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Synergistic plant–microbe interactions between endophytic bacterial communities and the medicinal plant *Glycyrrhiza uralensis* F.

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Abstract Little is known about the composition, diversity, and geographical distribution of bacterial communities associated with medicinal plants in arid lands. To address this, a collection of 116 endophytic bacteria were isolated from wild populations of the herb *Glycyrrhiza uralensis* Fisch (licorice) in Xinyuan, Gongliu, and Tekesi of Xinjiang Province, China, and identified based on their 16S rRNA gene sequences. The endophytes were highly diverse, including 20 genera and 35 species. The number of distinct bacterial genera obtained from root tissues was higher (n = 14) compared to stem (n = 9) and leaf (n = 6) tissue. Geographically, the diversity of culturable endophytic genera was higher at the Tekesi (n = 14) and Xinyuan (n = 12) sites than the Gongliu site (n = 4), reflecting the extremely low organic carbon content, high salinity, and low nutrient status of Gongliu soils. The endophytic bacteria exhibited a number of plant growth-promoting activities ex situ, including diazotrophy, phosphate and potassium solubilization, siderophore production, auxin synthesis, and production of hydrolytic enzymes. Twelve endophytes were selected based on their ex situ plant growth-promoting activities for growth chamber assays to test for their ability to promote growth of

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G. uralensis F. and *Triticum aestivum* (wheat) plants. Several strains belonging to the genera *Bacillus* (n = 6) and *Achromobacter* (n = 1) stimulated total biomass production in both *G. uralensis* and *T. aestivum* under low-nutrient conditions. This work is the first report on the isolation and characterization of endophytes associated with *G. uralensis* F. in arid lands. The results demonstrate the broad diversity of endophytes associated with wild licorice and suggest that some *Bacillus* strains may be promising candidates for biofertilizers to promote enhanced survival and growth of licorice and other valuable crops in arid environments.

Keywords Biodiversity · Plant growth-promoting bacteria · *Glycyrrhiza uralensis* · Endophytic bacteria · Arid lands-environmental microbiology

Introduction

Endophytic bacteria are ubiquitous microorganisms that reside within healthy plant tissues (Gasser et al. 2011; Malfanova et al. 2011). These microorganisms are often hypothesized to help their hosts by producing resources that afford protection and facilitate survival of the host plant (Sánchez-López et al. 2017). Therefore, a better understanding of plant–microbe interactions with respect to the physicochemical environment may provide insights into the microbial ecology of plant-associated endophytes (Islam et al. 2016; Patil et al. 2016). The plant-associated microbiome consists of distinct microbial communities living in roots, stems, leaves and other tissues. Symbioses between plants and endophytic bacteria are mutually beneficial through the exchange of metabolites and sharing of physiological processes (Reinhold-Hurek and Hurek 2011). The plant provides endophytes with a stable habitat while endophytic bacteria supply nutrients such as fixed nitrogen, soluble potassium, iron, and phosphate, and indole acetic acid (Liu et al. 2016, 2017; Sánchez-López et al. 2017).

In recent years, there has been an increasing interest in the characterisation and bioprospecting of growth-promoting endophytes, broadening the scope for innovative design of biofertilizers for different crops such as soy, canola, lentil, pea, radish, and wheat (Donate-Correa et al. 2005; Egamberdiyeva 2007). These endophytes are part of a broader strategy for sustainable agriculture because they may decrease environmental pollution due to fertilizers and other chemical applications. Endophytic bacteria associated with medicinal plants in arid lands can adopt sophisticated survival strategies to promote plant growth, alleviate abiotic stresses, aid in nutrient acquisition, and enhance systemic resistance and tolerance to disease (Boor 2006; Daffonchio et al. 2015). Nevertheless, despite the considerable body of knowledge of the associations between endophytes and crop plants (Wemheuer et al. 2017), much less is known of the diversity of bacterial communities associated with plants inhabiting arid lands, particularly medicinal plants.

Medicinal plants are receiving global attention because traditional medicines are often effective and easily available alternatives to pure pharmaceuticals. Medicinal plants are used worldwide as remedies for various diseases, including gastrointestinal symptoms, asthma, skin disorders, respiratory and urinary problems, and hepatic disease (Cushnie et al. 2014). It is well known that medicinal plants having an ethnobotanical history may harbor an endophytic microbiome that can synthesize a diverse array of bioactive compounds under conditions that are stressful to the host plant (Bajguz 2007). Bioactive compounds produced during endophyte-herb symbiosis can also affect plant-associated microbial communities and their physiological functions (Strobel et al. 2004).

The genus *Glycyrrhiza*, commonly known as licorice, comprises approximately 30 species including *Glycyrrhiza glabra*, *Glycyrrhiza uralensis, Glycyrrhiza inflata*, *Glycyrrhiza aspera*, and *Glycyrrhiza korshinskii* (Lewis et al. 2005). Licorice (*Glycyrrhiza uralensis* Fisch.) is one of the most widely used plants in food production, and it is also used as traditional Chinese medicine. Licorice contains bioactive compounds such as triterpene saponins, flavonoids, coumarins, and other phenolics (Zhang and Ye 2009), but little is known of their endophytic microflora. A better understanding of endophytic microorganisms adapted to arid lands is of broad importance in arid land ecology and can be exploited for biotechnological applications. Therefore, the objectives of our study were as follows: (1) isolate and identify endophytic bacteria associated with *G. uralensis* F.; (2) screen them for beneficial activities ex situ; and (3) evaluate their ability to stimulate

growth of the host plant and *Triticum aestivum*. To the best of our knowledge, this is the first report on the isolation, identification, and characterization of endophytic bacteria associated with the medicinal plant *G. uralensis* F. in an arid environment.

Materials and methods

Sample collection and chemistry analysis

Healthy-looking *G. uralensis* F. plants were randomly collected in the summer of 2015 from their natural arid habitats in Xinjiang province of China. The study - sites were Xinyuan (N 43°23′229″; E 83°50′248″), Gongliu (N 43°37′188″; E 81°48′787″), and Tekesi (N 43°19′205″; E 81°48′601″), each within Xinjiang Province (Fig. 1). Three healthy plants were collected from each site; plants were at least 2 m apart within an area of 100 m^2. Whole plants, including root systems (15–20 cm depth), were placed in Zip-loc bags, and stored at 4 °C during transportation to the laboratory. Plants were processed and screened for endophytic bacteria within 48 h of collection.

Soil samples were taken from each sample collection site and transported to the lab without temperature control and subsequently air dried with 48 h of collection. Soil organic matter was measured by the $K_2Cr_2O_7$ method (Nelson and Sommers 1996); total N was measured with Kjeltec system 1026 Distilling Unit (Tecator AB, Sweden); soil available phosphorus and potassium were measured by the standard methods used by (Olsen and Sommers 1982; Knudsen et al. 1982). Soil pH was measured in water (soil:water ratio = 1:5); electrical conductivity (EC) was measured using a portable conductivity meter (Cole-Parmer Instrument Company, USA). Soil soluble salts were analyzed using methods described by the Nanjing Institute of Soil Science, Chinese Academy of Sciences (1980).

Isolation, purification, and preservation of the endophytic bacteria

Each plant sample was thoroughly washed with running tap water to remove adhering epiphytes and soil debris. After washing, the samples were separated into leaves, stems, and roots, and then successively cut into 1–2-cm long pieces by using sterile scissors. Tissue samples were separately surface-sterilized in a laminar air flow chamber by immersing them sequentially, with shaking, in 0.1% Tween 20 for a few seconds, 70% ethanol for 3 min, and 5% NaOCl for 5 min. Subsequently, samples were rinsed in sterile distilled water 3–4 times for each step of surface sterilization. After 24 h of drying in a laminar air flow chamber, sterilized parts of the selected plants, including leaves, stems, and roots, were used for the isolation of endophytes. Samples were cut into 0.5 cm long fragments under aseptic conditions and ground in a sterilized blender. About 1 g of tissue homogenate was weighed aseptically and macerated with a sterile mortar and pestle, along with 9 mL sterile distilled water, and then transferred to a sterile polypropylene tube. After that, the tissue homogenate was centrifuged at $2200 \times g$ for 5 min. The supernatant was collected and serially diluted to a final concentration of 10^{-2} and 10^{-3} and then 100 μL of each dilution was plated in triplicate onto ten different isolation media (Table S2). The agar plates were incubated at 28 °C and monitored every 5 days for microbial growth. Colonies with distinct colony morphology were picked and re-streaked for purification.

The efficiency of surface sterilization was tested by plating 100 μL of the final rinse onto the ten selective isolation media. No microbial growth was detected on the isolation media after 7 days of incubation at 28 °C when the distilled water used in the final rinse of surface-sterilization was plated. This result indicated that the three-step surface sterilization protocol was successful in removing or killing epiphytic bacteria. The subsequent endophytic bacteria obtained during the isolation were, therefore, considered to be true endophytes.

The isolates were stored in the isolation medium with 30% glycerol at − 80 °C.

Genotypic characterization and identification

DNA was extracted from bacterial cells grown in ISP_2 broth for 2 days at 28 °C by using Chelex® 100 sodium following the manufacturer's instructions (Sigma, Shanghai, China). The extracted DNA was dissolved in 20 μL TE buffer and DNA concentration was determined using a NanoDrop2000 spectrophotometer (Thermo Scientific, Waltham, USA) and used as the template for PCR. PCR amplification of the 16S rRNA gene was performed by using primers 27F (5′-

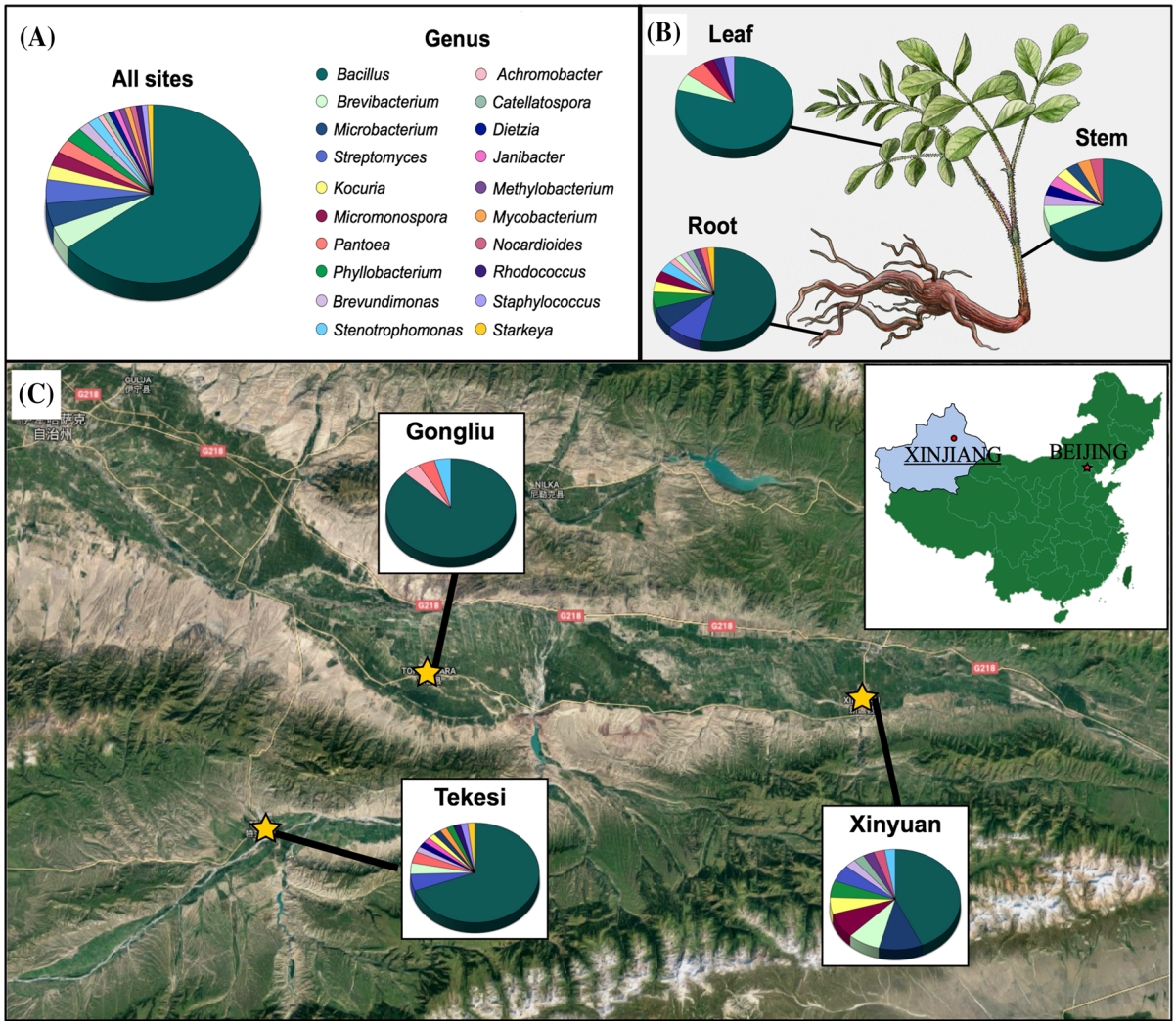

Fig. 1 The distribution and identity of 116 culturable endophytes from *Glycyrrhiza uralensis* F. based on 16S rRNA gene sequences. **a** A summary of genera present at all sites. **b** Genus assignments for isolates from different tissues, showing high diversity in the root and low diversity in the leaf. **c** Genus assignments according to location, showing the lower diversity of isolates from the most arid site, Gongliu. (Color figure online)

CAGAGTTTGATCCTGGCT-3′) and 1492R (5′-AGGAGGTGATCCAGCCGCA-3′) (Lane 1991). DNA was amplified in a BIORAD C1000 Thermal Cycler in a total volume of 25 µL consisting of 1 µL of genomic DNA (approximately 50 ng), 0.5 µL of each primer (10 pmol), 2.0 µL of deoxynucleotide triphosphates (2.5 mM each), 1× PCR buffer, and 1.0 U of Taq DNA polymerase. PCR was performed under the following conditions: initial denaturation step at 95 °C for 6 min, followed by 35 cycles of denaturation at 94 °C for 45 s, annealing at 57 °C for 1 min and extension at 72 °C for 1 min 30 s, with a final extension step at 72 °C for 10 min. The amplified PCR products were analyzed by agarose gel electrophoresis and sequenced using the Sanger method using primers 27F and 1492R. The near-complete 16S rRNA sequences were compared with the GenBank database via BLAST by using the EzBiocloud server (http://www.eztaxon.org) (Chun et al. 2007).

Characterization of potential plant-beneficial traits

Indole acetic acid (IAA) production assay

The ability of bacterial endophytes to produce indole-3-acetic acid (IAA) was tested from bacterial cultures grown in 25 mL of TYC broth (3 g L^{-1} yeast extract; 5 g L^{-1} tryptone; and 0.872 g L^{-1} $CaCl_2 \cdot 2H_2O$) with 0.1% (w/v) L-tryptophan for 5 days at 30 °C at 125 rpm, and were measured based on the colorimetric method. Stationary-phase cultures were centrifuged at 10,000 rpm for 5 min, and 1 mL of supernatant was mixed with Salkowski reagent (2 mL of 0.5 M $FeCl_3$, and 98 mL of 35% $HClO_4$) (1:1 v/v) and incubated in the dark for 30 min (Gordon and Weber 1951). Development of pink color indicated indole production. Results were confirmed by measuring the optical density at 530 nm and comparing with known amounts of IAA using Salkowski reagent and TYC broth with tryptophan and without endophytes as blanks (Li et al. 2012).

Detection of siderophores

Siderophore production was assayed based on competition for iron (Fe) between ferric complexes of chrome azurol S (CAS). The medium was prepared by adding 100 mL of MM9 salt solution (50 g L^{-1} NH_4Cl; 15 g L^{-1} KH_2PO_4; 25 g L^{-1} NaCl; 500 mL ddH_2O) to 750 mL of distilled water, followed by addition of piperazine-*N*, N^0-bis 2-ethanesulfonic acid (PIPES, 32.24 g), and adjusting the pH to 6.0. Then, this was followed by addition of 1.5% agar, and the medium was autoclaved at 121 °C for 20 min. After the medium was cooled to 50 °C, the following filter-sterilized solutions were added: 30 mL of filter-sterilized 10% (wt/vol) casamino acids, 10 mL of 20% (wt/vol) glucose, and 100 mL of dye solution, described below. The blue agar medium was aseptically poured into sterile plates and allowed to solidify. All the bacterial isolates were inoculated into the CAS medium and incubated at 30 °C for 5–7 days. Change of the blue color of the medium surrounding the bacterial growth and appearance of an orange/purple or purple/red halo zone around the colonies in the plates was scored as positive for production of siderophores (Alexander and Zuberer 1991).

The dye solution was prepared by mixing 50 mL of Chrome azurol S solution (0.06 g) with 9 mL of $FeCl_3 \cdot 6H_2O$ solution (0.0027 g dissolved in 10 mL of 10 mM HCl). The resulting solution was mixed with 0.073 g of hexadecyltrimethylammonium bromide in 40 mL of ddH_2O slowly along the flask wall with enough agitation to mix the solution.

Phosphate solubilization

All endophytic bacterial isolates were screened for solubilization of inorganic phosphate on solid Pikovskya's medium (0.5 g L^{-1} yeast extract; 0.5 g L^{-1} $(NH_4)_2SO_4$; 10 g L^{-1} glucose; 0.2 g L^{-1} NaCl; 0.1 g L^{-1} $MgSO_4 \cdot 7H_2O$; 0.2 g L^{-1} KCl; 0.002 g L^{-1} $FeSO_4 \cdot 7H_2O$; 0.002 g L^{-1} $MnSO_4 \cdot 2H_2O$) supplemented with $Ca_3(PO_4)_2$ (5 g L^{-1}) and bromophenol blue (0.025 g L^{-1}) (Pikovskaya 1948; Paul and Sinha 2017). After 7 days of incubation at 30 °C, the formation of yellow halos and/or clearing zones was evaluated. The change of color from blue to yellow or formation of a clear halo around the colonies was indicative of acid production.

Nitrogen fixation and potassium solubilization

The bacterial isolates were screened for the ability to fix nitrogen by using two nitrogen-free media: Ashby's mannitol agar (0.2 g L^{-1} KH_2PO_4; 0.2 g L^{-1} $MgSO_4$; 0.2 g L^{-1} NaCl; 5.0 g L^{-1} $CaCO_3$; 10.0 g L^{-1} mannitol; 0.1 g L^{-1} $CaSO_4$; 15.0 g L^{-1} agar; pH 7.0) and NFC medium (10.0 g L^{-1} mannitol; 0.2 g L^{-1} $MgSO_4 \cdot 7H_2O$; 0.2 g L^{-1} KH_2PO_4; 0.2 g L^{-1} NaCl; 0.2 g L^{-1} $CaSO_4 \cdot 2H_2O$; 5.0 g L^{-1} $CaCO_3$; 15.0 g L^{-1} agar; pH 7.2) (Sen and Sen 1965; Rao 1977; Liu et al. 2016). All the test organisms were incubated at 30 °C for 7 days. Nitrogen fixation activity was observed based on colony growth on the agar plates. For screening of potassium-solubilizing ability, bacterial strains were cultured in a synthetic medium containing 5.0 g L^{-1} sucrose; 0.5 g L^{-1} $MgSO_4 \cdot 7H_2O$; 2.0 g L^{-1} Na_2HPO_4; 0.5 g L^{-1} $(NH_4)_2SO_4$; 0.1 g L^{-1} $CaCO_3$; 0.005 g L^{-1} $FeCl_3$; 0.2 g L^{-1} yeast extract; 1.0 g L^{-1} waste mica (K source); 15.0 g L^{-1} agar; pH 7.5 (Basak and Biswas 2010). All endophytic bacterial strains were incubated at 30 °C for 7–10 days; a halo around the colonies was scored as positive.

Estimation of proteolytic, lipolytic, and cellulytic activity

Protease activity was assayed with YEM agar medium containing 5% (v/v) skim milk. After incubation for 5–7 days at 30 °C, a clear halo around the bacterial colonies due to hydrolysis of milk indicated a positive reaction (Brown and Foster 1970). Lipase enzyme activity was assayed with modified Sierra lipolysis agar supplemented with beef extract (3 g L^{-1}) and ferrous citrate (0.2 g L^{-1}) (Sierra 1957). After autoclaving, 50 mL of Victoria Blue B solution (0.1 g per 150 mL) and 10 mL of Tween 80 was added to the agar medium. After 5–6 days of incubation at 30 °C, white calcium precipitates around the bacterial colonies indicated a positive reaction (Li et al. 2012). Cellulase enzyme activity was assayed with modified DSMZ medium 65 (http://www.dsmz.de/microorganisms/media_list.php) without $CaCO_3$ and supplemented with carboxymethyl cellulose (5 g L^{-1}; Sigma, Shanghai, China) in place of glucose. After incubation for 5–6 days at 30 °C, plates were stained with a Congo red solution and destained with a NaCl solution (Teather and Wood 1982). A clear or lightly colored halo around the colonies indicated a positive reaction. For all the tests mentioned above, sterile nutrient agar was used as a control for bacterial growth. All experiments were performed twice with three replicates for each isolate.

Plant growth promotion assay

To investigate the direct effects of our strains on plant growth, *G. uralensis* F., the host plant, and *T. aestivum* (wheat) were used in this study. Seeds were sterilized by sequential washing in 95% ethanol for 15 min and 25% sodium hypochlorite (NaOCl) for 5 min, followed by rinsing three times in sterilized double distilled water. Sterilized seeds were germinated in agar Petri dishes at 28 °C for 48 h. Five seedlings were transplanted into each pot containing a very low nutrient soil mixture (sand, peatmoss, stone, perlite) (1:1:1:1), with ddH_2O used for plant watering (Chelius and Triplett 2000; Dong et al. 2003). A suspension of endophytic bacteria (10^8 cells/mL) was prepared using DensiCHEK Plus (Biomerieux, St. Louis, USA) and each seedling was inoculated with 5 mL of this suspension. Twelve strains that were positive for at least three plant-beneficial traits were tested individually. The *G. uralensis* F and *T. aestivum* seedlings were grown in a growth chamber with day and night temperatures of 25 and 18 °C, respectively, and with a 16/8-h light/dark cycle (BIC-400, Shanghai Boxun industry Co, Ltd Medical Equipment Factory, Shanghai, China). Plants were harvested 45 days after planting. All plants were separated into roots and shoots and rinsed with running tap water three times. Seedlings without inoculation of endophytes were considered as a negative control. Growth parameters such as root length, root dry weight, shoot length, shoot dry weight and total biomass were determined. All experiments were done in triplicate.

Statistical analysis

One-way ANOVA with Duncan's multiple range tests for multiple comparisons was used to compare the means of root length, root dry weight, shoot length, shoot dry weight and total biomass, each separately, between plants inoculated with different microbial species. Before analysis, data homogeneity was tested, and all data were homogeneous. All statistical analyses were conducted using SPSS statistical software (SPSS for Windows, Version 13, Chicago, USA).

Nucleotide accession numbers

Near full-length 16S rRNA gene sequences have been deposited in GenBank under Accession Numbers KY127308–KY127422.

Results

Isolation and Identification of Endophytic Bacteria associated with *G. uralensis* F.

Endophytic bacterial isolation and corresponding soil chemical analysis was conducted in three regions of Xinjiang Province, China. The three sites each hosted robust, wild populations of *G. uralensis* F. The soil at each location was sandy, with low organic matter content, low available N, P, and K, high total salt content, and alkaline pH (Table S1). The most extreme soil with regard to low organic matter content, low nutrient content, high salt concentration, and high pH, was at Gongliu.

A total of 116 bacterial isolates were isolated from roots, stems, and leaves of *G. uralensis* F based on their colony morphologies on different media and were further characterized by 16S rRNA gene sequencing. Based on 16S rRNA gene identity, the 116 isolates were assigned to 20 genera and 35 species (Figs. 1a, S1). All genera belonged to the *Firmicutes*, *Actinobacteria*, and *Proteobacteria*, including the classes *Alphaproteobacteria*, *Betaproteobacteria*, and *Gammaproteobacteria*. Most of the isolates belonged to the genus *Bacillus* (65% of total isolates) in the *Firmicutes*, but the highest genus-level diversity was in the *Actinobacteria* (n = 11). Other prevalent genera were *Brevibacterium*, *Microbacterium*, and *Streptomyces* (4% each); followed by *Kocuria, Micromonospora, Pantoea*, and *Phyllobacterium* (3% each); *Stenotrophomonas* and *Brevundimonas* (2% each); and subsequently *Achromobacter*, *Catellatospora*, *Dietzia*, *Janibacter*, *Methylobacterium*, *Mycobacterium*, *Nocardioides*, *Rhodococcus*, *Staphylococcus*, and *Starkeya* (1% each) (Table S3). The isolation media used in this investigation each supported growth of a high diversity of microorganisms (Fig. S2). The highest number of species was isolated on M10 (n = 13), including members of the genera *Bacillus*, *Brevibacterium*, *Brevundimonas*, *Kocuria*, *Microbacterium*, *Mycobacterium*, *Nocardoides*, *Pantoea*, and *Stenotrophomonas*. The lowest number was obtained on M4 (n = 5).

The diversity of culturable endophytic bacteria was strongly dependent on the plant tissue and sampling location. The highest number of distinct bacterial isolates, selected based on distinct colony morphology, was from root tissue (n = 54), compared to the leaves (n = 34) and stems (n = 28) (Table S3). Similarly, the number of genera associated with roots, stems, and leaves was 14, 9, and 6, respectively (Fig. 1b). Meanwhile, the highest number of isolates was from the Tekesi site (n = 55), followed by Xinyuan (n = 37), and Gongliu (n = 24) (Table S3). Similarly, the number of distinct genera was higher at Tekesi (n = 14) and Xinyuan (n = 12), compared to Gongliu (n = 4) (Fig. 1c). There was a similar drop in higher-level taxa at Gongliu. Almost all isolates from Gongliu belonged to the endospore-forming genus *Bacillus* (88% of isolates), whereas *Proteobacteria* were rare (12%), and *Actinobacteria* were absent (Fig. 1c; Table S3).

Beneficial plant traits of endophytic bacteria

All endophytes isolated from medicinal plant *G. uralensis* F. were screened for multiple plant growth-promoting traits in order to discover promising endophytic microorganisms (Figs. 2, S3; Table S4). Most strains exhibited one or more plant growth-promoting activities. Among all isolates, about 15% of them synthesized IAA, including members of the genera *Achromobacter*, *Bacillus*, *Brevibacterium*, and *Stenotrophomonas*. About 23% of the isolates produced siderophores, and these isolates belonged to various species within *Bacillus*, *Achromobacter*, and *Janibacter*. Similarly, about 25% of strains were able to solubilize potassium, including *Bacillus*, *Brevibacterium*, and *Phyllobacterium* isolates. In contrast, only 13% of isolated strains were able to solubilize phosphate, and phosphate solubilization was restricted to *Bacillus* and *Microbacterium* isolates. Most of the strains could fix nitrogen (76%), including members of the genera *Achromobacter*, *Bacillus*, *Brevibacterium*, *Microbacterium*, *Mycobacterium*, *Nocardiodes*, *Pantoea*, *Phyllobacterium*, *Rhodococcus*, and *Stenotrophomonas*.

In addition, the isolates were screened for the presence of hydrolytic enzymes. Most of the strains were able to produce one or more hydrolytic enzymes, but protease, cellulase, and lipase activities were only present in 65, 37, and 47% of isolates, respectively (Figs. 2, S3; Table S4). The strains that could produce all three hydrolytic enzymes belonged to various species within *Bacillus* and *Pantoea*.

Effect of selected endophytic bacteria on the growth of two plants

In the present work, all tested isolates showed at least one positive test for the plant growth-promoting properties. To investigate further, twelve strains with multiple plant beneficial traits were selected to evaluate growth enhancement in growth chamber experiments with *G. uralensis* F and *T. aestivum* under low-nutrient conditions (Table 1). Plants were harvested 45 days after inoculating the strains, and plant growth parameters were measured. For *G. uralensis* F. (Figs. 3, S4; Table S5), none of the strains increased root dry weight (Fig. 3a), but eight of the twelve strains significantly increased root length (Fig. 3b), including seven of the eight *Bacillus* strains, and

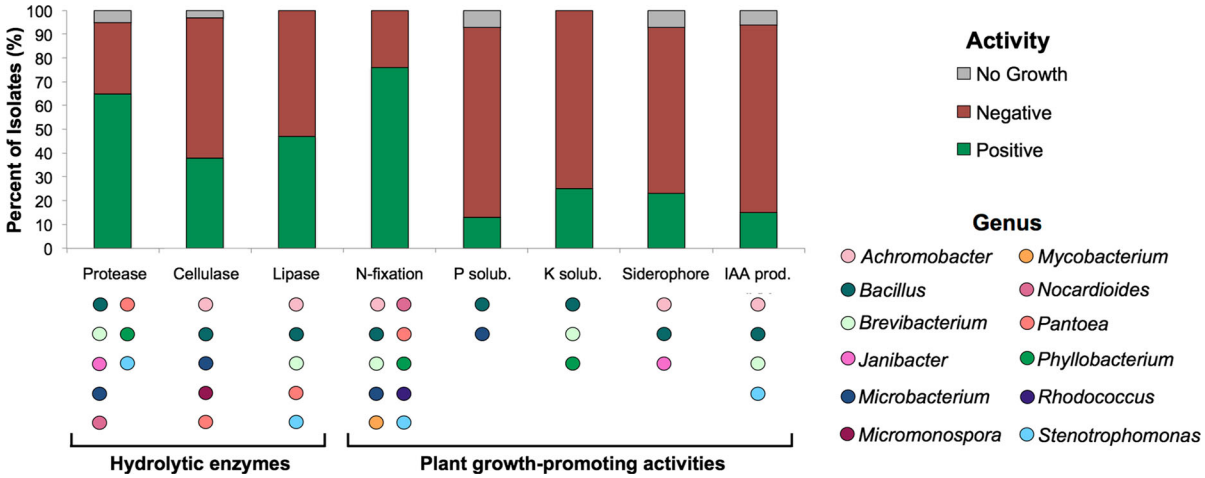

Fig. 2 Plant growth-promotion activities of endophytic bacterial isolates from *Glycyrrhiza uralensis* F. in vitro. *N-fixation* nitrogen fixation, *P solub.* phosphorous solubilization, *K solub.* potassium solubilization, *IAA prod.* production of the plant hormone indole acetic acid. (Color figure online)

Achromobacter spanius. For shoots, seven of the strains increased shoot dry weight (Fig. 3c), again including six of the seven *Bacillus* strains and *A. spanius*; the same strains, minus one *Bacillus* isolate, also stimulated shoot length (Fig. 3d). Seven of the strains increased overall biomass (Fig. 3e), including six *Bacillus* strains in the species *B. aryabhatti*, *B. atrophaeus*, *B. halotolerans*, and *B. mojavensis*, as well as *A. spanius*.

For *T. aestivum* (Figs. 4, S5; Table S5), four of the *Bacillus* strains, along with *Brevibacterium frigoritolerans* and *Stenotrophomonas rhizophila*, stimulated root dry weight (Fig. 4a), whereas all twelve strains significantly enhanced root length (Fig. 4b). Nine of the strains enhanced shoot dry weight (Fig. 4c), whereas eleven of the twelve strains stimulated shoot length (Fig. 4d), including *Bacillus* strains, *A. spanius, B. frigoritolerans*, and *S. rhizophila*. Ultimately, ten of the twelve strains increased overall biomass (Fig. 4e), again including seven *Bacillus* strains, *A. spanius, B. frigoritolerans*, and *S. rhizophila*.

Discussion

To provide greater insight into plant–endophyte interactions, plant growth-promoting activities were measured in a diverse population of endophytic bacteria isolated from the medicinal plant *G. uralensis* F. growing in different areas within arid regions in Xinjiang Province, northwest China. In this study, a total 116 strains of different colony morphology were isolated from surface-sterilized root, stem, and leaf tissues of *G. uralensis* F. The diversity of culturable endophytes differed based on both plant tissue and geographic location in Xinjiang province. The highest diversity was isolated from plant roots, in agreement with other studies (Ma et al. 2013; Liu et al. 2016; Wemheuer et al. 2017). The high bacterial diversity in plant roots may be related to the high bacterial population density in roots, estimated at 10^8 CFU g^{-1} in the rhizosphere, compared with 10^6 CFU g^{-1} in leaves (Rastogi et al. 2012; Jin et al. 2014). The determination of community structure in different tissues is essential for subsequent application of bacteria as biofertilizers (Szymańska et al. 2016). With regard to geography, a similar diversity of endophytes was obtained from the Xinyuan and Tekesi sites, whereas fewer species were isolated from the Gongliu site. Although all three sites are extreme with regard to low organic content, low N, P, and K content, and high salinity, the Gongliu site was the most extreme. Thus, as has been reported previously, environmental factors such as soil type, nutrient content, and salinity are primary factors influencing both vegetation patterns and the composition of plant-associated microorganisms (Sibanda et al. 2017).

In total, this study resulted in the isolation of 20 genera and 35 species, all belonging to the phyla *Firmicutes*, *Actinobacteria*, and *Proteobacteria*,

Table 1 The beneficial traits of the selected endophytic bacteria associated with *G. uralensis* F.

Strain code	Closest species in 16S rRNA gene sequences database	Hydrolytic enzymes			Plant growth-promoting traits					
		Protease[a]	Cellulase[b]	Lipase[c]	Nitrogen fixation[d]		Phosphorus[e]	Siderophore[f]	Potassium[g]	IAA[h]
					NFB	Ashby's				
G001	*Bacillus aryabhattai*	++	–	+	+	+	+	–	+	+
G002	*Brevibacterium frigoritolerans*	–	–	++	+	+	–	–	+	–
G015	*Brevibacterium frigoritolerans*	–	–	++	+	+	–	–	+++	–
G016	*Bacillus mojavensis*	++	+++	+++	+	+	+	+	+++	++
G022	*Bacillus halotolerans*	++	++	++	+	+	+	+	++	+++
G067	*Achromobacter spanius*	–	+	++	+	+	–	+	–	++
G071	*Stenotrophomonas rhizophila*	++	–	++	+	+	–	–	–	+++
G072	*Bacillus atrophaeus*	+++	–	+	+	+	+	+	–	–
G074	*Bacillus halotolerans*	++	+++	++	+	+	+	+	–	–
G078	*Bacillus atrophaeus*	++	–	+	+	+	+	+	+++	++
G083	*Bacillus halotolerans*	+++	+	+	+	+	+	+	++	+
G120	*Bacillus mojavensis*	+	+++	++	+	+	+	+	–	+

[a]Protease production: – no production, + weak halo around the colony (1.00–1.50 cm), ++ clear halo around the colony (1.50–2.00 cm), +++ strong halo around the colony (2.00–3.00 cm)

[b]Cellulose production: – no production, + weak halo around the colony (2.00–2.50 cm), ++ clear halo around the colony (2.50–3.00 cm), +++ strong halo around the colony (3.00–5.61 cm)

[c]Lipase production: – no production, + weak halo around the colony (1.00–1.50 cm), ++ clear halo around the colony (1.50–2.00 cm), +++ strong halo around the colony (2.00–2.50 cm)

[d]Nitrogen fixation: – no ability, + indicates that bacteria can grow on this medium

[e]Phosphorus solubilization: – no solubilization, + indicates to solubilize phosphate by producing clear zones or acid production by changing the color of media from blue to yellow

[f]Siderophore production: – no production, + indicates production of siderophores by changing of the blue color to an orange/purple or purple/red halo zone around the colonies

[g]Ability of dissolving potassium: – no ability, + weak halo around the colony (1.00–3.00 cm), ++ clear halo around the colony (3.00–5.00 cm), +++ strong halo around the colony (5.00–7.00 cm)

[h]IAA: production of the plant hormone indole acetic acid (IAA). Pink color indicated indole production. Values in parentheses refer to the absorbance at 530 nm: - no production (< 0.001), + weak (0.01–0.10), ++ moderate (0.10–0.127), +++ good (0.127–0.140)

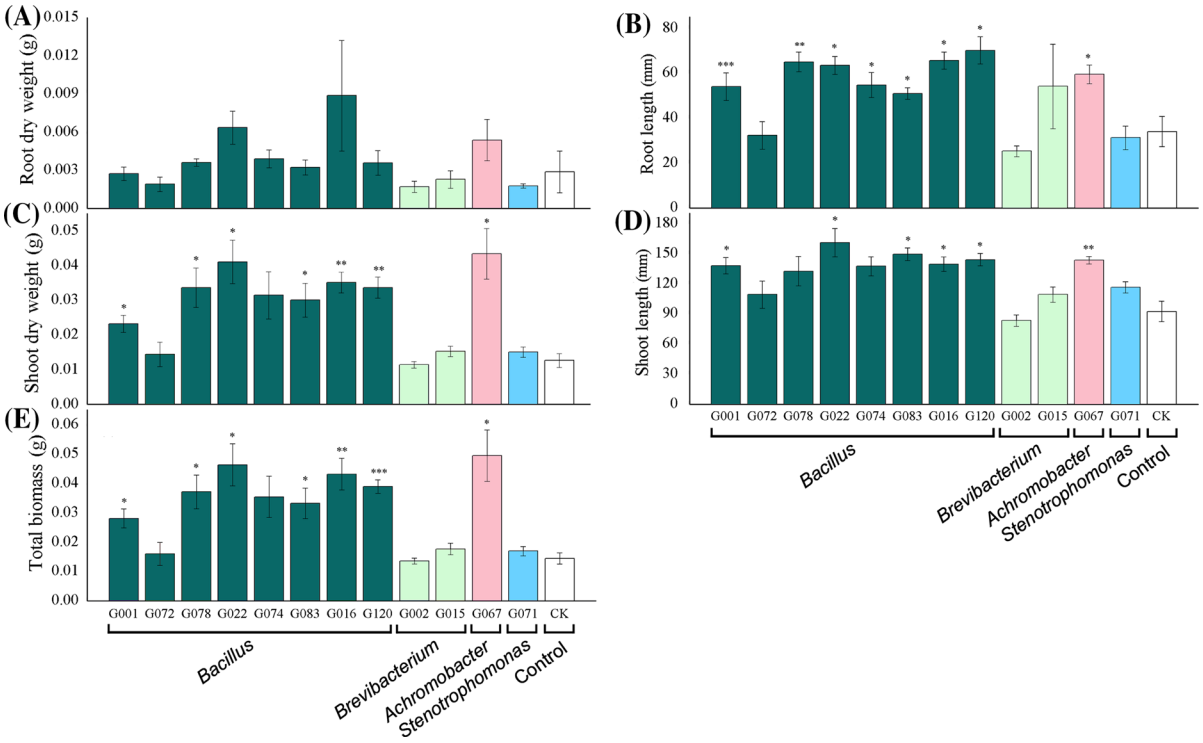

Fig. 3 The response of *Glycyrrhiza uralensis* F. 45 days after inoculation with the selected endophytic bacteria, compared with an uninoculated control plant. Asterisks represent p-values from a one-way ANOVA with Duncan's multiple range test for multiple comparisons. *p < 0.05; **p < 0.01; ***p < 0.001. (Color figure online)

which are abundant in soils worldwide. In a similar investigation conducted by our group in arid regions of Xinjiang, we isolated 27 and 29 genera from the medicinal plants *Ferula songorica* and *Ferula sinkiangensis*, respectively, belonging to the same three bacterial phyla (Liu et al. 2016, 2017). Similarly, Kaplan et al. isolated 31 endophytic strains belonging to four bacterial phyla, *Firmicutes*, *Actinobacteria*, *Proteobacteria,* and *Bacteroidetes*, and 20 genera from roots of two dominant shrubs found in the Negev Desert (Kaplan et al. 2013). These results confirm the rich microbial diversity in plants grown in arid lands, and the dominance of *Firmicutes*, *Actinobacteria*, and *Proteobacteria* among cultivable endophytes. Although the bacterial communities described in these studies were composed of the same phyla and shared some genera, such as *Bacillus*, *Kocuria*, and *Micrococcus*, they also differed somewhat in microbial composition and structure. This general conclusion is also supported by the finding that similar bacterial taxa have been observed using cultivation-independent techniques (Das et al. 2017; Liu et al. 2016; Jin et al. 2014).

Bacillus was the dominant bacterial genus in the tissus of *G. uralensis* F., which is consistent with other reports for endophytic bacteria isolated from medicinal plants (Kumar et al. 2012; Wei et al. 2014; Liu et al. 2016, 2017). *Bacillus* species are widely known for their metabolic activity and various beneficial effects on plant vigor and health. For example, the majority of the registered bacterial products in the European Union Pesticides Database, 2012 are based on species of *Bacillus*. The genus *Streptomyces*, which was the second most prevalent genus in our study, is also well known to grow endophytically within medicinal plants, in addition to its well-known capabilities producing novel secondary metabolites (Qin et al. 2012; Sibanda et al. 2017).

Endophytic bacteria are known to exhibit a wide variety of plant growth-promoting activities. Mechanisms of interactions between endophytic bacteria and host plants have been well-documented previously by (Li et al. 2012; Wong et al. 2014; Halo et al. 2015;

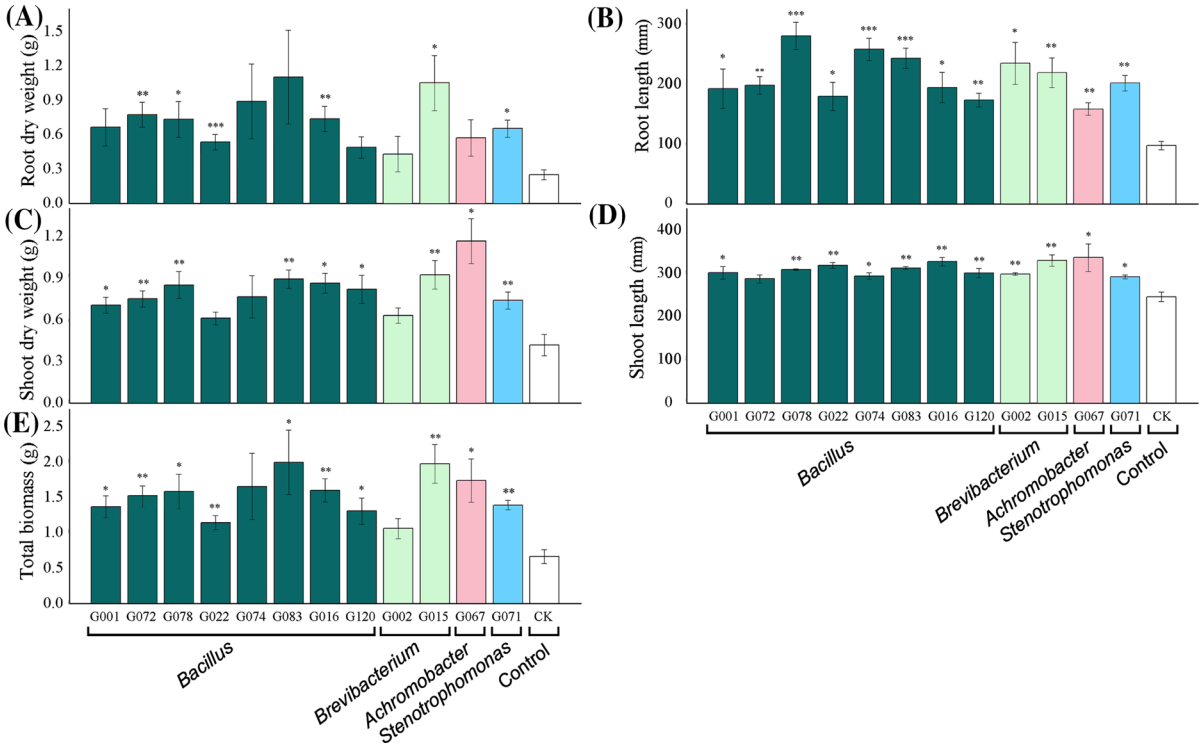

Fig. 4 The response of *Triticum aestivum* 45 days after inoculation with the selected endophytic bacteria, compared with an uninoculated control plant. Asterisks represent p-values from a one-way ANOVA with Duncan's multiple range test for multiple comparisons. *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$. (Color figure online)

Islam et al. 2016; Patil et al. 2016; Kandel et al. 2017), and include the production of phytohormones, siderophores, hydrolytic enzymes, phosphate and potassium solubilization, and diazotrophy. Some studies have reported that endophytic bacteria isolated from medicinal plants produced indole-3-acetic acid (IAA) (Khan et al. 2014), which is involved in stimulation of plant growth by stimulating cell enlargement, division, differentiation, and gene regulation (Leveau and Lindow 2005). In our studies, about 15% of the isolates produced IAA.

These endophytes also exhibited activities to solubilize mineral phases of important elements, specifically iron, phosphorous, and potassium. Iron is an abundant element in the earth's crust, primarily as ferric iron minerals that are not easily solubilized under oxic atmospheres and at circumneutral or alkaline pH, which are predominant in desert environments. About 23% of our isolates produced siderophores, which is important for the reduction of Fe^{3+} to Fe^{2+} and transport into the plant (Lemanceau et al. 2009). It has been shown previously that endophytic bacteria can produce different structural types of siderophores such as hydroxamates, catecholate, and citrate-based polycarboxylates (Ahmed and Holmström 2014), but the structures of the siderophores in our isolates were not determined. Many strains also acted as potassium-solubilizing microorganisms (KSM). In our study, about 25% of the isolates solubilized potassium. Potassium is an essential element for plant growth and development because it is responsible for adjusting cellular osmotic pressure, the transportation of compounds in plant tissues, activation of enzymes, and the synthesis of protein and sugar (Basak and Biswas 2010). Phosphorus plays a major role in numerous plant processes including nucleic acid synthesis, photosynthesis, respiration, energy generation, and cell signaling (Vance et al. 2003). Among the tested isolates, about 13% were capable of solubilizing bound phosphorus and making it available for the plant; therefore, these strains could potentially be used as inocula to increase phosphorus uptake by the plant and thus increase the crop yield (Hameeda et al. 2008; Trivedi et al. 2011).

A majority of isolates (76%) fixed nitrogen under the tested conditions, which is important because most arid soils have low fixed nitrogen content. Nitrogen is important for plant growth and productivity because it is an integral element in proteins, nucleic acids, and other essential biomolecules (Bøckman 1997; Zakry et al. 2012; Lin et al. 2012). Many of the endophytes isolated in this study produced hydrolytic enzymes such as protease, cellulase, and lipase. In similar work, it has been demonstrated that endophytic bacteria isolated from the medicinal plants *Ferula songorica*, *Hypericum perforatum*, and *Ziziphora capital* could secrete different hydrolytic enzymes including cellulase, protease, and amylase (Egamberdieva et al. 2017; Liu et al. 2016). Hydrolytic enzymes may play an important role in initial plant infection, allowing for further spreading within tissues. Additionally, these enzymes are industrially relevant, with their potential uses in various industries including pharmaceuticals, bioremediation, and agriculture having been well-established (Chand and Mishra 2003).

The assessment of the effect of selected endophytic bacteria on the growth enhancement of the host plant (*G. uralensis* F.) and wheat (*T. aestivum*) in pot experiments was conducted under low-nutrient conditions. Under these conditions, most of the tested *Bacillus* strains stimulated root length, shoot length, shoot dry weight, and total biomass in the host plant. In contrast, of the three other genera tested, only *Achromobacter* promoted growth of the host plant. Interestingly, although the endophytes were isolated from *G. uralensis* F., they stimulated growth of wheat under the tested conditions more effectively. In terms of total biomass, all strains except one significantly stimulated growth. We speculate that these results were probably a response of enhancement to microbial production of the plant hormone IAA. This hormone functions as an important signal molecule in the regulation of plant development by enlarging the root system (Leveau and Lindow 2005). In particular, they stimulate the formation of lateral roots and absorbent root hairs (Giassi et al. 2016). Moreover, it has been suggested that the combination of phosphate-solubilizing, nitrogen-fixing, and phytohormone producing bacteria could provide a balanced nutrition for plants and stimulate growth of various crops (Khan et al. 2014; Nimaichand et al. 2016). Our experiments show that the endophytes differ in their plant growth-promoting activities and implicate the genus *Bacillus* as a promising target for biofertilizers.

Conclusions

In summary, the present investigation represents the first report on the distribution and bioactivity of endophytic bacteria associated with the medicinal plant *G. uralensis* F. Our study has led us to conclude that G. *uralensis* F. represents a rich reservoir for diverse endophytic bacteria, which differ based on the specific tissue and geographic location. The results provide insights about plant beneficial traits of culturable endophytic bacteria associated with the medicinal plant licorice. The genus *Bacillus* appears to be the most promising bioinoculum for the two tested plants under growth chamber conditions, and they could be a cost-effective source for agro-based biofertilizer agents. These *Bacillus* strains have various abilities related to plant growth promotion, including solubilization of phosphate, siderophores, potassium, nitrogen fixation, protease, cellulase, lipase, and production of IAA, and were able to promote growth of both licorice and wheat in growth chamber experiments. In light of this broad activity, further evaluation of these strains to identify biologically active compounds and to assess their ability to promote growth of other plants (including medicinal plants) in arid environments is justified.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no indirect or direct conflict of interest.

Ethical approval This article does not contain any studies related to human participants or animals.

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