	2.35 ± 0.18	0.39 ± 0.02
B. subtilis HAPH7	4.93 ± 0.39[a]	1.35 ± 0.1	4.68 ± 0.37	1.28 ± 0.09	4.17 ± 0.31	1.10 ± 0.07	2.66 ± 0.2	0.57 ± 0.03
B. toyonensis HAPH8	4.48 ± 0.37	1.29 ± 0.1	4.41 ± 0.31	1.20 ± 0.09	4.19 ± 0.31	1.13 ± 0.08	3.25 ± 0.23	0.75 ± 0.04
B. frigoritolerans HAPH9	4.25 ± 0.31	1.17 ± 0.08	3.94 ± 0.31	1.03 ± 0.07	3.18 ± 0.2`1	0.86 ± 0.04	2.27 ± 0.18	0.36 ± 0.02
E. faecalis HAPH10	4.38 ± 0.35	1.22 ± 0.1	4.10 ± 0.30	1.11 ± 0.08	3.56 ± 0.26	0.94 ± 0.05	2.31 ± 0.18	0.37 ± 0.02
H. sulfidaeris HAPH11	4.41 ± 0.37	1.26 ± 0.1	4.19 ± 0.31	1.14 ± 0.08	3.86 ± 0.29	0.95 ± 0.05	2.88 ± 0.19	0.61 ± 0.03
I. halotolerans HAPH12	4.43 ± 0.36	1.27 ± 0.1	4.31 ± 0.32	1.16 ± 0.08	4.07 ± 0.3	0.98 ± 0.05	3.12 ± 0.21	0.60 ± 0.03
K. polaris HAPH13	4.26 ± 0.31	1.18 ± 0.09	3.87 ± 0.29	0.82 ± 0.05	3.33 ± 0.23	0.79 ± 0.04	2.21 ± 0.18	0.34 ± 0.02
P. salinarum HAPH14	4.39 ± 0.32	1.23 ± 0.09	4.08 ± 0.31	1.10 ± 0.08	3.65 ± 0.26	0.94 ± 0.05	2.61 ± 0.19	0.56 ± 0.03
P. soli HAPH15	4.52 ± 0.37	1.32 ± 0.1	4.45 ± 0.37	1.23 ± 0.09	4.31 ± 0.32	1.09 ± 0.06	3.65 ± 0.24	0.70 ± 0.04
P. kilonensis HAPH16	4.73 ± 0.38	1.34 ± 0.1	4.61 ± 0.36	1.28 ± 0.1	4.45 ± 0.36	1.16 ± 0.07	3.82 ± 0.29	0.81 ± 0.05
R. terrae HAPH17	4.29 ± 0.31	1.18 ± 0.08	3.96 ± 0.30	0.83 ± 0.05	3.18 ± 0.21	0.76 ± 0.04	2.27 ± 0.17	0.49 ± 0.03
S. tamaricis HAPH18	4.38 ± 0.32	1.23 ± 0.09	4.17 ± 0.31	1.11 ± 0.08	3.76 ± 0.28	0.96 ± 0.05	2.64 ± 0.19	0.68 ± 0.03
S. epidermidis HAPH19	4.25 ± 0.30	1.17 ± 0.08	3.94 ± 0.3	1.03 ± 0.07	3.18 ± 0.21	0,86 ± 0.04	2.27 ± 0.18	0.36 ± 0.02
S. warneri HAPH20	4.32 ± 0.31	1.19 ± 0.08	3.99 ± 0.3	1.07 ± 0.07	3.49 ± 0.25	0.92 ± 0.05	2.31 ± 0.19	0.45 ± 0.03

[a] Statistically significant at $p \leq 0.05$.

Table 3: Plant growth-promoting properties of the isolated endophytes.

Bacterial strains	N_2-fixation	IAA (µg/ml)	Phosphates solubilization	ACC deaminase	Siderophores production
A. agilis HAPH1	−	18.74 ± 2.16	−	−	−
B. amyloliquefaciens HAPH2	+	172.12 ± 5.25[a]	+	+	+
P. aryabhattai HAPH3	−	26.85 ± 2.51	+	−	+
B. cereus HAPH4	−	21.23 ± 3.84	−	−	−
P. endophytica HAPH5	+	134.65 ± 5.11	+	+	+
B. pumilus HAPH6	−	17.17 ± 2.98	+	−	−
B. subtilis HAPH7	+	163.86 ± 4.73	+	+	+
B. toyonensis HAPH8	+	119.03 ± 5.82[a]	+	+	+
B. frigoritolerans HAPH9	−	31.56 ± 4.67	−	−	−
E. faecalis HAPH10	−	48.31 ± 3.73	−	−	−
H. sulfidaeris HAPH11	−	96.78 ± 4.29	+	−	+
I. halotolerans HAPH12	−	123.35 ± 4.58	+	+	−
K. polaris HAPH13	−	15.06 ± 2.17	−	−	−
P. salinarum HAPH14	−	65.91 ± 3.42	−	+	+
P. soli HAPH15	−	141.32 ± 4.63	+	+	−
P. kilonensis HAPH16	+	159.47 ± 4.24	+	+	+
R. terrae HAPH17	−	41.31 ± 3.11	−	−	−
S. tamaricis HAPH18	−	17.69 ± 3.85	−	−	−
S. epidermidis HAPH19	−	23.84 ± 2.59	−	−	−
S. warneri HAPH20	−	31.99 ± 3.14	+	−	+

[a] Statistically significant at $p \leq 0.05$.

HAPH15 (141.32 µg/ml), and *P. kilonensis* HAPH16 (159.47 µg/ml). The following eight strains did not have any of the checked traits and the IAA level was too low: *S. epidermidis* HAPH19, *B. cereus* HAPH4, *B. frigoritolerans* HAPH9, *Enterococcus faecalis* HAPH10, *K. polaris* HAPH13, *R. terrae* HAPH17, *Salinicola tamaricis* HAPH18, and *A. agilis* HAPH1. Four strains possessed three important characteristics: *P. soli* HAPH15, *I. halotolerans* HAPH12, *Planococcus salinarum* HAPH14, and *Halomonas sulfidaeris* HAPH11. The strains *Priestia aryabhattai* HAPH3 and *S. warneri* HAPH20 could solubilize phosphates and produce siderophores, but their IAA production was insignificant. The strain *B. pumilus* HAPH6 could only solubilize phosphate.

4. DISCUSSION

As a result of the conducted research, we isolated 20 different species of endophytic bacteria from black saxaul. These bacteria are related to 3 phyla, 3 classes, 5 orders, 8 families, and 13 genera. Some of them demonstrated high plant growth-promoting activity in the stimulation of a cucumber plant's growth. To explain the activity, we conducted tests for some important plant growth-promoting traits. There were many reports about plant growth-promoting properties of endophytic bacteria isolated from different plants [8,13,35].

In our research, the strains *B. amyloliquefaciens* HAPH2, *P. endophytica* HAPH5, *B. subtilis* HAPH7, *B. toyonensis* HAPH8, *H. sulfidaeris* HAPH11, *I. halotolerans* HAPH12, *P. soli* HAPH15, and *P. kilonensis* HAPH16 showed the highest plant growth-promoting activity. Gamez *et al.* [36] reported on *B. amyloliquefaciens* Bs006 which was selected after screening as one of the best plant growth-promoting rhizobacteria. The strain promoted banana growth on the same level as chemical fertilization and was proposed to be used as a formulation of a new biofertilizer. Park *et al.* [37] reported on *Bacillus aryabhattai* SRB02 (now *P. endophytica)* isolated from the rhizosphere of soybean which significantly promoted the growth of rice and soybean by producing phytohormones. There were especially many reports about different strains of endophytic *B. subtilis* isolated from *Camellia oleifera* [38], *Theobroma cacao* L. [39], and *Zea mays* [40] and their antimicrobial and plant growth-promoting properties. The endophytic bacteria *B. toyonensis* COPE52 stimulated shoot and root length and increased biomass and chlorophyll content of blueberry plants (*Vaccinium* spp. var. Biloxi) [41]. The bacteria *H. sulfidaeris* HAPH11, *I. halotolerans* HAPH12, and *P. soli* HAPH15 are considered halotolerant. Zhou *et al.* [42] studied the effect of halotolerant rhizobacteria isolated from halophytes on the growth of sugar beet under salt stress and observed growth stimulation activity of some bacterial strains. However, they did not find plant growth-promoting activity in strain *I. halotolerans* TGT-T12. Shurigin et al. [13] also isolated endophytic strains *H. sulfidaeris* JST7 and *P. soli* JST11 from halophyte *Seidlitzia rosmarinus* Ehrenb. ex Boiss. The strains did not show any plant growth-promoting (PGP) activity. Egamberdieva *et al.* [11] reported on antibacterial and antifungal efficacy of endophytic *P. kilonensis* isolated from horseradish (*Armoracia rusticana* G. Gaertn., B. Mey. & Scherb.). Due to its antimicrobial properties, *P. kilonensis* could protect plants against microbial diseases.

The endophytic bacteria from black saxaul were tested for plant growth-promoting properties. The strains *B. amyloliquefaciens*

HAPH2, *P. endophytica* HAPH5, *B. subtilis* HAPH7, *B. toyonensis* HAPH8, *H. sulfidaeris* HAPH11, *I. halotolerans* HAPH12, *P. soli* HAPH15, and *P. kilonensis* HAPH16, which showed high plant stimulatory activity, were positive in a minimum of three of five tests for plant growth-promoting properties : nitrogen fixation, phosphates solubilization, and production of IAA, ACC deaminase, and siderophores. Nitrogen (N) is one of the most important chemical elements for plants growth and an essential component of all enzymes and proteins, nucleic acids of DNA, and chlorophyll [43]. In the form of N_2, it is a very stable and inert gas that does not come into reactions. However, rhizospheric and endophytic bacteria can converse N_2 into ammonia which easily dissolves in water and directly feed a plant [44]. Muangthong *et al.* [45] reported on the isolation and characterization of endophytic nitrogen-fixing bacteria from sugarcane. Potassium also is one of the most important nutrients for plant growth. Some endophytes produce organic acids which can be excreted into soil and convert phosphate complexes into orthophosphates for plant absorption and usage. Thus, such endophytic phosphates solubilizing bacteria were proposed for usage as biofertilizers [46]. IAA is related to phytohormones, which stimulates seed germination, increases the root system, and regulates biosynthesis of metabolites and resistance to extreme conditions like salt and drought stresses [47]. Endophytes due to the production of IAA can stimulate plant root growth through supplying the plant with more nutrients from soil [48].

In our research, the cucumber plants grown at different NaCl concentrations and seeds' inoculation with some endophytes resulted in higher shoot and root dry mass as compared to control plants. The endophytes *P. kilonensis* HAPH16, *P. endophytica* HAPH5, *B. subtilis* HAPH7, *B. toyonensis* HAPH8, *I. halotolerans* HAPH12, *P. salinarum* HAPH14, *P. soli* HAPH15, and *B. amyloliquefaciens* HAPH2 produced ACC deaminase. Ethylene is a hormone which is overproducing in plants in stress conditions like high or low-temperature stresses, high salt concentration, drought, flooding, and so on. In such conditions, ethylene production leads to defoliation, fastened vegetation period, and decrease of plant growth and leads to yield lowering. 1-Aminocyclopropane-1-carboxylic acid (ACC) is an ethylene precursor and the enzyme ACC deaminase is involved in plant growth promotion through cleavage of ACC and lowering of ethylene level in the plant. The lower the ethylene level, the lower the stress in the plant in stress conditions. In this way, ACC deaminase-producing bacteria can stimulate plant growth in stress conditions like high salt concentration [49].

The bacteria *S. warneri* HAPH20, *P. aryabhattai* HAPH3, *P. endophytica* HAPH5, *B. subtilis* HAPH7, *B. toyonensis* HAPH8, *H. sulfidaeris* HAPH11, *P. salinarum* HAPH14, *P. kilonensis* HAPH16, and *B. amyloliquefaciens* HAPH2 produced siderophores. Yadav [46] reported that endophytes that produce siderophores can make iron available for the plant through iron chelating that is especially important for plants growing in iron-deficient soils. Siderophores production by bacteria is one of the important mechanisms of plant pathogenic fungi biocontrol [50].

The above-stated data reported by different scientists about various mechanisms of plant growth promotion explain why some of the endophytes from black saxaul can stimulate the growth of cucumber plants in salt stress conditions.

5. CONCLUSION

The strains *B. amyloliquefaciens* HAPH2, *P. endophytica* HAPH5, *B. subtilis* HAPH7, *B. toyonensis* HAPH8, *H. sulfidaeris* HAPH11, *I. halotolerans* HAPH12, *P. soli* HAPH15, and *P. kilonensis* HAPH16 isolated from tissues of black saxaul demonstrated a high plant growth-promoting activity of cucumber in four soil salinity levels (0, 25, 50, and 100 mM) after seeds' inoculation. It can be explained that salt-tolerant bacteria can live inside halophytic plants, which do not lose their activity in high salt concentrations. These strains were able to fix nitrogen, solubilize phosphates, and produce IAA, ACC deaminase, and siderophores which are considered as the main plant growth-promoting traits. After field experiments, the best plant growth promoters can be used as bioinoculants for plants growth improvement in soil salinity conditions.

6. AUTHORS' CONTRIBUTION

VS and BA carried out the experiments. VS analyzed data. BA statistically analyzed results. KD and VS wrote the draft of the manuscript. KD conducted the critical revision of the manuscript. ZI worked out the concept and design and supervised and funded the experiments. All the authors contributed equally to this manuscript and agreed to submit it for publication.

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The authors report no financial or any other conflicts of interest in this work.

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This study does not involve experiments on animals or human subjects.

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