BMC Plant Biology

RESEARCH Open Access

Improved salinity tolerance in cucumber seedlings inoculated with halotolerant bacterial isolates with plant growth-promoting properties

Zeinab Fotoohiyan[1], Fatemeh Samiei[2], Ali Salehi Sardoei[3*], Fatemeh Kashi[4], Mansour Ghorbanpour[5*] and Khalil Kariman[6]

Abstract

To address salinity stress in plants in an eco-friendly manner, this study investigated the potential effects of salinity-resistant bacteria isolated from saline agricultural soils on the growth of cucumber (*Cucumis sativus*, cv. Royal) seedlings. A greenhouse factorial experiment was conducted based on a completely randomized design (CRD) with two factors, salinity at four levels and five bacterial treatments, with three replications ($n=3$). Initially, fifty bacterial isolates were screened for their salinity and drought tolerance, phosphate solubilization activity, along with production of auxin, siderophore and hydrogen cyanide. Isolates K4, K14, K15, and C8 exhibited the highest resistance to salinity and drought stresses in vitro. Isolates C8 and K15 demonstrated the highest auxin production capacity, generating 2.95 and 2.87 µg mL^{-1}, respectively, and also exhibited significant siderophore production capacities (by 14% and 11%). Additionally, isolates C8 and K14 displayed greater phosphate solubilization activities, by 184.64 and 122.11 µg mL^{-1}, respectively. The statistical analysis revealed that the selected four potent isolates significantly enhanced all growth parameters of cucumber plants grown under salinity stress conditions for six weeks. Plant height increased by 41%, fresh and dry weights by 35% and 7%, respectively, and the leaf area index by 85%. The most effective isolate, C8, was identified as *Bacillus subtilis* based on the 16 S rDNA amplicon sequencing. This study demonstrated that inoculating cucumber seedlings with halotolerant bacterial isolates, such as C8 (*Bacillus subtilis*), possessing substantial plant growth-promoting properties significantly alleviated salinity stress by enhancing plant growth parameters. These findings suggest a promising eco-friendly strategy for improving crop productivity in saline agricultural environments.

Keywords Cucumber, Salt stress, PGPR, Growth indicators, Auxin

*Correspondence:
Ali Salehi Sardoei
alisalehisardoei@gau.ac.ir
Mansour Ghorbanpour
m-ghorbanpour@araku.ac.ir
[1]Department of Plant Pathology, Jiroft Branch, Islamic Azad University, Kerman, Iran
[2]Department of Plant Pathology, Roudehen Branch, Islamic Azad University, Tehran, Iran
[3]Department of Horticultural and Crops Research, Southern Kerman Agricultural and Natural Resources Research and Education Center, AREEO, Jiroft, Iran
[4]Graduated with a master's degree in statistics from Allameh Tabataba'i University, Tehran, Iran
[5]Department of Medicinal Plants, Faculty of Agriculture and Natural Resources, Arak University, Arak 38156-8-8349, Iran
[6]UWA School of Agriculture and Environment, The University of Western Australia, 6009 Perth, WA, Australia

Introduction

Cucumber (*Cucumis sativus* L.) is a major vegetable crop worldwide that is sensitive to salt stress due to its shallow root system [1]. Environmental stresses such as drought and salinity are the major factors affecting global agriculture and food security [2]. Salinity decreases the soil water potential and consequently mineral absorption by plant roots [3]. Salinity also changes Na^+/K^+ ratio and osmotic stress, and inhibits a multitude of biochemical and physiological processes involved in plant growth and development [4, 5]. The accumulation of ions (Na^+ and Cl^-) beyond threshold limits adversely affects plant metabolism, transpiration system, photosynthesis, and most importantly, ionic balance and nutrient uptake [6, 7]. Plant breeding efforts are vital for developing salt-tolerant varieties, while genetic engineering to enhance plant resilience, is sometimes discouraged due to its time-intensive nature and concerns regarding the safety/acceptance of genetically modified products. Hence, in addition to diverse breeding and agronomic approaches as well as established desalination techniques, embracing strategies that harmonize with nature, e.g. biofertilizers, is vital for achieving sustainable agricultural productivity [8]. In recent years, there has been a significant interest to employ beneficial microorganisms that boost plant growth, nutrition and tolerance against environmental stresses such as drought and salinity, i.e. an eco-friendly strategy to address key global challenges in agriculture [9, 10]. Numerous studies have demonstrated that biofertilizers can enhance crop yields [11, 12]. Sustainable strategies to increase the ability of plants to tolerate salinity stress include the use of plant growth-promoting rhizobacteria (PGPR) that are able to provide cross-protection against multiple stress factors [13]. PGPR are a group of bacteria inhabiting the rhizosphere, that can either directly or indirectly facilitate plant growth under optimal or stressful conditions through diverse mechanisms such as improving the bioavailability of mineral nutrients for plants, aiding in osmotic adjustment, and accumulate organic solutes [14, 15]. By promoting overall plant growth and nutrition, PGPR can reduce crop reliance on synthetic fertilizers [16, 17]. PGPR can facilitate plant growth and development through various mechanisms, including nitrogen fixation within the rhizosphere, production of phytohormones such as auxins, cytokinins, gibberellins, polyamines, and enhanced nutrient uptake [18].

Bacterial isolates obtained from stressful environments demonstrate greater effectiveness in enhancing plant resilience against stresses due to their high stress adaptation [19]. Qi et al. [20] reported that *Bacillus licheniformis* and *B. subtilis* promoted the growth of cucumber seedlings, enhanced the availability of nutrients, and alleviated the adverse effects of salt stress. Similarly, investigations into the effects of PGPR isolates on pistachio (*Pistacia vera* L.) seedlings demonstrated improvements in various growth parameters and physiological indicators [21]. In another study, inoculation with *Pseudomonas fluorescens* and *B. subtilis* strains led to mitigation of salinity stress in cucumber plants through altering their osmolytes and antioxidants profiles [22]. Moreover, research exploring the impact of *B. subtilis*, *P. putida*, and *Enterobacter cloacae* isolates on hazelnut (*Corylus avellana*) seedlings revealed positive effects on nutrient uptake, collar diameter, height, and various morphological indicators [23]. *Pseudomonas* spp. and *Bacillus* spp. can produce auxin (IAA) and siderophores, and exhibit 1-aminocyclopropane-1-carboxylic acid (ACC) deaminase activity, leading to improved growth of plants subjected to salinity stress [24].

However, the effects of salinity stress on plant growth depend on the species susceptibility, growth conditions as well as the ionic composition of the given salinity stress [25]. In regions with saline soils (e.g. Jiroft, Iran), there is a significant crop yield loss due to soil salinity [26]. PGPR hold significant potential for agricultural applications. This approach has not only enhanced crop nutrition and protection but also mitigated environmental contamination and reduced farmers' production costs. Numerous bacterial species, especially those in the genus *Bacillus*, have been found to possess plant growth-promoting traits [27]. While numerous studies have investigated the rhizosphere of salinity-tolerant plants across distinct geographical regions, to our knowledge, this is the first study to isolate indigenous salt-tolerant bacteria from the saline soils of Jiroft. This study aimed to isolate locally adapted, salinity-resistant, and growth-promoting PGPR isolates from crop fields with saline soils located around Jiroft, Iran, and ii) conduct a greenhouse study to investigate the effects of potent halotolerant PGPR strains on growth parameters of cucumber seedlings under salinity conditions.

Materials and methods

Isolation of bacteria

Field soil samples were selected from a depth of 30 cm in the arid and saline parts of Jiroft (Kanarsandal, Jazsaleh, Anbarabad and Karimabad areas; Kerman province, Iran) in 2020 [28]. To isolate bacteria, ten grams of each soil sample was transferred into vials containing 90 ml of sterile distilled water and placed on a shaker at 100 rpm (revolutions per minute) for one hour. Subsequently, serial dilutions (up to five) were prepared from the soil suspensions. Finally, 60 µl of each dilution was added to plates containing the minimal base culture medium [29], and spread with a sterile glass rod. Bacterial isolates were cultured on tryptic soy agar (TSA) medium in screw-cap gradient tubes for 24 h at 30 °C. Subsequently, the isolates

were classified based on their phenotypic and staining characteristics, named, and stored at 4 °C for future analysis [30].

Screening of salt-resistant bacteria

To check the salinity resistance of the isolates, initially, fifty isolates were cultured in nutrient broth medium with NaCl concentrations of 0 (control), 5%, 10%, 15%, 20%, 25%, 35% and 40% at 30 °C for 24 h. Growth rates of the isolates were evaluated through optical density measurements at 660 nm using a spectrophotometer [16]. Subsequently, the salinity tolerance of eleven isolates was assessed in media containing 40% NaCl.

Screening of drought-resistant bacteria

The salt-resistant bacterial isolates were screened for drought resistance by subjecting them to different water potentials in the presence of polyethylene glycol 6000 at 30 °C for 24 h. Different water potentials (0.50, -0.15, -0.03, -0.49 and −0.73 MPa) were prepared by adding calculated amounts of polyethylene glycol 6000 per liter of nutrient broth medium, according to the following formula [31]:

$$\text{Water potential (wp)} = -(1018e\text{-}2)c - (1.18e\text{-}4)c^{8}2 + (2.67e\_4)^{ct} + (8.39e\text{-}7)c^{8}2T.$$

T: Kelvin temperature **c**: polyethylene glycol concentration **t**: ambient temperature **e**: constant coefficient **wp**: water potential.

Then, 0.1 mL of bacterial liquid culture was added to media with different water potentials, and three replicates were prepared for each concentration. The inoculated vials were incubated at 30±2 °C for 24 h, shaken at 120 rpm. Bacterial growth was determined by measuring the optical density at 600 nm using a Jenway spectrophotometer (model 6505, UK) [31].

Investigation of plant-growth promoting characteristics of selected isolates

Quantitative estimation of auxin

Estimation of auxin content was conducted following the Beick et al.'s method [32] and spectrophotometry. First, bacterial isolates were inoculated in liquid nutrient broth medium supplemented with 0.5% L-tryptophan and kept for 48 h on a rotary shaker incubator (Biological Oxygen Demand, BOD) at 27±2 °C temperature. After 48 h of growth, the bacterial cultures were centrifuged at 10,000 rpm for 10 min and 1 mL of supernatant was taken in a test tube mixed with 1 mL of Salkowski's reagent (35% of perchloric acid, 1 mL 0.5 M $FeCl_3$) solution. The reaction mixture was incubated in dark condition for 20 min. Appearance of a reddish-pink colour in the test tube indicated the production of indole acetic acid (IAA), and the optical absorption of the reactions was read at a wavelength of 530 nm [33].

Measurement of siderophore production

The siderophore production ability of the isolates was determined using the Casschatel method [34]. This assay operates on the principle of chrome azurol S (CAS) changing color from blue to orange as siderophores remove iron from the dye. The selected isolates were cultured in a minimum iron medium at 30 °C. Then, the culture samples were centrifuged for 15 min at 27,000 rpm and the CAS solution was added to the supernatant in an equal proportion, and incubated at 30 °C for 20 min. In the presence of siderophores, iron was extracted from the dye complex, leading to a decrease in the intensity of the blue color. The color intensity was subsequently measured at 630 nm using a spectrophotometer (Jenway, model 6505, UK).

Measurement of hydrogen cyanide production

To assess the hydrogen cyanide (HCN) production by isolates, we employed a method utilizing picric acid salts. Bacterial isolates were grown in nutrient broth medium supplemented with 4.4% glycine. Subsequently, filter papers (Whatman No. 1) were soaked in a solution containing 2% sodium carbonate and 0.5% picric acid on the surface of the culture medium. The incubation was carried out at a temperature of 28±2 °C for a duration of 96 h. The presence of HCN was indicated by a distinct color change observed on the filter paper, shifting from yellow to an orange-brown, as described by Castric [35].

Evaluation of phosphate solubilization activity

Bacterial isolates were cultured on Picosky solid culture medium [36], containing 10 g of glucose, 5 g of tricalcium phosphate, 0.5 g of yeast extract, 0.5 g of ammonium sulfate, 0.2 g of potassium chloride, 0.2 g of sodium chloride, 0.1 g of magnesium sulfate, 0.0003 g of iron sulfate, 0.0003 g of manganese sulfate and 10 g of agar per liter, with pH 7, and incubated at 30±2 °C for five days. To detect the phosphate solubilization activities, the formation of clear zones around the bacterial colonies was monitored. The isolates were subsequently inoculated into the Picosky liquid medium and incubated for 100 h on shaker (100 rpm) at 30±2 °C. After incubation, 2 mL of the culture medium was removed and transferred to 2-mL vials. To remove bacterial cells and solid materials from the culture medium, the vials were centrifuged (12,000 rpm) for 10 min at 25 °C. The clear supernatant solution of each vial was transferred to new vials. Finally, concentration of the solubilized phosphorus in the supernatant was quantified by the method described by Watanabe and Olsen [37].

Greenhouse experiments

The plant growth-promoting ability of the four most effective isolates (K4, K14, K15, and C8) was evaluated based on their effect on the growth indices of cucumber seedlings (plant height, fresh and dry weight, and leaf area). A factorial experiment was carried out in a greenhouse, based on a completely randomized design with two factors, salinity at four levels (control, 4, 8 and 16 dS/m created through addition of NaCl to the irrigation water) and five bacterial treatments (without inoculation, and individual inoculation with four salt-resistant bacterial isolates) with three replications. The cucumber seeds (Royal cultivar) were obtained from the Agricultural and Natural Resources Research Center of Southern Kerman (Iran). For surface-sterilization, seeds were submerged in 5% sodium hypochlorite solution for 3 min and rinsed 10 times with sterile distilled water, and subsequently treated with the fungicide Captain (Wp50%) for 3 min [38].

Clean pots (21×16×16 cm) were filled with 2 kg soil, and eight pre-germinated seeds were planted in each pot at a depth of 2 cm. When placing seeds in the soil, three mL of bacterial suspension (containing 10^8 cells per mL) were added onto each seed [39]. In order to prevent the initial severe shock to the plants, salinity treatments were gradually applied in 3 applications. In order to prevent the salt leaching, we used sealed pots (without drainage) and the soil moisture content was kept at 70% of field capacity (FC) [40]. Plants were grown for six weeks and the vegetative parameters including plant height, fresh and dry weight and leaf area were measured. The height of the seedlings was measured using a mm-precision graduated ruler. The plant fresh weight was measured with digital scale with accuracy of 0.001 g. Plant samples were dried in oven at 72 °C for 48 h and the dry weights were measured, subsequently. To measure the leaf area, the leaves were scanned using the leaf scanner, converted to images, and analyzed using the Photoshop graphic software [41].

Biochemical identification of the potent isolates

Biochemical characterization of the bacterial isolates was carried according to the standard protocols described by Nezami et al. [42], and included Gram staining, catalase, lactose, urease, oxidase, motility, indole, and H_2S production tests.

Molecular identification of the most effective isolate

The genomic DNA of the C8 isolate was extracted from the cultures growing on nutrient agar for 18 h, according to the method described by Weisburg et al. [43]. The extracted genomic DNA was detected by gel electrophoresis and then stored at -20 °C until further use. The gene encoding 16 S rRNA was amplified using the universal primers 27 F: 5′-AGAGTTTGATCCTGGCTCAG-3′, and the 1492R: 5′-CTACGGCTACCTTGTTACGA-3′. The PCR reaction mixture was made in a total volume of 50 µL, containing 25 µL of master mix (Promega, USA), 2 µL of template DNA, 2 µL each of forward and reverse primers, and 19 µL of nuclease-free water. The PCR amplification conditions were as follows: an initial denaturation step of 96 °C for 3 min followed by 27 cycles of 96 °C for 30 s, annealing of 56 °C for 25 s, and elongation at 72 °C for 15 s, followed by a final extension step at 72 °C for 10 min [44]. The PCR products were detected by gel electrophoresis using a DNA ladder (Intronbio, South Korea) as a marker. The bands were visualized under UV light and photographed. The amplified PCR products of 16 S rDNA fragments were sent to Macrogen company (South Korea) for purifying and sequencing. The obtained 16 S rDNA gene sequence of the C8 isolate was edited using the MEGA6 software and aligned with the nucleotide sequences databases of NCBI using BLAST tools. Phylogeny analysis and phylogenetic tree construction was carried out based on the Neighbor-joining phylogeny algorithm [45, 46].

Statistical analyses

Graphs were generated using the Excel 2018 software. The data analysis was carried out using the SPSS statistical software version 26, and means were compared using Duncan's multi-range test at a 5% significance level.

Results

Isolation of bacteria

Based on morphological characteristics of the colonies grown on TSA medium, different bacterial isolates were selected and coded (Table 1). A total of 50 isolates were obtained from soil samples collected from various saline crop fields in different areas of Jiroft, Kerman Province, Iran. These soil samples were collected from Jazsaleh (8 isolates), Kanar Sandal (16 isolates), Karimabad (16 isolates), and Anbarabad (10 isolates) during the 2020 cropping year. The codes of bacterial isolates obtained from each region are provided in Table 1.

Screening of salt-resistant bacteria

Salt resistance screening of fifty isolates revealed that all strains could tolerate salinity levels of 0.5%, whereas, only 11 isolates were able to grow in media containing NaCl up to 40% (Table 2). The isolates K4, K10, K12, K14 and K15 (from Kanar Sandal), C8, C10 and C11 (from Karimabad), A2, A3 and A4 (from Anbarabad) were able to grow in media containing NaCl up to a concentration of 40% salt and were selected for subsequent studies. The isolates that did not grow at the highest salt medium after 24 h of incubation at 30 °C are designated with a negative symbol in Table 2. According to the results, the

Table 1 Codes of bacterial isolates obtained from different areas of Jiroft, Iran

Bacterial code	Collection area	Bacterial code	Collection area	Bacterial code	Collection area	Bacterial code	Collection area
J1	Jazsaleh	K6	Kanar Sandal	C3	Karimabad	C16	Karimabad
J2	Jazsaleh	K7	Kanar Sandal	C4	Karimabad	A1	Anbarabad
J3	Jazsaleh	K8	Kanar Sandal	C5	Karimabad	A2	Anbarabad
J4	Jazsaleh	K9	Kanar Sandal	C6	Karimabad	A3	Anbarabad
J5	Jazsaleh	K10	Kanar Sandal	C7	Karimabad	A4	Anbarabad
J6	Jazsaleh	K11	Kanar Sandal	C8	Karimabad	A5	Anbarabad
J7	Jazsaleh	K12	Kanar Sandal	C9	Karimabad	A6	Anbarabad
J8	Jazsaleh	K13	Kanar Sandal	C10	Karimabad	A7	Anbarabad
K1	Kanar Sandal	K14	Kanar Sandal	C11	Karimabad	A8	Anbarabad
K2	Kanar Sandal	K15	Kanar Sandal	C12	Karimabad	A9	Anbarabad
K3	Kanar Sandal	K16	Kanar Sandal	C13	Karimabad	A10	Anbarabad
K4	Kanar Sandal	C1	Karimabad	C14	Karimabad		
K5	Kanar Sandal	C2	Karimabad	C15	Karimabad		

maximum NaCl tolerance was shown by isolates K12 and C8. As shown in Fig. 1, the selected isolates were capable of growing at a stable OD under the 40% NaCl stress condition, thus, the salinity level of 40% was adopted to study the role of the bacterial isolates in promoting plant growth. A significant positive correlation was identified between the OD and bacterial concentrations.

Screening drought-resistant bacteria

The drought resistance screening trial revealed that all 11 isolates were able to grow in the media with the water potential of 0.5 MPa (Table 3), whereas, K4, K14, K15 and C8 isolates had the highest growth in media with the highest water potential (-0.73 MPa), i.e. the highest drought resistance (Fig. 2). Based on their high resistance to salinity and drought stresses, these isolates were selected as the potent isolates for further studies.

Plant growth-promoting properties of the potent isolates

Results showed that all the selected isolates (K4, K14, K15, and C8) were able to produce auxin, hydrogen cyanide, siderophore and they also possessed phosphate solubilization activity (Fig. 3). The amount of auxin production by isolates ranged from 0.17 to 2.95 μg mL^{-1}. The highest auxin production capacity was observed for the C8 isolate (2.95 μg mL^{-1}), while K15 and K4 isolates had the lowest auxin production (Fig. 3A). Furthermore, all isolates were able to produce siderophore (Fig. 3B). Additionally, C8 and K15 isolates produced the highest amount of siderophore with values of 14% and 11% (Fig. 3B). Results of the phosphate solubilization test showed that all four isolates were able to solubilize the water-insoluble inorganic phosphate compound, with C8 and K14 isolates showing the highest phosphate solubilization activities by 184.64 and 122.11 μg mL^{-1}, respectively (Fig. 3C). In terms of hydrogen cyanide production, the C8 isolate was able to produce the highest amount of hydrogen cyanide (0.74) whereas the K14 isolate produced the lowest amount (0.08) (Fig. 3D).

Effect of potent isolates on growth of cucumber seedlings in a greenhouse

According to the ANOVA results, salinity, bacterial strains and their interactions had significant effects on all growth characteristics of cucumber plants (Table 4). The salinity stress (up to 16 dS/m) significantly decreased plant height, fresh and dry weights and leaf area by 29%, 20%, 42% and 42%, compared to the control, respectively (Fig. 4). The statistical analysis revealed that the selected potent isolates significantly enhanced all growth parameters including plant height (41.47%), fresh and dry weights (34.97% and 7.49%), and leaf area (85.07%) indices of cucumber plants grown under salinity stress condition for six weeks (Fig. 4). Regardless of bacterial strains, plant height increased compared to the uninoculated control for all four isolates. The maximum plant height increase was related to the C8 isolate, by 47.58% (Fig. 4A). Inoculation with all four isolates increased fresh and dry weights of plants subjected to salinity, compared to control (Fig. 4B and C). Also, inoculation with each of four isolates led to increased leaf area compared to the control, the maximum of which belonged to the C8 isolate, by 42% (Fig. 4D). The C8 isolate was shown to have the greatest plant growth-promoting properties, which can be attributed to certain factors such as its higher potency of auxin production compared to the other isolates. Accordingly, molecular identification of the C8 isolate was carried out.

Biochemical identification of potent isolates

Biochemical identification of the four potent isolates (K4, K14, K15 and C8) was carried out via conducting diverse analyses (Table 5). All the isolates were positive for Gram staining, catalase, lactose, motility and H_2S production and they were negative for oxidase, urease and indole

Table 2 The optical absorption (660 nm) for bacterial isolates obtained from different areas of Jiroft, cultured in media with 5%, 10%, 15%, 25%, 35%, and 40% concentrations of NaCl at 30 °C for 24 h

NaCl concentration Bacterial Code	5%	10%	15%	20%	25%	35%	40%	Control
J1	0.103	0.003						0.114
J2	0.131							0.133
J3	0.358	0.056	0.040					0.372
J4	0.594	0.258	0.088					0.595
J5	0.009	0.008						0.215
J6	0.265	0.131	0.098					0.182
J7	0.189							0.216
J8	0.009							0.049
K1	0.320	0.115	0.053	0.019				0.339
K2	0.478	0.207	0.107	0.012				0.889
K3	0.009	0.008				0.008	0.009	0.273
K4	0.295	0.118	0.095	0.089	0.063	0.024	0.004	0.328
K5	0.352	0.110	0.207					0.400
K6	0.067	0.060	0.057					0.149
K7	0.116	0.097	0.059					0.185
K8	0.213	0.090	0.003					0.305
K9	0.119	0.49	0.19					0.288
K10	0.391	0.310	0.233	0.152	0.082	0.062	0.047	0.389
K11	0.163	0.100						0.413
K12	0.148	0.112	0.711	0.389	0.235	0.198	0.135	0.193
K13	0.058	0.013	0.012					0.168
K14	0.362	0.152	0.120	0.078	0.054	0.034	0.024	0.434
K15	0.123	0.114	0.067	0.051	0.028	0.023	0.018	0.175
K16	0.074	0. 051	0.036					0.193
C1	0.051	0. 009	0.006					0.262
C2	0.361	0.125	0.026					0.177
C3	0.111	0.083	0.058	0.045	0.018	0.009		0.157
C4	0.056							0.158
C5	0.034							0.156
C6	0.018							0.110
C7	0.086							0.156
C8	0.148	0.112	0.711	0.389	0.235	0.198	0.135	0.193
C9	0.058	0.013	0.012					
C10	0.372	0.162	0.130	0.088	0.064	0.044	0.034	0.334
C11	0.123	0.114	0.067	0.051	0.028	0.023	0.018	0.175
C12	0.074	0.051	0.036					0.193
C13								0.174
C14	0.066	0.009						0.165
C15	0.082							0.197
C16	0.009	0.001						0.210
A1								0.195
A2	0.320	0.274	0.174	0.164	0.076	0.063	0.028	0.339
A3	0.878	0.407	0.400	0.342	0.331	0.049	0.045	0.889
A4	0.009	0.008	0.231	0.196	0.187	0.265	0.069	0.273
A5	0.115							0.199
A6								0.234
A7	0.111	0.098	0.005					0.184
A8	0.186	0.102	0.006					0.193
A9	0.196	0.115	0.091					0.213
A10	0.214	0.126	0.060					0.245

Bacterial codes: **J**: Jazsaleh, **K**: Kanar Sandal, **C**: Karimabad and **A**: Anbarabad

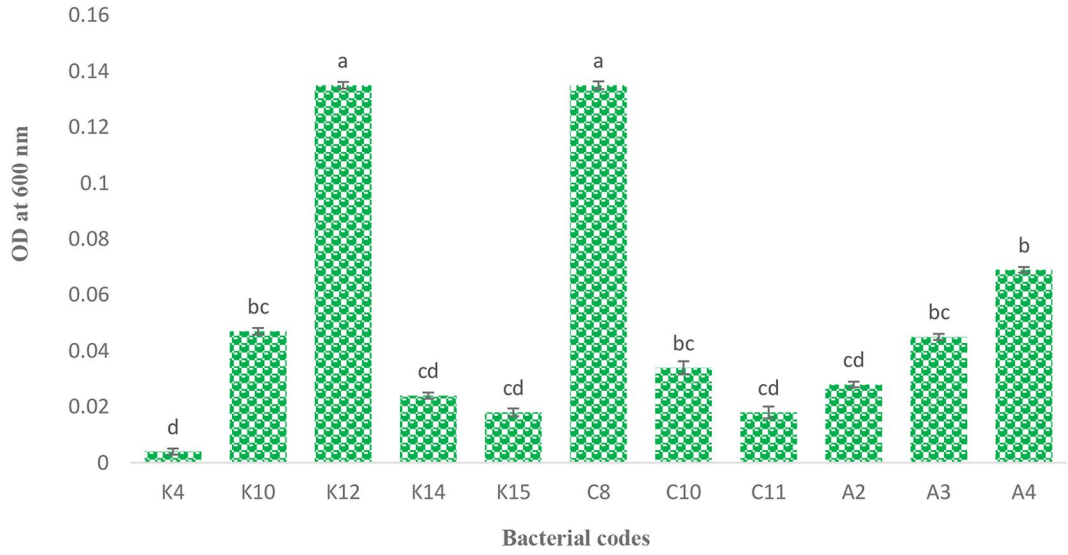

Fig. 1 Salinity tolerance in bacterial isolates from different areas of Jiroft, exposed to 40% NaCl concentration **(J**: Jazsaleh, **K**: Kanar Sandal, **C**: Karimabad and **A**: Anbarabad). Bacterial isolates showing high optical density (OD) value at 600 nm wavelength under salinity stress were considered as salt-tolerant bacterial strains

Table 3 The optical absorption (600 nm) for bacterial isolates cultured in media with different water potentials

Water potential (MPa) Bacteria code	0.50	-0.15	-0.03	-0.49	-0.73	Control
K4	0.026	0.029	0.029	0.144	0.038	0.328
K10	0.023	0.017	0.011			0.389
K12	0.019	0.017	0.012	0.010	0.008	0.193
K14	0.059	0.057	0.055	0.023	0.019	0.434
K15	0.013	0.025	0.041	0.155	0.013	0.175
C8	0.056	0.056	0.046	0.044	0.043	0.193
C10	0.034	0.007	0.004	0.003		0.334
C11	0.040	0.039	0.014			0.175
A2	0.012	0.009				0.339
A3	0.050	0.011				0.889
A4	0.030					0.273

Bacterial codes: **(K**: Kanar Sandal, **C**: Karimabad and **A**: Anbarabad)

production. On the basis of these observations, we propose that all isolates could belong to the *Bacillus* genus.

Molecular identification of the most effective bacterial isolate (C8)

The isolate C8 was identified as the most effective isolate based on its substantial tolerance against salinity and drought stresses as well as its remarkable plant growth-promoting properties. Approximately, 1500 bp band was observed in agarose gel following the PCR amplification of 16 S rDNA. The amplicon sequencing was performed by the Bioneer Corporation (South Korea). After the sequencing of the amplified products, BLAST alignment was performed on NCBI to obtain a strain with a high degree of homology. The molecular weight of these sequence fragments was consistent with that of the bacterial 16 S rDNA fragment. Based on the sequence BLAST results and the phylogenetic tree (Fig. 5), the C8 isolate was identified as *Bacillus subtilis* because its amplicon sequence shared 99–100% similarity with *Bacillus subtilis* gene sequences.

Discussion

Cucumber (*Cucumis sativus* L.) is a major vegetable crop worldwide, and it is sensitive to salt stress. Salinity and drought stresses are primary environmental factors that hamper the growth and yield of crops [47]. Jiroft, a city in Kerman Province (Iran), is a prominent agricultural center, which has also been impacted by salinity factors, prompting this study. Recently, researchers have been interested in determining how halophilic bacteria can aid plants in saline soils. The key mechanisms underpinning such microbe-mediated adaptations to different stresses include modulation of phytohormones biosynthesis

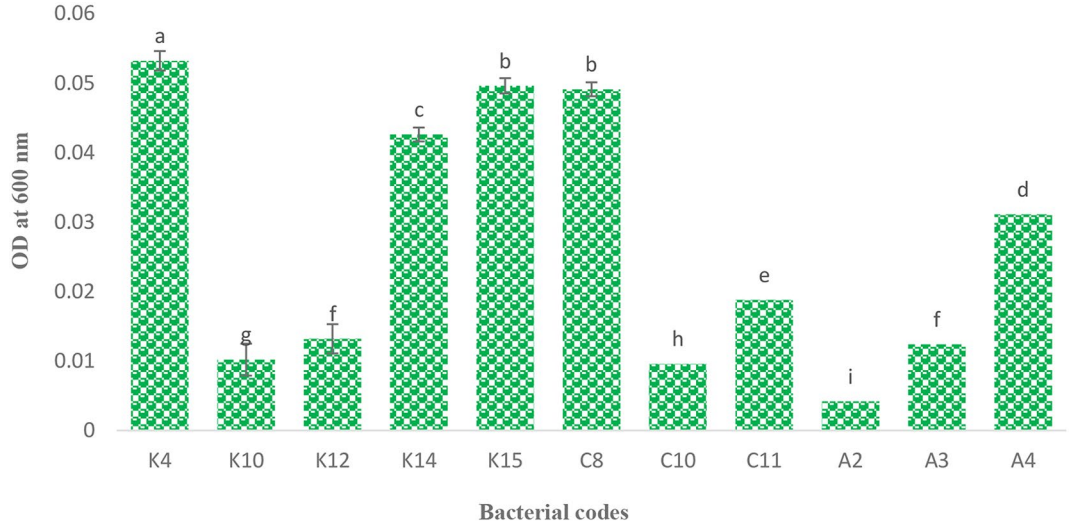

Fig. 2 Drought tolerance in bacterial isolates from different areas of Jiroft, grown in -0.73 MPa of water potential **(K**: Kanar Sandal, **C**: Karimabad and **A**: Anbarabad). Bacterial isolates showing high optical density (OD) value at 600 nm wavelength under drought stress were considered as drought-tolerant bacterial strains

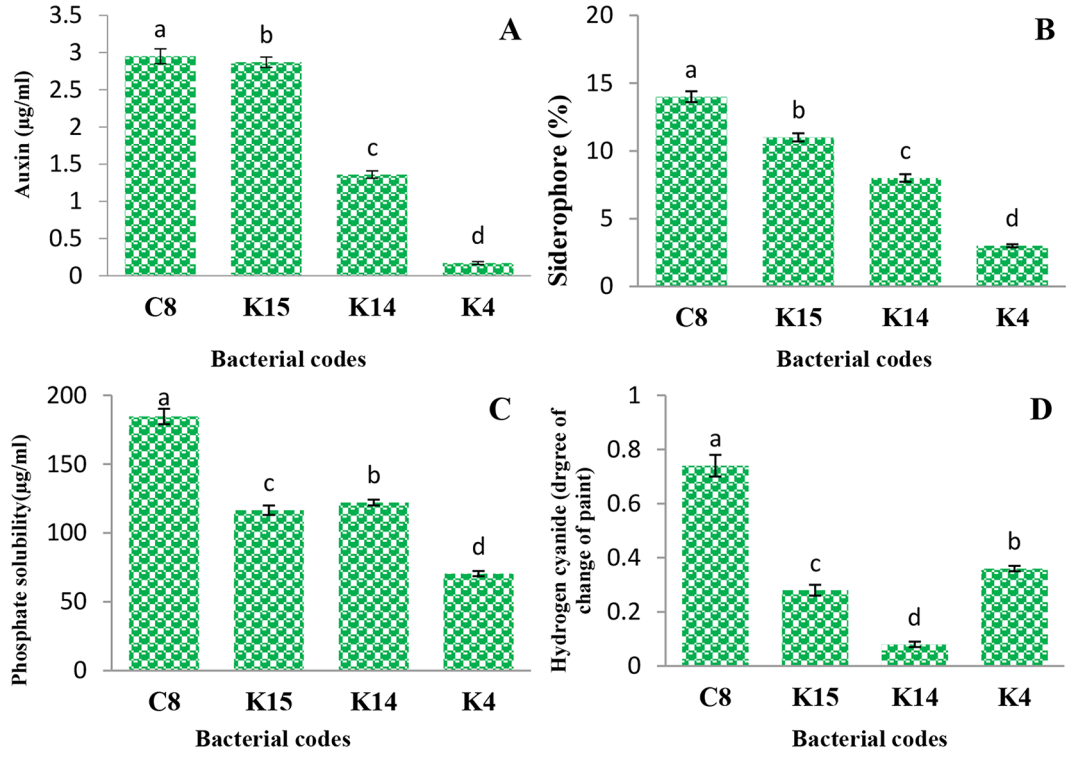

Fig. 3 Comparison of mean production of auxin (**A**), siderophore (**B**), phosphate solubilization activity (**C**) and hydrogen cyanide (**D**) by the four potent isolates, which were isolated from different areas of Jiroft (Iran) **(K**: Kanar Sandal and **C**: Karimabad)

(auxins, cytokinins, gibberellins, abscisic acid, ACC deaminase (1-aminocyclopropane-1-carboxylate deaminase), brassinosteroids, and ethylene, accumulation of osmoprotectants (betaines, proline, and soluble sugars), upregulation of different plant defense-related genes, and production of secondary metabolites [48, 49]. Salinity stress disrupts plant physiology and metabolism through osmotic disturbances, cytoplasmic toxicity from excessive ion absorption (e.g., sodium), nutrient imbalances, and oxidative stress due to the accumulation of reactive oxygen species. This disruption leads to reduced photosynthesis, growth, and ultimately diminished crop yield [50]. Therefore, the current study evaluated the potential of bacterial isolates from saline regions of Jiroft, and

Table 4 ANOVA analysis of the effect of salinity, bacterial strains and their interactions on the growth characteristics of cucumber seedlings under salinity stress after six weeks of growth

ANOVA	df	Plant Height (cm)	Fresh weight (g)	Dry weight (g)	Leaf area (cm^2)
Salinity	3	246.178**	335.588**	13.770**	2360.956**
Bacteria	4	1233.475**	58.206**	40.099**	1590.371**
Salinity × Bacteria	12	31.608**	48.760**	1.643**	60.725**
Error	40	0.617	0.081	0.003	0.068
Cv.		1.87	0.87	1.28	0.30

** indicates a significant difference at 1% significance level ($p \leq 0.01$)

examined their growth under varying salt (NaCl) concentrations and different water potentials. One innovative approach to combat salinity in plants and mitigate its adverse effects is the introduction of salt-tolerant bacteria [51]. Plants and bacteria have evolved symbiotic interactions to overcome abiotic stresses [52]. Among the 50 isolates tested initially, we found that 11 isolates had high salt tolerance, and four isolates exhibited the highest resistance to both salinity and drought stresses. Salt-tolerant bacteria, owing to their high osmotic pressure and the accumulation of organic solutes in their cytoplasm, can help counteract salinity stress. They achieve this through hydraulic conductivity, osmotic adjustments, elimination of toxic sodium ions, enhanced osmotic conductivity, improved photosynthesis, soil fertility enhancement, and bolstered plant resistance [31].

Moreover, the average growth of salt-resistant isolates from the Anbarabad and Kenarsandel areas exceeded those of isolates from the other regions. In a similar study, Albdaiwi et al. [8] isolated halotolerant plant growth-promoting rhizobacteria from durum wheat field. We also investigated the production of auxin, hydrogen cyanide, siderophore, as well as the phosphate solubilization activities of the selected isolates. Results showed that C8 and K15 isolates exhibited the highest levels of auxin and siderophore production. Moreover, C8 and K14 isolates displayed the highest phosphate solubilization activities. Bacteria isolated from saline regions that promote plant growth achieve this through various mechanisms, such as the production of plant hormones, siderophores, and phosphate solubilization activities [53]. Plant hormones play a crucial role in regulating plant growth and development. The production of auxin is a significant criterion for measuring the beneficial effects of PGPR isolates. This hormone significantly influences plant cell elongation, stimulates cell division, and promotes plant differentiation [54]. The ability to produce siderophores is another key characteristic observed in PGPR. Siderophores are secreted in response to iron deficiency stress and facilitate the conversion of iron into a soluble chelate, making it available to cells through specific membrane receptors [55]. Notably, Bhatt and Vyas, [56] reported that 40% of isolates from plant rhizospheres in a semi-arid region could produce siderophores. Phosphorus is one of the least mobile and least available nutrients for plants due to its high reactivity with soil constituents [57]. Plant growth-promoting bacteria enhance phosphorus availability to plants by producing enzymes such as phosphatases, as well as organic acids like gluonic acid and citric acid, which convert water-insoluble forms of phosphorus into soluble and bioavailable forms [58]. Additionally, the production of hydrogen cyanide by PGPR, primarily known for limiting pathogens' growth, can indirectly increase phosphorus availability through chelation with metal elements and phosphorus release in the rhizosphere [59].

We evaluated the growth-promoting potential of four potent salinity-resistant isolates on cucumber growth indices under salinity stress condition. Inoculation with all four bacterial isolates led to increased growth indices in each salinity level compared to their respective controls, and the C8 isolate exhibited the greatest increase. Treatment with each of the four bacterial isolates under salinity stress conditions increased plant height, along with the fresh and dry weights of plants; the highest increases were observed for C8 and K15 isolates. Leaf area ratio increased significantly due to bacterial inoculation, with the C8 isolate exhibiting the most pronounced effect, resulting in a 42% increase. Among the various plant growth-promoting properties of bacteria, auxin production plays a crucial role in facilitating plant growth under stress conditions. Therefore, the outstanding performance of the C8 isolate in enhancing cucumber growth in this study can be attributed to its higher auxin production capacity compared to the other three isolates, in addition to its ability to produce siderophore, hydrogen cyanide, and the highest phosphate solubilization capacity. Similar studies have reported the positive impact of auxin produced by growth-promoting bacteria on the shoot and root weight of wheat under salinity stress [60]. Furthermore, the findings of this study align with results from studies on the growth improvement of *Pinus halepensis* [61], pistachio (*Pistacia vera*) [62], and hazelnut (*Corylus avellana*) seedlings [23]. Similarly, Zhu et al. [63] isolated 22 *Bacillus* isolates from tomato seeds, all of which had the capacity to produce ACC deaminase enzyme. Among these, *B. subtilis* exhibited the highest ACC deaminase production, leading to increased seed germination rates and improved plant growth upon inoculation of tomato seeds. The increase in plant dry weight observed in this study reflects the improved efficiency of photosynthesis due to bacterial activity. Reduced photosynthetic efficiency is a significant factor hindering plant growth under high salinity conditions [64]. Osmotic stress leads to stomatal closure, reducing carbon dioxide

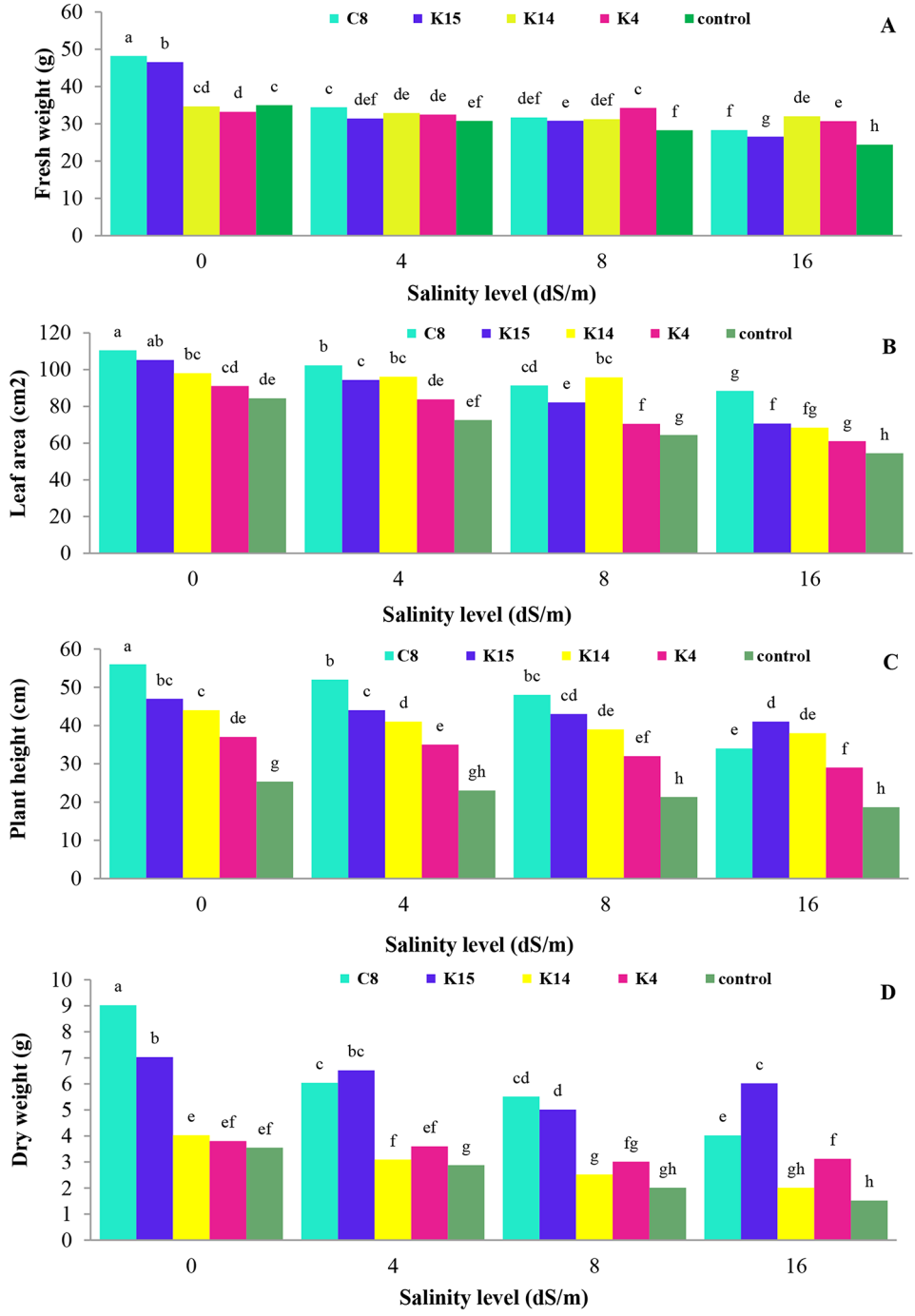

Fig. 4 Effects of potent bacterial isolates on the fresh weight (**A**), leaf area (**B**), plant height (**C**), and dry weight (**D**) of cucumber seedlings under salinity stress after six weeks of growth. **(K**: Kanar Sandal and **C**: Karimabad)

Table 5 Results of biochemical tests of the four potent bacterial isolates isolated from different areas of Jiroft (Iran)

Biochemical tests Isolates	Gram staining	Catalase	Oxidase	Urease	Lactose	Motility	H_2S production	Indole production
C8	positive	positive	negative	negative	positive	positive	positive	negative
K4	positive	positive	negative	negative	positive	-	positive	negative
K14	positive	positive	negative	negative	positive	-	positive	negative
K15	positive	positive	negative	negative	positive	positive	positive	negative

Bacterial codes: **(K**: Kanar Sandal and **C**: Karimabad)

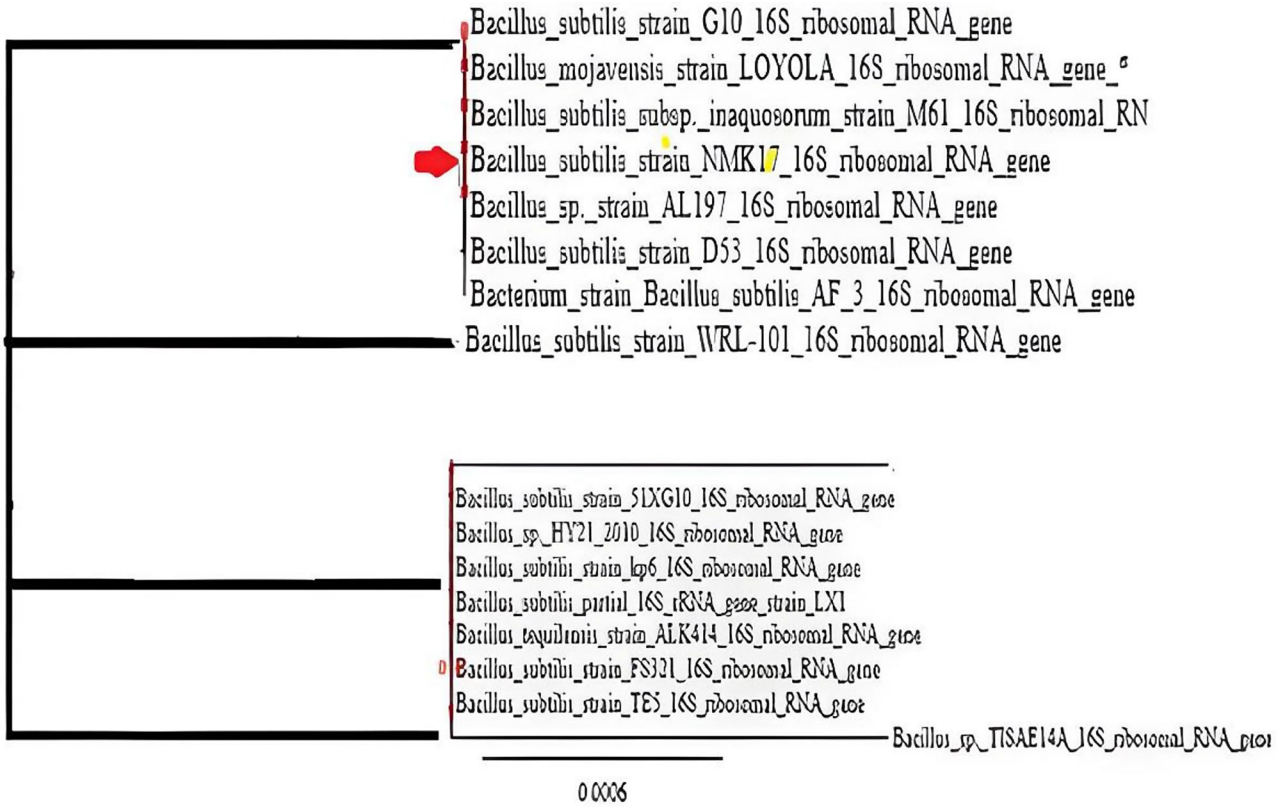

Fig. 5 Phylogenetic tree of the bacterial isolate C8 based on its 16 S rDNA gene sequence

absorption and photosynthesis. Plant growth-promoting bacteria counteract the effects of ethylene by enhancing root growth and providing micronutrients through siderophore and ACC deaminase production [65]. Other research has also highlighted the growth-stimulating effects of salinity-resistant bacteria isolated from saline soils, making them valuable components for sustainable agriculture [66]. In this study, among the four salinity-resistant isolates with substantial growth-promoting characteristics, the C8 isolate emerged as the most effective one. The DNA sequencing of this isolate confirmed its identity as *Bacillus subtilis*. *Bacillus* bacteria are particularly effective in enhancing plant growth indices. *Bacillus* genus members are Gram-positive and aerobic bacteria [67–69]. Also, *Bacillus* bacteria possess advantageous physiological characteristics such as multilayer cell walls, endospore production ability, phosphorus solubilization, production of hormones, antibiotics, peptide signal molecules, and extracellular enzymes [70, 71]. These characteristics provide *Bacillus* bacteria with favorable conditions for survival and distribution in stressful environments. Their ability to produce spores, is another factor contributing to their uniqueness and popularity as biofertilizers [72]. Additionally, *Bacillus* bacteria have a relatively higher sporification capacity than other growth-promoting bacteria, making them suitable for use as bioinoculants [73, 74]. Also, a previous study by Chen et al. [75] found that the positive effects of *Bacillus* on soil N availability and plant N uptake could be due to its N-fixing ability. The incorporation of *Bacillus*-based biofertilizers, as well as novel approaches such as the combination of biochar and *Bacillus* species, highlights the possibility for restructuring soil fertility and nutrient management systems. Various mechanisms employed by the *Bacillus* isolates to mitigate stresses caused by abiotic and biotic agents are reported (Supplementary Figure S1). Direct mechanisms include nitrogen fixation, siderophore production, phytohormone production, and nutrient solubilization. Indirect mechanisms include the production of hydrogen cyanide, exo-polysaccharides, biofilm formation, and lytic enzymes. The C8 isolate, identified as *Bacillus subtilis*, demonstrated significant potential to promote cucumber growth in saline soils.

Conclusions

Environmental stresses such as drought and salinity pose significant challenges to sustainable agricultural production in regions like Iran, where approximately 15–20% of the total land area comprises saline lands. Bacteria possess the capacity to thrive under salinity and drought conditions, coexisting harmoniously with plants. The plants, in turn, rely on bacteria and their metabolites,

including auxin, siderophore, cyanide acid, vitamins, for their growth. In this study, the isolation of salinity-tolerant bacteria from Jiroft's crop fields led to the identification of a potent PGPR isolate, C8. This exceptional isolate exhibited resilience to high salinity and drought and its repertoire of plant growth-promoting capabilities. These key traits, collectively, resulted in enhanced tolerance of cucumber seedlings subjected to salinity stress. Molecular identification confirmed the identity of the C8 isolate as *Bacillus subtilis*. *Bacillus*-based biofertilizers are a major step toward ecologically sound farming. This research underscores the importance of harnessing indigenous PGPR such as the C8 isolate to address the challenges posed by salinity and drought in local agriculture. However, there is an urgent need to conduct more studies related to improving screening techniques, isolation and identification of plant growth-stimulating compounds and antimicrobial compounds from bacterial isolates, and clarifying the molecular basis of the mechanisms involved. Therefore, further research is recommended to investigate the effects of these bacteria on cucumber yield under field conditions, their impact on other regional crops, and their potential for biological control of plant diseases.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12870-024-05538-y.

Supplementary Material 1

Supplementary Material 2

Acknowledgements
The authors would like to thank Islamic Azad University Jiroft branch for technical support during the research work.

Author contributions
A.S.S. supervised the research. Z.F. and F.S. performed the experiment and collected the data. F.K. revised the text critically, and contributed in interpretation of the obtained results. M.G. and K.K. provided advice on the research and revised the manuscript, critically. All authors read and approved the final manuscript.

Funding
Not applicable.

Data availability
All the data generated/ analyzed during the study are available with the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate
Not applicable.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

Received: 8 June 2024 / Accepted: 22 August 2024
Published online: 02 September 2024

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